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 $22 \pm 2\,^\circ$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

of writhes observed in control animals. Dose–response curves were obtained for EMAT1 and ETMAT1autumn extracts, and a single dose effect for GME, ETMAT1summer (200 mg/kg), using groups of 8 animals for a single dose and groups of 16 control animals were treated similarly, but they did not receive the samples. Another group of eight animals was pretreated with ibuprofen used as reference drug. The ibuprofen doses were 3, 1, 0.3 and 0.1 mg/kg. Least squares linear regression analysis of the log-dose–response curves allowed the calculation of the dose that produced 50% of antinociception (ED_{50}) for each extract and reference drug (Delporte et al., 2007).

2.3.2. Tail flick test

A radiant heat automatic tail flick algesiometer (U. Basile, Comerio, Italy) was used to measure response latencies according to a previous report (Miranda et al., 2003). The intensity of the light beam was focused on animal tails 2–2.5 cm from the tip and adjusted for baseline readings between 2 and 3 s. An 8 s cut-off was imposed to avoid tail damage by heat. Control reaction was recorded twice with 20 min intervals between readings. After oral or topical administration of the extracts, the reaction time was tested at 30 min (time of peak effect) and the difference reaction time was recorded (Δlatency). For topical administration, the mouse-tail was immersed for 3 min in DMSO containing the extracts at different concentrations (5, 2.5, 1.25 and 0.625%, w/v). For oral administration, a gastric catheter was used for the different doses (200, 100, 50 and 12.5 mg/kg) of EMAT1, GME, ETMATsummer, ETMATautumn extracts. Tail flick latencies were converted to maximum possible effect (MPE), according to the following formula: MPE (%) = $100 \times$ (post-extract latency – preextract latency)/(cut-off time – preextract latency). Dose–response and concentration–response curves for p.o. and topical administration, respectively were obtained for EMAT1, GME, ETMATsummer, ETMATautumn extracts and for EMAT1, ETMATautumn extracts, only a single dose for comparison was tested for GME and ETMAT-summer. Groups of 8 animals for a single dose and groups of 16 control animals were treated similarly, but they did not receive the extracts. Another group of eight animals were pretreated with ibuprofen-reference drug. For topical administration, 30, 15, 7.5 and 3.75% (w/v) concentrations were used. For oral administration, the reference drug doses were 30, 10, 3 and 1 mg/kg. Least squares linear regression analysis of the log-dose (p.o.)– or log-concentration (topical)–response curves allowed the calculation of the dose or concentration that produced 50% of antinociception (ED_{50} or ED25 and EC_{50} or EC25, respectively) for each extract and reference drug (Delporte et al., 2007).

2.3.3. Tail formalin test

A modified formalin test was used, as described by Koleniskov et al. (2004). The different extracts at 5% (w/v) concentration were applied by topical administration as described in the tail flick test, and mice were immediately intra-dermally injected 20 μL of a formaldehyde solution to 10% into dorsal surface of their tail, using a tuberculin syringe. Then the mouse was located into a chamber, with mirrors walls to enable clear observation of animal tails for 5 min. The nociceptive behavior is directly proportional to the licking time of the tail, which is a monophasic process (Koleniskov et al., 2004). The time-course observation was restricted to 5 min, the length of time during which pain occurs. Antinociceptive activity (A) was expressed according to the following formula: A (%) = $100 - [(T1 \times 100)/T2]$, where $T1$ stands for the mean licking time post-extract and $T2$ is the mean licking time control (Delporte et al., 2007).

All the different extracts were evaluated at 5% (w/v) as single concentration. For each, the topic analgesic activity was evaluated using groups of 8 animals for a single dose and groups of 16 control animals were similarly treated, but they did not receive the extracts. Another group of eight animals were pretreated with ibuprofen, as reference drug at 5, 2.5, 1.2 and 0.6% (w/v) concentrations.

2.3.4. Statistical analysis

Results are presented as $ED_{50} \pm$ S.E.M. or $EC_{50} \pm$ S.E.M. except for the tail formalin test. The statistical difference between theoretical and experimental values was assessed by Wilcoxon test for independent data and p values less than 0.05 ($p \leq 0.05$) were considered significant.

2.4. Pharmacological characterization of antinociception induced by Buddleja globosa extracts

To elucidate the mechanism of action of *Buddleja globosa* extracts several antagonist drugs were administered and the thermal model for nociception – tail flick test – was used.

2.4.1. Buddleja globosa extracts

Two extracts GME and EMAT1 were selected for this study, based in their different chemical composition, and administered topically. The base or control effect of each extract was obtained by administering a dose equivalent to the CE_{25tt} (5.0% and 1.1%, respectively) previously calculated according to data shown in Table 1. The effect at these concentrations for each extract was $23.38 \pm 3.43\%$ and $27.81 \pm 2.08\%$, respectively (Table 2) effect that will be considered as the maximum in the experiments with several antagonists.

2.4.2. Antinociceptive antagonists

In order to characterize pharmacologically the antinociception induced by *Buddleja globosa* extracts, the following drugs were used: naltrexone (NTX), a non-selective antagonist of mu, delta and kappa opioid receptors (MOR-R, DOR-R, KOP-R, respectively); naltrindole (NTI), a selective antagonist of DOP-R; nor-binaltorphimine (nor BNI), selective antagonist of KOP-R; tropisetron, a selective antagonist of the 5-HT$_3$ receptors; prazosin, antagonist of α_1-adrenoceptors; yohimbine, of α_2-adrenoceptors; atropine, a non-selective cholinergic muscarinic receptors and *N*-nitro-L-arginine methyl ester (L-NAME), a known non-selective NOS inhibitor. The doses used (Table 2), were adapted or modified from previously published studies that showed pharmacological activity of each individual receptor subtypes and were administered intraperitoneal (i.p.) and the reaction time was tested at time of peak effect (30 or 60 min) (Rang et al., 2000; Miranda et al., 2008).

2.4.3. Tail flick test

This test was applied as described above. After reaching time peak effect of each administered antagonist (30 min or 60 min), the mouse-tail was immersed for 3 min in DMSO containing EMAT or GME extracts at CE_{25tt} (1.1 and 5%, w/v) and reaction time was recorded after 5 min.

DMSO solution provides an effective way for solubilization of the different extracts and facilitating their transport through skin. DMSO has no effect on topical tail flick and formalin assays.

3. Results

3.1. Plant extraction and major constituents

As described above, no significant chemical differences were observed when extracting plant material with methanol or ethanol for sequential extracts. The major constituents were verbascoside and 7-*O*-luteolin glycoside. Even though significant differences were shown between ETMAT summer collection and ETMAT

Table 1
Antinociceptive effect of *Buddleja globosa* extracts, isolated compounds and reference drug (ibuprofen) in thermal and chemical pain models

Sample	Doses (p.o.mg/kg topic %)	Tail flick p.o.		Tail flick topic		Formalin topic (% effect)	Writhing p.o.	
		% Effect	ED_{25} (mg/kg)	% Effect	EC_{25} (%, w/v)		% Effect	ED_{50} (mg/kg)
EMAT1	200	56.3 ± 6.0	59.0 ± 6.1				79.8 ± 14	53.5 ± 6.0
	5			54.0 ± 4.2	1.1 ± 0.1	73.6 ± 10.9		
ETMAT (summer)	200	77.2 ± 9.3	50.8 ± 1.3				85.5 ± 4.2	n.d.
	5			51.6 ± 4.7		90.8 ± 3.3		
ETMAT (autumn)	200	32.7 ± 5.4	133.5 ± 34.8				59.5 ± 5.4	149.0 ± 16.3
	5			40.5 ± 4.5	2.5 ± 0.2	68.9 ± 7.8		
GME	200	47.0 ± 4.7	83.3 ± 1.3				64.1 ± 3.2	n.d.
	5			26.8 ± 5.1	4.8 ± 0.9	85.7 ± 4.2		

Sample	Doses (mg/kg–mmol/kg) (%d w/v–mM)	Tail flick p.o.		Tail flick topic		Formalin topic (% effect)	Writhing p.o.	
		% Effect	ED_{25} (mg/kg)	% Effect	EC_{25} (%, w/v)		% Effect	ED_{50} (mg/kg)
Ibuprofen	45.4–0.22	25.0 ± 2.3	45.4 ± 10.7					
	14.4–0.07						50.0 ± 4.1	14.4 ± 4.3
	0.8%–40			25.0 ± 0.1	0.8 ± 0.1			
	5%–240					76.5 ± 6.3		
Verbascoside	137.6–0.22	27.5 ± 3.8	n.d.					
	30.1–0.05					n.a.	67.6 ± 2.3	n.d.
	1%–16			24.5 ± 3.6	n.d.			
Luteolin-7-*O*-gl	98.6–0.22	28.2 ± 4.9	n.d.					n.d.
	22.0–0.05					n.a.	27.0 ± 9.1	
	1%–22.3			30.3 ± 7.3	n.d.			

$p \leq 0.05$; $ED_{50} =$ dose (mg/kg) that produces 50% antinociception in acetic acid writhing test (p.o. administration). $ED_{25} =$ dose that produces 25% antinociception in tail flick test (p.o. administration). $EC_{25} =$ concentration (%, w/v) that produces 25% antinociception in tail flick test for topical administration. EMAT1: serial methanol extract, ETMATsummer and ETMATautumn: serial ethanol extract from leaves collected in summer and autumn, respectively, GME: global methanol extract. Each group represents the mean ± S.E.M. of eight animals pretreated with extracts or reference drug. n.d., not determined; n.a., not assayed.

autumn collection, as the last presented nearly half the amount of verbascoside, mean while flavonoids concentration was not affected (data still not published).

Global methanol extract (GME) differs from EMAT1 and ETMAT as it contains the mixture of all the components already described in *Buddleja globosa*: triterpenoids, steroids, flavonoids, phenylethanoids (Backhouse et al., 2008).

3.2. Writhing test

The p.o. administration of the serial methanol and ethanol extracts of *Buddleja globosa* leaves induced a dose-dependent antinociceptive activity and the ED_{50} values ± S.E.M. for the extracts

Table 2
Influence of nociceptive antagonists in *Buddleja globosa* extracts analgesic effects in the tail flick model

Extract (antagonist + Extract(EC_{25tt}))	EMAT1 (% MPE_{EMAT})	GME (% MPE_{GME})
Antinociceptive activity		
Extract (EC_{25tt})	27.81 ± 2.08	23.38 ± 3.43
$NTX_{i.p.}$ + extract	$57.52^* \pm 10.94$	$43.57^* \pm 3.25$
$NTI_{i.p.}$ + extract	$45.98^* \pm 2.46$	25.73 ± 2.92
$norBNI_{i.p.}$ + extract	$40.46^* \pm 1.85$	24.53 ± 2.14
$TROPI_{i.p.}$ + extract	$58.15^* \pm 4.21$	$39.88^* \pm 4.21$
$PRAZ_{i.p.}$ + extract	$54.10^* \pm 6.72$	$51.95^* \pm 6.81$
$YOH_{i.p.}$ + extract	31.91 ± 5.43	27.70 ± 3.86
$ATRO_{i.p.}$ + extract	34.93 ± 3.08	22.58 ± 2.29
L-$NAME_{i.p.}$ + extract	$50.23^* \pm 6.55$	$45.66^* \pm 9.18$

Extract: methanol serial extract (EMAT) or methanol global extract (GME) at EC_{25} (1.1 and 5% (w/v), respectively); %MPE_{EMAT} and %MPE_{GME}: percentage of maximum effect observed with the samples: extracts alone (EMAT or GME) and extracts previous i.p. administration of antagonists. $NTX_{i.p.}$: naltrexone$_{i.p.}$ (1 mg/kg), $NTI_{i.p.}$: naltrindole (1 mg/kg), $norBNI_{i.p.}$: nor-binaltorphimine (0.5 mg/kg) $TROPI_{i.p.}$: tropisetron (1 mg/kg), $PRAZ_{i.p.}$: prazosin (0.1 mg/kg), $YOH_{i.p.}$: yohimbine (0.01 mg/kg) $ATRO_{i.p.}$: atropine (1 mg/kg), L-$NAME_{i.p.}$: (5 mg/kg).

* $p < 0.05$ extract (EMAT or GME) vs. antagonist + extract.

and isolated compounds are shown in Table 1. Methanol and ethanol extracts from leaves collected in summer time are the most active when compared with those collected in autumn (ED_{50} 59.5 and 149.0 mg/kg, respectively). This activity could be in great part attributed to verbascoside that showed higher effect than ibuprofen at similar doses (0.07 and 0.05 mmol/kg with 50 and 67.6% of effect). Also luteolin 7-*O*-glucoside is contributing to analgesia. No significant pharmacological solvent influence was found, analgesic effect was similar for EMAT and ETMATsummer extracts. This can be explained by the presence of the same active metabolites in both and in similar concentration (data still not published). The GME also showed important analgesic effect, though weaker than the serial extracts, due to the complex composition of this extract and smaller amount of verbascoside. The higher effect observed for EMAT1 in this assay in comparison with our previous work (Backhouse et al., 2008) is due to the modifications of the method as writhes were count only during the first 5 min after the i.p. injection of acetic acid.

3.3. Tail flick test

The antinociceptive activity induced by oral or by topical administration of the extracts was dose-dependent; Table 1 shows the ED_{25} (p.o.) and EC_{25} (topical administration) values using the tail flick test. The ETMATautumn showed the weakest activity when administered *per os* (ED25: 133.5 mg/kg) confirming seasonal pharmacological influence. The most active extracts were EMAT1 and ETMAT1summer, both by oral and topic administration, and no significant differences were observed when comparing their ED25 (oral); nevertheless at the maximum doses assayed p.o. (200 mg/kg) the ethanol extract was more active. Moreover, the results obtained for GME confirmed the importance in considering a serial purified alcohol extract (EMAT1 or ETMAT1summer) for future therapeutic formulations.

3.4. Tail formalin test

Topical administration induced a strong antinociceptive activity for all extracts at the single concentration assayed (5%) shown in Table 1. No important differences were observed, though in this assay GME effect was slightly higher than EMAT1; moreover GME and ETMATsummer showed higher activity (90.8 and 85.7%, respectively) than the reference drug (76.5%).

3.5. Pharmacological characterization of antinociception induced by Buddleja globosa extracts

The pretreatment (30 min before the extract) with NTX, TROPI, PRAZ or L_NAME significantly increased the effect of EMAT and GME (at CE_{25}). Previous administration of Nor-BNI or NTI also increased antinociceptive effect of EMAT, nevertheless no influence was observed on the analgesic activity of GME. Moreover, the analgesic effect induced by both *Buddleja globosa* extracts was not affected with pretreatment with either YOH or ATRO.

The present study allowed us to propose a selective effect of MOR, DOR and KOR antagonists over EMAT activity. Similar response was obtained with 5-HT$_3$ receptor antagonists: α_{1A} and non-selective NOS inhibitor. On the contrary, GME was modified only by MOR non-selective antagonist, by receptors 5-HT$_3$ inhibition; α_{1A} and non-selective NOS inhibition. The ACh and α_2 receptors participation was not significant for both EMAT and GME extracts.

4. Discussion

The present study demonstrated that methanol or ethanol sequential extracts (EMAT1 and ETMAT1) of *Buddleja globosa* leaves produce a dose-dependent antinociceptive activity, as observed through different algesiometric tests. The major metabolites involved in sequential extracts antinociception mechanism are verbascoside and 7-O-luteolin glycoside; while, triterpenoids, sterols, flavonoids and phenylethanoids, in the global methanol extract nociception mechanism.

Verbascoside and luteolin glycoside showed similar topical analgesic effects, but verbascoside exhibited a much stronger oral analgesic effect than the flavonoid. This could be due to the poor intestinal absorption of luteolin glycoside which must be previously metabolized enzymatically to increase intestinal absorption (Shimio et al., 1998, 2001). Flavonoid skin penetration studies have demonstrated that they can be absorbed from the dermis to penetrate deeper cutaneous layers (Merfort et al., 1994), justifying their stronger topical activity.

Verbascoside, the major component of EMAT1 and ETMAT1 has been reported for its antinociceptive and anti-inflammatory activity (Küpeli et al., 2007), together with its ability for inhibiting the formation of PGE2, the tumor necrosis factor-α (TNF-α), nitric oxide (NO), suppressing the inducible nitric oxide synthase (iNOS), the enzymatic activity of cyclooxygenase (COX-2) and for exerting a protective action against oxygen free radical-induced peroxidative damage to biomembranes (Lee et al., 2005). Moreover, these not only can explain its anti-inflammatory activity and protective endothelial function but also the analgesic effect (inhibition of PGE2).

Luteolin and luteolin-7-O-glucoside also suppress iNOS and COX-2 inducible enzymes responsable for nitric oxide production and PGE2 release that are increased in human tumor tissues and inflammation disorders. The inhibition of PGE2 production can explain the analgesic effect here found (Hu and Kitts, 2004). Moreover, the antinoceciptive effect of *Balbisia calcyna* and of *Wedelia*

paludosa has been attributed to the presence of luteolin (Block et al., 1998; Miño et al., 2002).

Sequential and global extracts possess both central and peripheral antinociception and the mechanism of action of the active principles of these extracts could be partially related to lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade and/or opioid receptors inducing antinociception by multiple neurotransmisers (Deraedt et al., 1980; Le Bars et al., 2001).

The thermal model of the tail flick tests considered to be a spinal reflex, could also involve higher neural structures identifying mainly central analgesic (Jensen and Yaksh, 1986; Le Bars et al., 2001). Thus the results provide direct evidence that the EMAT antinociceptive activity can be increased by selective blocking MOR, DOR and KOR opioid receptors. In contrast, the antinociception developed by GME seems modulated only by naltrexone. The reasons for these differences are unclear, but in part it might be due to the diverse chemical constituents and reflect their different sites of action, related to the distribution of opioid receptors. In addition, these interactions may be explained by the paradoxical bimodal effect of opioid antagonist, to induce analgesia in mice by blocking the presynaptic autoinhibition of endogenous opioid peptides release thus causing an exaggerated release of endogenous opioids (Gourlay, 2005).

In relation to the modulatory effects induced by selective serotonergic antagonist tropisetron, increasing the antinociceptive activity of both, EMAT and GME, is concordant with the fact that serotonergic administration has been reported to be antinociceptive or facilitatory (Millan, 1999). Moreover, it has been reported that 5-HT3-receptors antagonist, such as tropisetron, are involved in peripheral and central perception and processing of pain as well in inflammation (Riering et al., 2004). Besides, ligand binding at the 5-HT3 receptor causes manifold effects on other neurotransmitter and neuropeptides systems. In particular, diminish serotonin-induced release of susbstance P from C-fibers and prevent unmasking of NK2-receptors in the presence of serotonin (Wolf, 2000).

The augmented modulatory effect of prazosin over EMAT and EMG seems to be according the reported activation of α_1-adrenoceptors during the nociceptive transmission (Miranda et al., 2001). In addition it has been reported that prazosin reduced or suppressed nocifensive responses (e.g. flinching) induced by local administration into the plantar hind-paw of the combination of a ligand for P2X3/P2X2/3 receptors with adrenaline (Meisner et al., 2007).

5. Conclusion

The present study demonstrated that methanol or ethanol sequential extracts of *Buddleja globosa* leaves produce a dose-dependent antinociceptive activity, as observed through different algesiometric tests.

Differences in analgesic oral effects between autumn and summer sequential ethanol extracts could be attributed to verbascoside concentration.

Our results corroborate the analgesic effects of *Buddleja globosa* not described by folk medicine, and it is an important finding for a traditional species used for treating wound healing.

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