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Tracheal relaxation of five medicinal plants used in Mexico for the treatment of several diseases

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ABSTRACT

Objective: To assess the relaxant effect of several organic extracts obtained from *Agastache mexicana* (*A. mexicana*), *Cochlospermum vitifolium* (*C. vitifolium*), *Cordia morelosana* (*C. morelosana*), *Lepechinia caulescens* (*L. caulescens*) and *Talauma mexicana* (*T. mexicana*) used in Mexican traditional medicine for the treatment of several diseases. **Methods:** Extracts were obtained by maceration at room temperature using hexane, dichloromethane and methanol for each plant material. The organic extracts were evaluated *ex vivo* to determine their relaxant activity on the contractions induced by carbachol (cholinergic receptor agonist, 1 μ mol/L) in isolated rat tracheal rings. **Results:** A total of 15 extracts were evaluated (three for each species). All test samples showed significant relaxant effect, in a concentration-dependent manner, on the contractions induced by 1 μ mol/L carbachol, with exception of extracts from *C. morelosana*. Active extracts were less potent than theophylline [phosphodiesterase inhibitor, EC_{50}: (28.79±0.82) μ g/mL] that was used as positive control. Concentration-response curves revealed that the extracts with more significant effects were dichloromethanic extracts of *T. mexicana* [E_{max}: (103.03±3.32)% and EC_{50}: (159.39±3.72) μ g/mL) and *C. vitifolium* [E_{max}: (106.58±2.42)% and EC_{50}: (219.54±7.61) μ g/mL]. Finally, hexanic and dichloromethanic extracts from *A. mexicana* were fully effective but less potent than *T. mexicana* and *C. vitifolium*. **Conclusions:** Less polar extracts obtained from *A. mexicana*, *T. mexicana* and *C. vitifolium* exhibited greater relaxant effect on tracheal rat rings, which allows us to suggest them as sources for the isolation of bioactive molecules with potential therapeutic value in the treatment of asthma.

1. Introduction

Asthma is a chronic inflammatory disease of the lung characterized by episodic airway obstruction and increased bronchial responsiveness[1]; the structural features include epithelial shedding, increased airway smooth muscle mass, mucus gland hyperplasia, sub epithelial fibrosis and infiltration of the bronchial wall whit inflammatory cells[2]. Bronchial obstruction is the most important symptom because of the potential risk of severe asthma attacks; however, other non-obstructive symptoms are frequent and may also cause severe suffering and poor quality of life[3]. The current treatment does not resolve the disease and only focuses on countering episodes of bronchospasm and controlling the underlying inflammation[4]. Together, the high level tolerance and resistance of asthmatic patients, as a result of frequent drug use, increase the interest and

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need to develop new therapeutic strategies with specific, unexplored targets acting on the mechanisms that generate the asthmatic episode.

Mexico has over 4 000 species of medicinal plants, that have been used empirically by 80% of the indigenous population to treat health problems like respiratory diseases[5]. Current research was carried out in order to screen the pharmacological properties of *Agastache mexicana* (*A. mexicana*), *Cochlospermum vitifolium* (*C. vitifolium*), *Cordia morelosana* (*C. morelosana*), *Lepechinia caulescens* (*L. caulescens*) and *Talauma mexicana* (*T. mexicana*) to find candidates for further bio-guided studies, which allows the discovery of novel molecules with therapeutic properties for treating asthma, in particular, those which involve a contractile process of the smooth muscle cells.

2. Materials and methods

2.1. Chemicals and drugs

Carbamylcholine (carbachol), teophylline, dimethylsulfoxide (DMSO) and ethyl ether were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents were of analytical grade from local sources. Stock solutions of extracts were made using distilled water and DMSO and freshly prepared at the same day of experimentation.

2.2. Plant material

Plant species were selected using an ethnomedical criterion[6] and were collected and identified by Dr. Patricia Castillo España of the Center for Research in Biotechnology, UAEM. A sample of each specimen was deposited in the HUMO-herbarium of the Center for Research and Environmental Studies "Sierra de Huautla"(CEAMISH) of Morelos State University. Details of the collection, traditional uses, and voucher number of each species, among others, are mentioned in Table 1.

2.3. Extraction of plant material

The air-dried plant material was ground into powder. Crude extracts were prepared by successive room temperature-maceration of 30 g of dried plant material in 600 mL of hexane, dichloromethane and methanol for 72 h, respectively. After filtration, extracts were concentrated in vacuo at 45 ℃. Finally, a yield was obtained for each extract. To carry out the experiments, all extracts were dissolved in a mixture of dimethylsulfoxide:water (50:50, v:v).

2.4. Pharmacological evaluations

2.4.1. Rats

Healthy male Wistar rats (250-300 g) were used and maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted in accordance with our Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, Mexico) and approved by the Institutional Animal Care and Use Committee based on US National Institute of Health publication (No. 85-23, revised 1985). All experiments were carried out using six animals per group. Animals used were euthanized by cervical dislocation.

2.4.2. Rat trachea ring test

Previous protocol was used[7]. The trachea was dissected, cleaned out of connective tissue and mucus, and immediately cut into 4-5 mm long rings. Then, tissue segments were mounted in stainless steel hooks, under optimal tension of 2 g, in 10 mL organ baths containing warmed (37 ℃) and oxygenated (O_2/CO_2, 95:5, v:v) Krebs solution (118.0 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, 25.0 mmol/L $NaHCO_3$, 0.026 mmol/L EDTA, and 11.1 mmol/L glucose, pH 7.4). Changes in tension were recorded by Grass-FT03 force transducers (Astromed, West Warwick, RI, USA) connected to a MP100 analyzer (BIOPAC Instruments, Santa Barbara, CA, USA) as previously described[7]. After equilibration, rings were contracted by 1 μmol/L carbachol and washed every 40 min for 2 h. After contraction with carbachol, the test samples (organic extracts, vehicle and positive control) were added to the bath in a volume of 100 μL; then cumulative concentration-response curves were obtained for each ring (3.03-1 000.00 μg/mL). The relaxant effect of extracts and positive control (theophylline, an inhibitor of phosphodiesterase, 1.67-550.00 μmol/L) were determined by comparing the muscular tone of the contraction before and after addition of the test materials. Muscular tone was calculated from the tracings, using Acknowledge software (BIOPAC®).

2.5. Data analysis

The experimental results are expressed as mean of six experiments ± standard error of mean (SEM). Concentration-response curves were plotted and experimental data of

the concentration-response curves were adjusted by the nonlinear, curve-fitting program MicrocalTM origin 8.0 (Microcal Software Inc., USA). Significance was evaluated using the ANOVA[8]. Values of $P<0.05$ imply significance of the pharmacological effects in the experiments.

3. Results

The plant material was subjected to extraction process by maceration, and details of their yields are shown in Table 2. A total of 15 extracts were evaluated (three for each species). All test samples showed significant relaxant effect in a concentration-dependent manner on the contractions induced by 1 μ mol/L carbachol, with exception of extracts from *C. morelosana* (Table 2). Active extracts were less potent than theophylline [phosphodiesterase inhibitor, EC_{50}: (28.79±0.82) μ g/mL] used as positive control. Concentration-response curves revealed that the extracts with more significant effects were dichloromethanic extracts of *T. mexicana* [E_{max}: (103.03±3.32)% and EC_{50}: (159.39±3.72) μ g/mL] and *C. vitifolium* [E_{max}: (106.58±2.42)%; EC_{50}: (219.54±7.61) μ g/mL] (Figure 1). Finally, hexanic and dichloromethanic extracts from *A. mexicana* were fully effective but less potent than *T. mexicana* and *C. vitifolium* (Figure 2).

Table 1
Detailed information about plant species screened.

Plant name	Family	Common name	Voucher number	Traditional uses	Place of collection
A. mexicana subsp. xolocotziiana Bye, Lin & Ram.	Lamiaceae	Toronjil	26336	Anxiety, stress, antihypertensive, heart disease, antispasmodic, stomachache, vomit, kidney inflammation, asthma	Felipe Neri, Morelos State
C. vitifolium (Willd)	Bixaceae	Pánicua	14628	Antihypertensive, diabetes, hepatobiliary disorders	Sierra de Huatla, Morelos State
C. morelosana Standley	Boraginaceae	Anacahuite	Pending	Respiratory diseases	Amatlán, Morelos State
L. caulescens (Ortega) Epling	Lamiaceae	Bretónica, Brenilla	20386	Kidney diseases, diarrhea, vomit, diabetes	Amatlán, Morelos State
T. mexicana (DC.) G. Don	Magnoliaceae	Yolloxóchitl	1986-012 A	Anxiety, sleeplessness, heart disease, stress	Jalapa, Veracruz

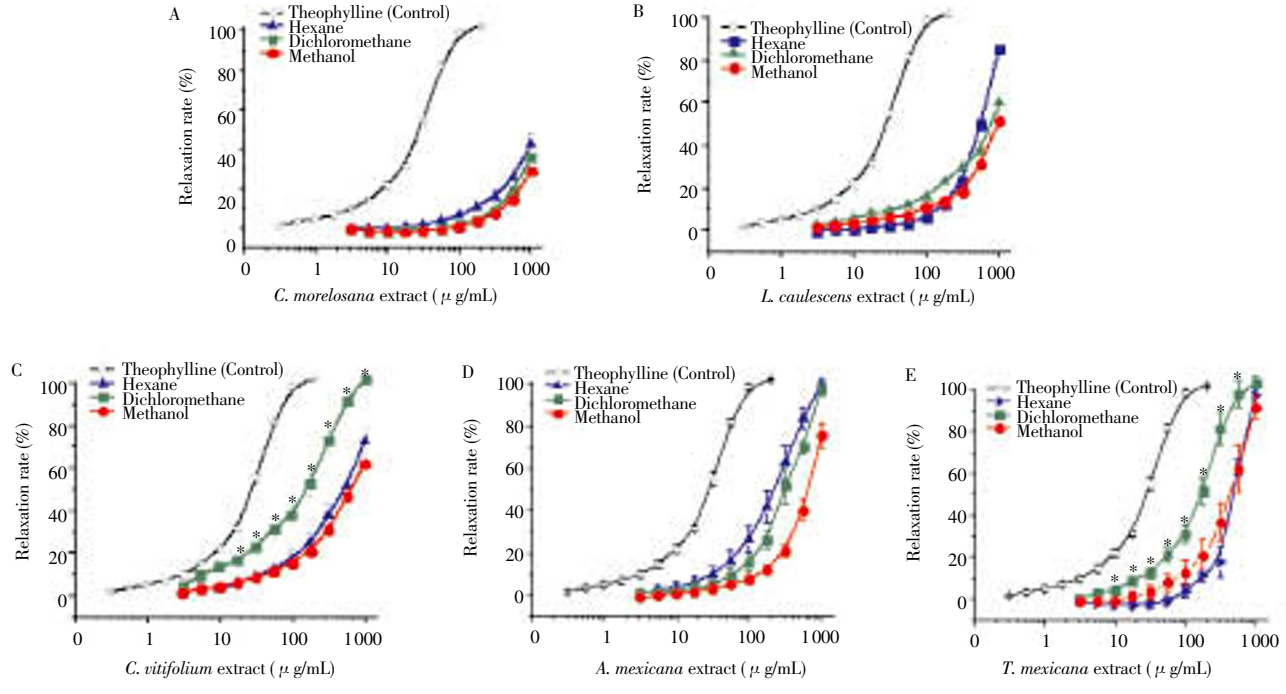

Figure 1. Concentration-response curves of the relaxant effect of organic extracts on trachea rings pre-contracted with 1 μ mol/L carbachol. All results are expressed as the mean±SEM of six experiments (*$P<0.05$).

Table 2

Extraction yield and relaxant effect of plant extracts on rat tracheal rings pre-contracted with carbachol 1 μ mol/L.

Plant name	Extract	Extraction yield (%)	E_{max} (%)	EC_{50} (μ g/mL)
Positive control	Theophylline		106.85±0.89	28.79±0.82
A. mexicana	Hexane	1.68	100.16±1.71	219.00±7.40
	Dichloromethane	2.15	97.78±3.34	320.87±5.72
	Methanol	6.38	75.54±6.03	644.44±4.53
C. vitifolium	Hexane	0.45	72.71±6.63	507.14±5.30
	Dichloromethane	0.49	106.60±2.42	152.83±7.61
	Methanol	1.23	61.91±3.03	631.52±3.42
C. morelosana	Hexane	2.93	42.46±5.56	ND
	Dichloromethane	1.89	35.80±1.54	ND
	Methanol	9.59	29.07±1.71	ND
L. caulescens	Hexane	0.81	85.51±1.42	516.69±3.72
	Dichloromethane	0.70	59.81±4.39	801.23±4.53
	Methanol	2.51	51.67±5.03	ND
T. mexicana	Hexane	4.00	97.45±1.04	477.28±5.19
	Dichloromethane	6.50	103.03±3.32	159.39±8.7
	Methanol	7.00	91.77±5.19	430.71±5.04

E_{max} and EC_{50} indicate efficacy and potency of the extract, respectively. ND: Not determined.

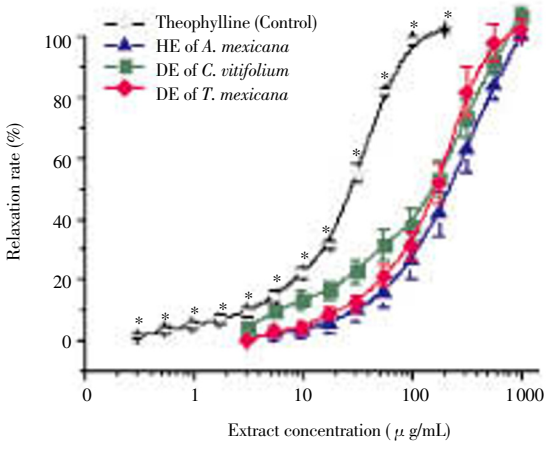

Figure 2. Concentration-response curves of the relaxant effect of the most potent organic extracts on trachea rings pre-contracted with 1 μ mol/L carbachol.

All results are expressed as the mean±SEM of six experiments (*P<0.05). HE: Hexane extract; DE: Dichloromethane extract; ME: Methanol extract.

4. Discussion

Medicinal plants *A. mexicana*, *C. vitifolium*, *C. morelosana*, *L. caulescens* and *T. mexicana* are used in Mexican traditional medicine for the treatment of several diseases[6]. These plants were selected based on ethnomedical criterion[6] and previous reports related with relaxation of several smooth muscle tissues[9-15], with exception of *C. morelosana* which is used as an anti-asthmatic agent and related respiratory diseases in traditional medicine. Current strategies for anti-asthmatic drug development include anti-inflammatory and/or bronchodilator drugs. Thus, we decided to explore the direct airway relaxant effects of hexanic, dichloromethanic and methanolic extracts from these selected plants using carbachol-precontracted rat tracheal rings, an emergent model related to the widely used guinea-pig tracheal model[16].

In this framework, the extracts were obtained by maceration at room temperature and, as we expected, the methanolic extracts were those that showed higher yields in the extraction process for all plant species, because the metabolic content of medicinal plants are mainly compounds with medium to high polarity[17]. All extracts evaluated showed significant relaxant effect in the range of concentrations used, with exception of extracts of *C. morelosana*. However, we cannot discard the anti-asthmatic properties of *C. morelosana*, since this species may act through modulating the inflammatory process in asthmatic episodes[18], modulating airway hyperresponsiveness and hypersensitivity development as a result of asthmatics' status, or inhibiting inflammatory mediator release[19]. It is important to mention that *C. morelosana* is a plant species that has been traditionally used for the treatment of respiratory diseases and/or asthma, and it is necessary to accomplish other pharmacological tests to confirm its traditional use in the population, such as the evaluation of the asthmatic effect in mice sensitized with ovalbumin[4]. On the other hand, extracts from *L. caulescens* showed moderated relaxant effect being hexane extract of *L. caulescens* the most active extract from this plant. Last suggest that previous isolated low polarity bioactive compounds from *L. caulescens* may be responsible for the tracheal relaxant effect such as ursolic and oleanolic acids, spathulenol, 9 α ,13 α -epidioxyabiet-8(14)-en-18-oic acid methyl ester, dehydroabietic acid, and 9 β -hydroxydehydroabietyl alcohol which showed relaxant effect on various smooth muscle tissue[9,20]. On the other hand, *C. vitifolium*, *A. mexicana* and *T. mexicana* are used in traditional Mexican medicine for the treatment of cardiovascular diseases. These species were the most

active tracheal relaxant agents, with the dichloromethanic extracts from *C. vitifolium* and *T. mexicana* being the most active samples, following by the dichloromethanic and hexanic extracts from *A. mexicana*. Previous reports showed that *C. vitifolium* and *T. mexicana* have significant vasorelaxant effect on partially endothelium-dependent and concentration-dependent manners on aorta rat rings. Flavonoids and triterpenic derivatives were found to be the responsible bioactive agents, with NO/cGMP system involved as main vasorelaxant mode of action[12-15]. Moreover, there is an evidence demonstrating that flavonoids have relaxing properties over smooth muscle of the rat and guinea pig trachea[21,22]. Finally, *T. mexicana* has been used since the 15th century for the treatment of cardiovascular diseases, but this species has few pharmacological studies that support the traditional uses of the plant[23]. Further phytochemical experiments are necessary to isolate the responsible of the relaxant effect on tracheal rat rings.

In conclusion, we have shown that various extracts of *T. mexicana*, *C. vitifolium* and *A. mexicana* induced a relaxant effect on rat-isolated trachea, which may propose them as potential sources for the isolation of anti-asthmatic agent(s).

Conflict of interest statement

We declare that we have no conflict of interest.

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