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Vasorelaxant effect of *Laelia speciosa* and *Laelia anceps*: Two orchids as potential sources for the isolation of bioactive molecules

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ABSTRACT

The aim of the current study was to investigate the vasorelaxant effect of several extracts from *Laelia speciosa* and *Laelia anceps*, on an *ex vivo* method using aorta rat rings with and without endothelium pre-contracted with norepinephrine (0.1 µM), in order to establish them as a real source for the isolation of bioactive compounds with potential use as antihypertensive agent. All extracts caused concentration-dependent relaxation in -precontracted aortic rings with and without endothelium; the most active extracts were the hexanic and dichlorometanic extracts from roots of *L. anceps* and *L. speciosa* (HERLanc, DERLanc, HERLspec and DERLspec, respectively), and were less potent than positive controls used (carbachol and sodium nitroprusside). These results suggest that secondary metabolites, responsible for the vasorelaxant activity, belong to a group of compounds of medium and low polarity, and the roots were the main tissues of the plant where the vasorelaxant compounds are stored. In conclusion, both orchids represent an ideal source for obtaining lead compounds for designing new therapeutic agents, with potential vasorelaxant and antihypertensive effects.

INTRODUCTION

The use of orchids in traditional medicine is widely described throughout history (Bulpitt, 2005; Castillo-España and Monroy-Ortiz, 2007; Conzatti, 1981; Estrada *et al.*, 1999; Gutiérrez, 2010; Kong *et al.*, 2003; Smith and Ashiya 2007). In Mexico, *Laelia speciosa* and *Laelia anceps* (orchids) are used to avoid the abortion process and for the treatment of hypertension (Castillo-España and Monroy-Ortiz, 2007). Moreover, it had been reported a large number of secondary metabolites isolated from orchids with a large structural diversity such as triterpenes, stilbenoids, phenanthrenes and cumarins, among others (Bulpitt, 2005; Gutiérrez, 2010; Kong *et al.*, 2003; Smith and Ashiya, 2007). From these, stilbenoids and phenanthrenes have reported to posses various biological activities such as antiallergic, anti-inflammatory, antimicrobial, antiplatelet aggregations, cytotoxicity, spasmolytic, vasorelaxant effects, and others (Gutierrez, 2010; Kovács *et al.*, 2008; Xiao *et al.*, 2008). Consequently, the aim of this study was to investigate the vasorelaxant effect of *L. speciosa* and *L. anceps*, with the purpose of offering them as potential sources for the isolation of lead compounds for designing new therapeutic agents with vasorelaxant and antihypertensive effects, since, currently hypertension is a cardiovascular disease with the highest epidemiological impact in the world, and also represents a major risk factor for developing other diseases such as endothelial dysfunction, metabolic syndrome, renal dysfunction, congestive heart failure, coronary artery disease and stroke.

MATERIALS AND METHODS

Chemicals

All reagents used were analytical grade and purchased from Sigma-Aldrich™. For *in vitro* experiments, extracts were dissolved in distilled water and dimethylsulfoxide (DMSO, 1% v/v), and other reagents were dissolved in distilled water and sonicated just before use.

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Plant material and extraction

L. speciosa and *L. anceps* plant material were collected in Coatepec, Veracruz, México. The identification was carried out by Dr. Patricia Castillo-España. The voucher specimens (No. 22025 and 26337) were deposited at the HUMO-Herbarium of Morelos University. Briefly, the plants materials were separated in roots, and pseudobulbs; and later on, they were subjected to successive maceration with hexane, dichloromethane and methanol (3 times for 72 h at room temperature). After filtration, extracts were concentrated *in vacuo* at 40 °C.

Animals

Male Wistar rats (250–350 g) were used. They were maintained under standard laboratory conditions with free access to food and water. Rats were maintained under standard laboratory condition swith free access to food and water.

All animal procedures were conducted in accordance with the Mexican Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, México), and approved by the Institutional Animal Care and Use Committee (Universidad Nacional Autónoma de México) basedon a U.S. National Institutes of Health publication (No. 85-23, revised 1985).

Preparation of rat aortic rings and effect of extracts on the contraction induced by NE

The experimental design was performed as described by Vergara-Galicia *et al.,* 2010. The aortic rings with and without endothelium were precontracted with norepinephrine (NE, 0.1 μM). Once the plateau was attained, concentration–response curves of extracts-induced relaxation (0.15 μg/mL to 50 μg/mL) were obtained by adding cumulative concentrations to the incubation bath.

Data analysis

Data were analysed using ANOVA with repeated measures. Statistical significance was set *a priori* at P<0.05 for all comparison. Data were expressed as means ± standard error of the mean.

RESULTS

Hexane, dichloromethane and methanolic extracts from roots (HERLspec, DERLspec and MERLspec, respectively) and pseudobulbs (HEPLspec, DEPLspec and MEPLspec, respectively) of *L. speciosa* relaxed NE (0.1μM)–precontracted aortic rings, with and without endothelium in a concentration-dependent manner (Table 1 and Fig. 1A-C). Similarly, hexane and dichloromethane extracts from roots (HERLanc and DERLanc, respectively), pseudobulbs (HEPLanc and DEPLanc, respectively), and leaves (HELLanc and DELLanc) of *L. anceps* induced a vasorelaxant effect in aortic rings with and without endothelium, in a concentration-dependent fashion (Table 1 and Fig. 2A-B).On the other hand, hexanic and methanol extracts from *L. Speciosa* were the most active test samples evaluated. Thus, MERLspec was the most potent and efficient of all extracts evaluated (Table 1 and Fig 1A). Finally, all test samples were less active than positive controls (SNP and carbachol, respectively) and their effect was endothelium-independent.

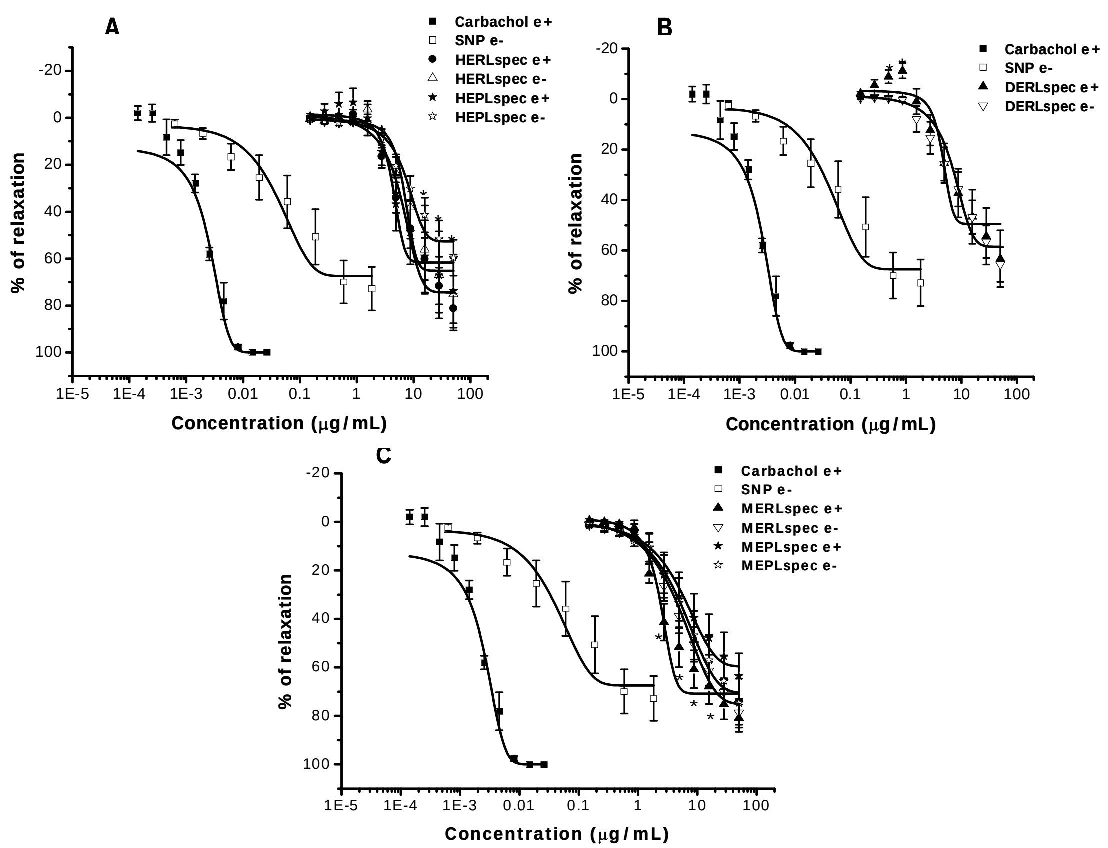

Fig. 1: Inhibitory effects of hexane, dichloromethane and methanolic extracts obtained from *L. speciosa* on the contraction induced by NE (0.1 μM) in endothelium intact and denuded aortic rings. Results are presented as mean±S.E.M. n=6. P*<0.05 compared with aortic rings without endothelium.

Table. 1: Relaxant effects induced by extracts obtained from *L. speciosa and L. anceps* on the contraction induced by NE 0.1 µM.

Vasorelaxant agent	Whith endothelium (E+)		Without endothelium (E-)	
	EC_{50} (µg/mL)	E_{max} (%)	EC_{50} (µg/mL)	E_{max} (%)
Carbachol	0.002	100.00±1.01	ND	ND
SNP	ND	ND	0.044	72.8±9.24
HERLspec	8.78	81.21±6.28	7.01	75.17±15.45
HEPLspec	4.91	73.82±15.58	8.75	59.60±7.66
DERLspec	5.85	63.25±11.26	5.65	65.54±6.90
MERLspec	2.75	80.89±5.63	4.76	78.54±5.10
MEPLspec	5.12	63.55±9.34	6.85	74.71±10.11
HELLanc	15.68	34.78±7.41	28.00	49.79±8.2
HEPLanc	18.15	54.79±6.77	17.36	57.03±2.18
HERLanc	ND	-32.65±6.97	14.28	81.35±8.25*
DELLanc	16.07	34.10±4.22	28.79	51.92±5.52*
DERLanc	6.78	68.45±3.89	7.32	70.04±5.91
DEPLanc	12.13	61.82±3.65	17.15	89.26±3.66*

Results are presented as mean ± S.E.M., n=6. P*<0.05 compared with aortic rings with endothelium.

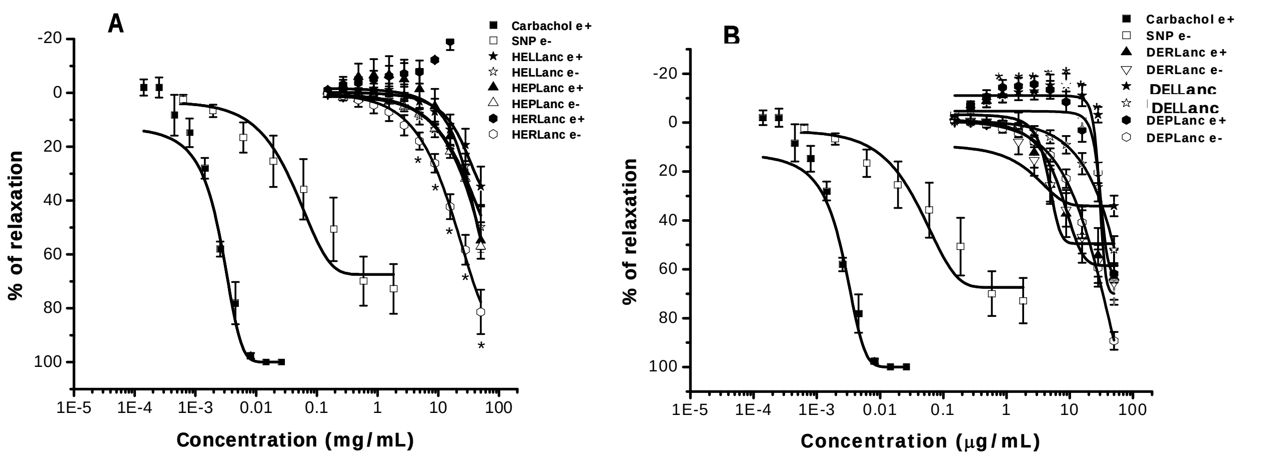

Fig. 2: Inhibitory effects of hexane and dichloromethane extracts obtained from *L. anceps* on the contraction induced by NE (0.1 µM) in endothelium intact and denuded aortic rings. Results are presented as mean±S.E.M. n=6, P*<0.05 compared with aortic rings without endothelium.

DISCUSSION

In previous investigations we have showed the vasorelaxant effect of some orchid species (Aguirre-Crespo *et al.,* 2005; Corson and Crews, 2007; Kwana *et al.,* 2004; Vergara-Galicia *et al.,* 2008; Vergara-Galicia *et al.,* 2010). In this framework, the hexane and dichloromethanic extracts from pseudobulb (HEPLanc) and leaf (HELLanc) of *L. anceps*, promoted relevant vasorelaxant effect ina concentration-dependent and endothelium-independent manners, being HERLanc the most vasorelaxant active extract evaluated.Thus, the fact that all extracts induced their effect in an endothelium-independent manner suggest that theyare acting on molecular targets located on smooth muscle cells, which are involved in the regulation of the contraction/relaxation of the blood vessels (such as an augment of intracellular cGMP concentration, antagonism of adrenergic receptors, calcium channel blockade, and activation of potassium channels, among others). However, HERLanc and DEPLanc induced a slight contraction in a concentration- and endothelium-dependent manners, suggesting that they have compounds that promote contraction due torelease of contractile factors, and/or reduced production of relaxing factors derived from endothelium (Cotran *et al.,* 2000; Katzung, 2004; Hill *et al.,* 2001; Lam *et al.,* 2006; Lam *et al.,* 2007; Lincoln *et al.,* 2001; Murad, 2006; Soares *et al.,* 2006; Somlyo and Somlyo, 2000). Further experiments are necessary to validate this hypothesis. On the other hand, all extracts obtained from roots and pseudobulbs of *L. speciosa* showed a concentration-dependent and endothelium-independent vasorelaxant effect. The hexane and methanol extracts obtained from the root (HERLspec and MERLspec) were the most active extracts evaluated. It is important to mention that the relaxant effect showed by extracts agrees with previous relaxant effects observed from several orchids where the presence of stilbenoids derivatives was confirmed, which are presumably responsible of the relaxant effect (Estrada *et al.,* 1999; Estrada-Soto *et al.,* 2006; Vergara-Galicia *et al.,* 2010).In conclusion, the results prove that the extracts from *L. anceps* and *L. speciosa* are important sources for theisolation of vasorelaxant agents with a potential antihypertensive uses. In this sense, further experiments are in progress in order to isolate and characterize secondary metabolites responsible for the vasorelaxant activity and to elucidate their mechanism(s) of action.

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