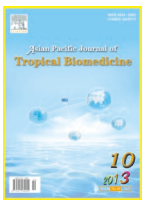
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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



Document heading doi:10.1016/S2221-1691(13)60154-9

Vasorelaxant activity of extracts obtained from *Apium graveolens*: Possible source for vasorelaxant molecules isolation with potential antihypertensive effect

Vergara-Galicia Jorge[1*], Jimenez-Ramirez Luis Ángel[1], Tun-Suarez Adrián[1], Aguirre-Crespo Francisco[1], Salazar-Gómez Anuar[2], Estrada-Soto Samuel[3], Sierra-Ovando Ángel[4], Hernandez-Nuñez Emmanuel[5]

[1] *Division of Health Sciences, University of Quintana roo, Chetumal, Quintana Roo, Mexico*

[2] *Department of Biotechnology, Polytechnic University of the State of Morelos, Jiutepec, Morelos, Mexico*

[3] *College of Pharmacy, University of the State of Morelos, Cuernavaca, Morelos, Mexico*

[4] *Division of Health Sciences, Juarez Autonomous University of Tabasco, Villahermosa, Tabasco, Mexico*

[5] *School of Engineering, University of Yucatan, Merida, Yucatan, Mexico*

PEER REVIEW

Peer reviewer

Adam Hermawan, Cancer Chemoprevention Research Center, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Sekip Utara II, Yogyakarta, 55281, Indonesia.
Tel/Fax: +62-274 6492662/543120
E-mail: adam_apt@ugm.ac.id

Comments

In this good study, the authors performed antihypertensive activity of *A. graveolens* in *in vitro* model based on relaxation of aorta, and mechanism of extracellular calcium influx blocking. *A. graveolens* was found to be a promising anti hypertensive agent with mechanism of vasorelaxant of aorta.
Details on Page 779

ABSTRACT

Objective: To investigate vasorelaxant effect of organic extracts from *Apium graveolens (A. graveolens)* which is a part of a group of plants subjected to pharmacological and phytochemical study with the purpose of offering it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects.
Methods: An *ex vivo* method was employed to assess the vasorelaxant activity. This consisted of using rat aortic rings with and without endothelium precontracted with norepinephrine.
Results: All extracts caused concentration-dependent relaxation in precontracted aortic rings with and without endothelium; the most active extracts were Dichloromethane and Ethyl Acetate extracts from *A. graveolens*. These results suggested that secondary metabolites responsible for the vasorelaxant activity belong to a group of compounds of medium polarity. Also, our evidence showed that effect induced by dichloromethane and ethyl acetate extracts from *A. graveolens* is mediated probably by calcium antagonism.
Conclusions: *A. graveolens* represents an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects.

KEYWORDS

Aortic ring, *Apium graveolens*, Medicinal plant, Vasorelaxant

1. Introduction

There are reports describing the use in various forms of *Apium graveolens (A. graveolens)* in traditional medicine of Morelos state to avoid the toothache and treat diarrhea, hypertension, and broncho pulmonary diseases[1]. Iso, the

*Corresponding author: Jorge Vergara Galicia, Division of Health Sciences, University of Quintana Roo, Chetumal, Quintana Roo, Mexico.
Tel/Fax: +52 983 8350300
E-mail: vgjorge7@uqroo.mx

Foundation Project: This study was financed by grants from "Promotion of generation or innovative application of knowledge and promotion of applied research or technological development" (PROMEP-SEP 2012-2013, oficio PROMEP/103.5/12/8308), "Programme for Strengthening Research" (PROFI-UQROO 2012) and P/PIFI-2012-23MSU0140Z-09DCS.

Article history:
Received 29 Jun 2013
Received in revised form 16 Jul, 2nd revised form 22 Jul, 3rd revised form 28 Jul 2013
Accepted 17 Sep 2013
Available online 28 Oct 2013

reports have reported that to date a large number of secondary metabolites with a large structural diversity have been isolated from *A. graveolens*[2,3]. Consequently, this study was performed in order to investigate vasorelaxant effect of extracts obtained from *A. graveolens* with the purpose to offer it as an ideal source for obtaining lead compounds for designing new therapeutic agents with potential vasorelaxant and antihypertensive effects, since the hypertension is a cardiovascular disease with the most epidemiological impact in the world and also represents a major risk factor for developing other diseases such as endothelial dysfunction, metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary artery disease and stroke.

2. Materials and methods

2.1. Chemicals

All reagents used were grade analytical 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 used.

2.2. Plant material and extraction

A. graveolens was collected and identified by Dr. Patricia Castillo-España in Cuernavaca, Morelos, Mexico. A voucher specimen was deposited at the Herbarium of Morelos State University. Briefly, the plant material was dried at room temperature and subjected to successive maceration with hexane, dichloromethane, ethyl acetate and methanol (3 times for 72 h at room temperature). After filtration, the extracts were concentrated at 40 °C.

2.3. Animals

Male Wistar rats (250-350 g) were used. They were maintained under standard laboratory conditions with free access to food and water. The study was reviewed and approved by the local institutional review board.

2.4. Preparation of rat aortic rings and effect of extracts on the contraction induced by norepinephrine (NE)

The experimental design was performed according to the method described by Rendon-Vallejo[4]. The aortic rings with and without endothelium were precontracted with NE (1×10^{-7} mol/L). Once the plateau was attained, concentration-response curves of extracts-induced relaxation (0.15-50 μg/mL) were obtained by adding cumulative concentrations to the bath.

2.5. Effect of ethyl acetate extract from A. graveolens (EAEAg) on the cumulative contraction induced by NE

Endothelium-denuded aortic rings were incubated with 62, 110 and 200 μg/mL of EAEAg for 15 min, and then NE was added at different concentrations (1×10^{-11} to 3.16×10^{-6} mol/L). Finally, the contractile effect induced by NE was compared in the absence (control group) and presence of the extract.

2.6. Effect of EAEAg on extracellular Ca^{2+}-induced contraction activated by KCl

To determine whether the inhibition of extracellular Ca^{2+} influx was involved in EAEAg-induced relaxation, the experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded aortic rings were washed with Ca^{2+}-free solution (approximately 20 min) and then rinsed with Ca^{2+}-free solution containing KCl (0.08 mol/L). The cumulative concentration-response curves for $CaCl_2$ (7×10^{-4} to 0.02 mol/L) were obtained in the absence of EAEAg (control group) or after a 15 min incubation with the extract (110 and 200 μg/mL). Finally, the contractile effect induced by $CaCl_2$ was compared in the absence (control group) and presence of EAEAg.

2.7. 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±SEM.

3. Results

Hexane, dichloromethane, ethyl acetate and methanol extracts relaxed NE (1×10^{-7} mol/L) -precontracted aortic rings with and without endothelium in a dose-dependent manner (Table 1), suggesting that vasodilatation is motivated by the interference on a common pathway that several receptor agonists exert, such as the augment of free cytosolic Ca^{2+} levels[5,6]. In this context, EAEAg (62, 110 and 200 μg/mL) inhibited the concentration-response contraction of NE in a nonparallel manner and depressed the maximal response (Figure 1a), suggesting that extract might block voltage-dependent and receptor operated Ca^{2+} channels[7]. Moreover, we found that 110 and 200 μg/mL of the extract significantly inhibited $CaCl_2$-induced contraction of control group in a parallel manner and depressed their maximal responses (Figure 1b), supporting the idea that EAEAg possesses a Ca^{2+} entry blocking activity[7,8].

Table 1

Relaxatory effects induced by ethanol extracts obtained from *A. graveolens* on the contraction induced by NE (1×10^{-7} mol/L).

Vasorelaxant agent	With endothelium (E+)		With out 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	72.80±9.24
MEAg	7.32	58.39±7.39	47.72	35.10±2.45
HEAg	51.90	26.09±3.93	40.42	34.64±5.45
EAEAg**	15.05	69.79±6.58	43.15	79.92±6.67*
DEAg**	47.60	69.49±6.28	49.45	66.58±3.67

Results are presented as mean±SEM, n=6. *: P<0.05 compared with aortic rings with endothelium, **: P<0.05 compared with MEAg and HEAg. MEAg: Methanol extract from *A. graveolens*. HEAg: Hexane extract from *A. graveolens*. EAEAg: Ethyl Acetate extract from *A. graveolens*. DEAg: Dichloromethane extract from *A. graveolens*. ND: Non determinate.

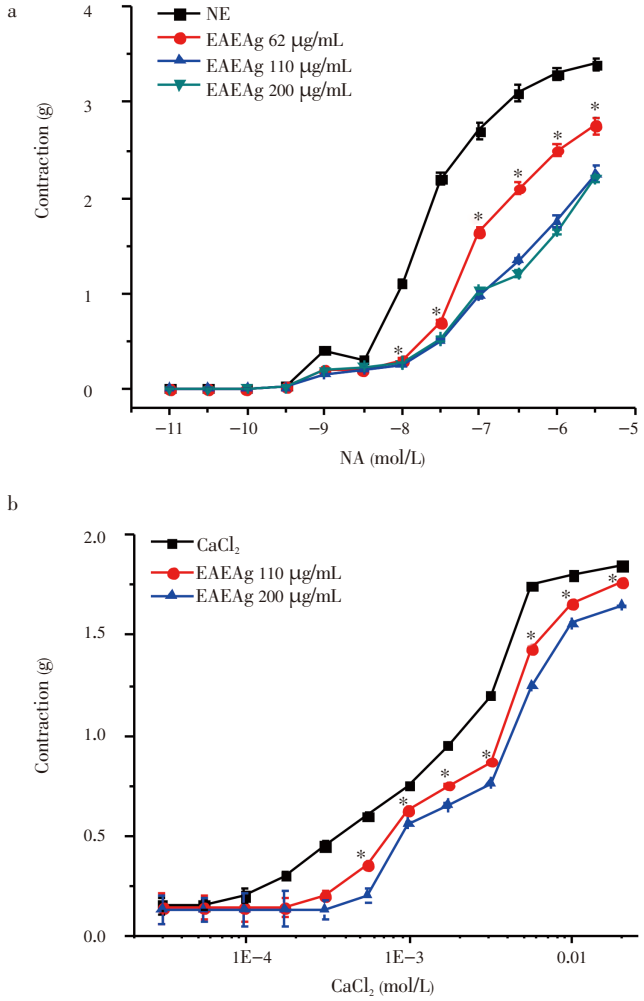


Figure 1. Inhibitory effects of EAEAg on the contraction and cumulative-contraction.

a: Inhibitory effects of EAEAg on the contraction induced by NE (1×10^{-11}–3.16×10^{-6}mol/L) in endothelium-denuded aortic rings; b: Inhibitory effects of EAEAg on the cumulative-contraction curve dependent on extracellular Ca^{2+} influx induced by 80 mmol/L KCl in Ca^{2+}-free solution. Results are presented as mean±SEM, n=6. *: P<0.05 compared with control.

4. Discussion

The current investigation represents the first effort to describe the vasodilator effect of different extracts from medicinal plants used in Mexico. Hexane, dichloromethane, ethyl acetate and methanol extracts relaxed NE (1×10^{-7} mol/L) -precontracted aortic rings with and without endothelium, suggesting that vasodilatation is motivated by the interference on a common pathway that several receptor agonists exert, such as the augment of free cytosolic Ca^{2+} levels[5,6]. In this regard, in smooth muscle cells there are two kinds of Ca^{2+} channels: voltage-dependent Ca^{2+} channels (high KCl-induced contraction due to membrane depolarization, leading to increased Ca^{2+} influx through voltage dependent channels) and receptor operated Ca^{2+} channels (contraction induced by NE in Ca^{2+}-free medium is due to intracellular Ca^{2+} release through sarcoplasmic reticulum Ca^{2+} channels activated by IP_3)[7,9]. Therefore, agents acting directly on the vascular smooth muscle cells may alter tone by three mechanisms: altering intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$), varying the sensitivity of the contractile regulatory apparatus to $[Ca^{2+}]i$ or modulating the sensitivity to other vasoactive inputs[6]. On the other hand, EAEAg inhibited the concentration-response contraction of NE in a nonparallel manner and depressed the maximal response, suggesting that extract might block voltage-dependent and receptor operated Ca^{2+} channels[7]. In addition, we found that 110 and 200 μg/mL of the extract significantly inhibited $CaCl_2$-induced contraction of control group in a parallel manner and depressed their maximal responses, supporting the idea that EERMv possesses a Ca^{2+} entry blocking activity[7,8,10]. It is important to mention that the relaxant effect showed by extracts obtained from this vegetal species is in accord with previous relaxant effects produced by apigenin isolated from *A. graveolens*. In addition, there are reports describing the isolation and characterization of several secondary metabolites such as apigenin, luteolin, kaempferol, caffeic acid, ferulic acid and coumaric acid from *A. graveolens*, which could be presumably responsible for the relaxant effect[2,3]. Therefore, it is necessary to direct the attention to compounds present in organic extracts. In conclusion, the present results provide pharmacological support for the use of *A. graveolens* in ethnomedical practices as antihypertensive in Mexico. Moreover, present efforts are directed to isolate the active constituents from extracts of this species to allow us to understand its mechanism(s) of action and design new therapeutic agents with potential antihypertensive effects.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This study was financed by grants from "Promotion of generation or innovative application of knowledge and promotion of applied research or technological development" (PROMEP-SEP 2012-2013, oficio PROMEP/103.5/12/8308), "Programme for Strengthening Research" (PROFI-UQROO 2012) and P/PIFI-2012-23MSU0140Z-09DCS.

Comments

Background

A. graveolens, a traditional medicine of Morelos state for several diseases *e.g.* hypertension, and broncho pulmonary. Hypertension is the most common cause of death in the world. This study was carried out in order to investigate vasorelaxant effect of extracts obtained from *A. graveolens*.

Research frontiers

The present study performs anti hypertensive effect of several *A. graveolens* extract in *in vitro* model using aorta contraction, and its mechanism on inhibition of extracellular Ca^{2+} influx.

Related reports

Animal experiment using aortic rings with and without endothelium has been widely established.

Innovations and breakthroughs

A. graveolens is a traditional medicine of Morelos state for treating hypertension. In the present study, authors have demonstrated the antihypertensive activity of *A. graveolens* in *in vitro* models based on aorta relaxation and extracellular calcium influx blocking.

Applications

From the previous study, *A. graveolens* is widely used as a traditional medicine in Morelos state. This present study supports scientific evidence of the plant as anti hypertensive agent.

Peer review

In this good study, the authors performed antihypertensive activity of *A. graveolens* in *in vitro* model based on relaxation of aorta, and mechanism of extracellular calcium influx blocking. *A. graveolens* was found to be a promising anti hypertensive agent with mechanism of vasorelaxant of aorta.

References

[1] Castillo-España P, Monroy-Ortiz C. *Medicinal plants used in Morelos, Mexico*. Cuernavaca: Morelos State University; 2007, p. 58.

[2] Yang Y, Wei S, Mengjie Z, Guixing R. Phenolic composition and antioxidant activities of 11 celery cultivars. *J Food Sci* 2010; **75**(1): 9-13.

[3] Zhou K, Zhao F, Liu Z, Zhuang Y, Chen L, Qiu F. Triterpenoids and flavonoids from celery (*Apium graveolens*). *J Nat Prod* 2009; **72**(9): 1563-1567.

[4] Rendon-Vallejo P, Hernandez-Abreu O, Vergara-Galicia J, Millan-Pacheco C, Mejía A, Ibarra-Barajas M, et al. *Ex vivo* study of the vasorelaxant activity induced by phenanthrene derivatives isolated from *Maxillaria densa*. *J Nat Prod* 2012; **75**(12): 2241-2245.

[5] Jadhav A, Liang W, Papageorgiou PC, Shoker A, Kanthan SC, Balsevich J, et al. Catharanthine dilates small mesenteric arteries and decreases heart rate and cardiac contractility by inhibition of voltage-operated calcium channels on vascular smooth muscle cells and cardiomyocytes. *J Pharmacol Exp Ther* 2013; **345**(3): 383-392.

[6] Vergara-Galicia J, Ortiz-Andrade R, Rivera-Leyva J, Castillo-España P, Villalobos-Molina R, Ibarra-Barajas M, et al. Vasorelaxant and antihypertensive effects of methanolic extract from roots of *Laelia anceps* are mediated by calcium-channel antagonism. *Fitoterapia* 2010; **81**(5): 350 -357.

[7] Zhu XM, Fang LH, Li YJ, Du GH. Endothelium-dependent and-independent relaxation induced by pinocembrin in rat aortic rings. *Vascul Pharmacol* 2007; **46**(3): 160-165.

[8] Hernandez-Abreu O, Castillo-España P, Leon-Rivera I, Ibarra-Barajas M, Villalobos-Molina R, Gonzalez-Christen J, et al. Antihypertensive and vasorelaxant effects of tilianin isolated from *Agastache mexicana* are mediated by NO/cGMP pathway and potassium channel opening. *Biochem Pharmacol* 2009; **78**(1): 54 -61.

[9] Cunha GH, Moraes MO, Fechine FV, Frota Bezerra FA, Silveira ER, Canuto KM, et al. Vasorelaxant and antihypertensive effects of methanolic fraction of the essential oil of *Alpinia zerumbet*. *Vascul Pharmacol* 2013; **58**(5-6): 337-345.

[10] Torres-Piedra M, Figueroa M, Hernández-Abreu O, Ibarra-Barajas M, Navarrete-Vázquez G, Estrada-Soto S. Vasorelaxant effect of flavonoids through calmodulin inhibition: *Ex vivo*, *in vitro*, and *in silico* approaches. *Bioorg Med Chem* 2011; **19**(1): 542-546.