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# Vasoactivity of Mantonico and Pecorello grape pomaces on rat aorta rings: an insight into nutraceutical development

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## **Abstract**

The valorisation of agrochemical wastes has become a low cost and sustainable tendency for the development of functional food. In particular, these enriched products seem to be enjoyable tools to treat metabolic disorders. In this field, Calabrian autochthonous white grape pomaces (Mantonico and Pecorello cultivar) were firstly investigated for their vasoactive properties. Skins and seeds were extracted and characterized by NMR spectroscopy, revealing the presence of numerous vasoactive compounds. The effects of extracts were analyzed in in vitro experiments on rat aorta rings (with and without endothelium), contracted by phenylephrine or KCl. Seeds extracts showed, differently from skins, an appreciable endothelium-dependent, eNOS-mediated vasodilation in the range 1-100  $\mu\text{g/mL}$ . From a food market point of view, all the extracts were enclosed into a pectin polymer matrix. In the same experimental conditions, the polymers demonstrated the persistence of vasodilator activity only for Pecorello seed extract, which presented the most rich chemical profile.

## **Keywords**

Vasoactivity; white grape pomaces; catechin; pectin; rat aorta rings.

## 1. Introduction

Grape (*V. vinifera* subsp. *Vinifera*) is one of the most abundant crops cultivated in Europe, particularly in France and Italy, which are the first wine producers worldwide (*oiv.int*). In Italy, the Mediterranean area represents an historical soil of grape cultivation; in particular, Calabria region is known with the antique denomination Enotria – Terra del vino (Land of wine). Here, several autochthonous, black and white berries, grape cultivars grow. The most famous black berries are Gaglioppo and “its brothers”, named Magliocco Canino, Mangiaguerra, Greco Nero, and Arvino, while autochthonous white berries are Greco Bianco, Mantonico, Malvasia, and Pecorello, as recently confirmed by genetic analysis (*Sunseri et al., 2018*). Winemaking wastes of Calabria region are commonly used for agronomic (fertilizers) and energetic uses or relegated as amino acid-rich food for livestock. Interestingly, several studies reported the beneficial effects of red grape pomaces from all over the world in human health (*Toscano et al., 2018; Rasines Perea et al., 2018; Balea et al., 2018*) and, particularly, in cardiovascular diseases (*Asgary, Rastqar, & Keshvari, 2018*). Clinical trials demonstrated the efficacy of red grape pomaces in reducing systolic blood pressure and heart rate and, as a consequence, in treating hypertension (*Feringa et al., 2011; Ras et al., 2013*). Several mechanisms have been proposed to underlie this beneficial effects: a pronounced vasodilatation, due to endothelial nitric oxide synthase (eNOS) activation (*Frota Madeira et al., 2009; Pons et al., 2016*); an enhanced oxygen delivery, a general decrease in peripheral vasoconstriction along with a reduced cardiac activity in prehypertensive males during exercise (*Kim et al., 2018*). That is why, nowadays several researchers are focussing their interest on diet-based prevention of chronic diseases, such as hypertension, diabetes, and obesity. The bioactive components, mainly polyphenols other than aminoacids, sugars, found in agrochemical waste as well as in edible materials, better known as nutraceuticals, are frequently used as functional ingredients to fortify food and beverage products. In addition, they are sold as Over The Counter (OTC) natural supplements (*McClements, 2012*). Most of these nutraceuticals, however, are highly hydrophobic molecules, difficult to incorporate into foods

by simple mixing. Polymers are commonly employed to circumvent this issue. Recently, for example, pectin was derivatised by the grafting procedure with the seed and skin extract of Mantonico grape pomaces to produce a functional pear jam. This showed improved antioxidant performances, lasting for at least 15 days, in comparison with its non-functional counterparts (*Restuccia et al., 2018*). Polymer carriers, in fact, reduce phytochemical interactions with food ingredients, thus preserving their quality attributes (*Gibis, Vogt, & Weiss, 2012*). Noticeably, vegetable and fruit fiber (with pectin), tea, fish oil, and soy proteins show blood lipid level–lowering effects in humans, as they inhibit fat absorption and suppress cholesterol synthesis (*Asgary, Rastqar, & Keshvari, 2018*). To our knowledge, no studies have yet evaluated the potential, beneficial effects of white berries Mantonico and Pecorello grape pomaces toward the cardiovascular system. Therefore, the in vitro vasoactivity of skin and seed extracts, obtained by ultra-sound-assisted method in green conditions, was investigated on aorta rings under various experimental conditions. NMR analysis was performed to identify the different compounds present in the extracts in an attempt to identify the bioactive molecules. Results demonstrate that all the extracts incorporate vasoactive molecules whose activity, however, is negatively influenced by polymer vehicolation with pectin. Since extracts-induced vasodilation is observed also in the absence of a functional endothelium, these pomaces can be considered promising nutraceuticals for patients with endothelial dysfunction accompanied by vasoconstriction.

## **2. Materials and Methods**

### *2.1 Waste materials and preparation*

Mantonico and Pecorello white grape pomaces were provided from Le Moire srl (ctr Strivillati Motta Santa Lucia, Catanzaro, Italy) of Dr. Paolo Chirillo (Latitude: 39°05'28" N - Longitude: 16°17'35" E - Altitude: 527 m) and harvested in September and October 2017. All the samples were stored at -18°C until use.

## 2.2 Chemicals

Ethanol 96° (pharmaceutical grade), deuterium oxide (D<sub>2</sub>O), 3-(trimethylsilyl)-propionic-2,2,3,3,-d<sub>4</sub> acid sodium salt (TSP) and hydrochloric acid 37% (v/v) (HCl), pectin (esterified 55-70%) from citrus fruit were purchased from Merck (Darmstadt, Germany, Europe). Water used throughout experiments was purified using a Milli-Q system from Millipore Corp. (Bedford, MA, USA). Phenylephrine, acetylcholine, sodium nitroprusside, pinacidil, N $\omega$ -nitro-L-arginine methyl ester (L-NAME), and nifedipine were from Sigma Chemical Co. (Milan, Italy). Phenylephrine was dissolved in 0.1 M HCl and sodium nitroprusside in distilled water. Nifedipine dissolved in ethanol and pinacidil dissolved in DMSO, were diluted at least 1000 times before use.

## 2.3 Sample extraction

Skins and triturated seeds (30 g) were extracted using an ultrasound-bath Branson model 3800-CPXH (Milan, Italy) at 30°C (10 cycles/sec) for 15 minutes, at an ultrasonic frequency of 40 kHz, by adding to raw material 200 mL of a hydro-alcoholic mixture (ethanol:water 50:50) acidified at pH=2 with HCl 37% (v/v) (*Carrera et al., 2012*). The obtained extracts were named as follow: MBS and PBS (Mantonico and Pecorello skins, respectively), MSS and PSS (Mantonico and Pecorello seeds).

## 2.4 Synthesis of the pectin-embedded polymers

A free-radical grafting procedure was performed to synthesize “enriched-extracts” pectin polymers as reported (*Restuccia et al., 2018*). The obtained conjugates were named: PB (blank pectin polymer, used as positive control), MBS pec, PBS pec, MSS pec, PSS pec. Polymers were characterized by IR spectroscopy via PerkinElmer Spectrum Version 10.4.00 Spectrum Two 96417 Software NIOS2 Main 00.02.0009.

## 2.5 Contractility experiments

### 2.5.1 Animals

All animal care and experimental protocols conformed the European Union Guidelines for the Care and the Use of Laboratory Animals (European Union Directive 2010/63/EU) and were approved by the Italian Department of Health (666/2015-PR).

### 2.5.2 Aorta ring preparation

Rat aorta rings (2.5-mm wide), either endothelium-intact or -denuded, were prepared from male Wistar rats (250-350 g, Charles River Italia, Calco, Italy) anaesthetized (i.p.) with a mixture of Zoletil 100® (7.5 mg/kg tiletamine and 7.5 mg/kg zolazepam; Virbac Srl, Milan, Italy) and Rompun® (4 mg/kg xylazine; Bayer, Milan, Italy), decapitated and exsanguinated (*Fusi et al., 2008*). A PowerLab data acquisition system and LabChart 7.3.7 Pro (Power Lab; ADInstruments, Castle Hill, Australia) were used to record and analyse contractile isometric tension. In rings precontracted with 0.3  $\mu$ M phenylephrine, an acetylcholine-induced relaxation  $\geq 75\%$  denoted the presence of a functional endothelium.

### 2.5.3 Effect of extracts on either phenylephrine- or $K^+$ -induced contraction

The same sample of SS and BS, and their conjugated with pectin, was used during the course of the experiments. Extracts were added on the plateau of 0.3  $\mu$ M phenylephrine-induced contraction in rings, either endothelium-intact (in the absence or presence of L-NAME) or -denuded (*Fusi et al., 2017*). A similar protocol was performed in rings deprived of endothelium and depolarised with low (20-30 mM) or high (60 mM)  $K^+$  (*Saponara et al., 2016*). Sodium nitroprusside (100  $\mu$ M) and/or nifedipine (10  $\mu$ M) were used to prove smooth muscle functional integrity at the end of each concentration-response curve. Vasoactivity was calculated as percentage of the active tone induced by either phenylephrine or  $K^+$  (taken as 100%) (*Fusi et al., 2016*).

#### 2.5.4. Data analysis

Values are shown as mean  $\pm$  SEM; n is the number of independent rings analysed (indicated in parentheses), isolated from at least three animals. Analysis of data and Student's t-test for unpaired samples (two-tail), were performed using GraphPad Prism version 5.04 (GraphPad Software Inc., San Diego, CA, USA).  $P < 0.05$  was considered significant. The pharmacological response to the extracts, described in terms of  $IC_{50}$ , was calculated by nonlinear regression analysis.

#### 2.6 Chemical Characterization

The substances contained in the extracts were determined by  $^1H$ -NMR,  $^1H$ - $^1H$  TOCSY homonuclear bidimensional experiments. Samples were re-suspended in 600  $\mu$ l of  $D_2O$  containing TSP (2 mM) as internal chemical shift and concentration standard. Mono-dimensional  $^1H$ -NMR spectra were performed by collecting 64 scans for each spectrum on a Bruker Avance 400 spectrometer (Bruker Spectrospin, Karlsruhe, Germany) operating at 9.4 T at 298 K using a  $90^\circ$  detection pulse and acquiring the FIDs in 64 K points, the spectral width was 6009.15 Hz. The solvent signal was suppressed by means of a presaturation scheme and the relaxation delay was set to 6.55 s in order to achieve a 15 s total acquisition time to avoid relaxation effects. Homonuclear  $^1H$ - $^1H$  TOCSY experiments were performed in order to ensure signal assignment. TOCSY experiments were acquired with a spectral width of 6009.15 Hz in both dimensions, a data matrix of 8 K $\times$ 256 points, a mixing time of 110 ms and relaxation delay of 2 s. Monodimensional  $^1H$  spectra were analysed with 1D- NMR Manager software ver. 12 (ACD/Labs, Toronto, Canada): FIDs underwent exponential multiplication (LB=0.09 Hz), were Fourier transformed, and phase and baseline corrected. Bidimensional spectra were processed with Bruker Topspin ver. 2.1. The assignment of the resonances was performed by analysing  $^1H$ -NMR characteristics and cross-correlated signals in 2D spectra and by comparison with the literature compilations (*Wishart et al., 2013*). Quantification of



the identified compounds was performed by comparison of the signal integral with the reference one, and quantities were expressed in mg of compound normalized for the aliquot weight expressed in g.

### **3. Results and Discussion**

#### *3.1 <sup>1</sup>H-NMR characterization of the extracts*

MBS extract was previously characterized, (*Restuccia et al., 2018*) while the other extracts were characterized in order to evaluate the presence of substances likely responsible for the vasoactivity. In particular, it was found that MSS showed a little amino-acidic component, while there were interesting amounts of ascorbic acid and catechin. The corresponding MBS extract showed an excessive amount of glucose and interestingly the presence of hydroxymethylfurfural. PBS extract showed a substantial acidic component, with high levels of glucose. On the other hand, PSS showed the most interesting chemical composition due to an interesting amino-acidic component, including GABA and tryptophan and considerable amounts of catechin and organic acids, in particular p-coumaric and gallic acids.

#### *3.2 Effects of SS and BS on phenylephrine-induced contraction*

In a first series of experiments, the effects of both SS and BS were assessed on rings pre-contracted with the  $\alpha_1$  adrenergic agonist phenylephrine, either in the presence or absence of endothelium. In endothelium-intact rings, SS from both cultivar caused a concentration-dependent relaxation that, at higher concentrations, was followed by a return to basal values (Fig 1A). Pecorello appeared more efficacious than Mantonico as a vasorelaxant extract. In rings challenged with MSS, removal of the endothelium abolished only vasoconstriction and significantly shifted the vasorelaxant activity to the right (Table 2). Conversely, PSS showed a biphasic vasorelaxant effect, which was more pronounced at concentrations  $>300 \mu\text{g/ml}$  (Figure 1A). These data demonstrate that PSS and MSS simultaneously stimulate the release of endothelium-derived relaxant and contracting factors, the former being

preponderant for PSS, the latter for MSS. In endothelium-denuded rings, myorelaxant activity occurred at higher concentrations as compared to endothelium-intact specimens. This supports the hypothesis that both extracts are capable to directly target the smooth muscle cell and activate vasorelaxant mechanisms thus ensuring a persistent decrease of active muscle tone. Taking into account the chemical composition of the extracts, MSS vasoactivity is likely due to the presence of the vasodilating agents catechin and ascorbic acid (Ghayur, Khan & Hassan Gilani, 2007; Duffy et al., 1999). On the other hand, GABA, which inhibits noradrenaline release from sympathetic nerves thus exerting an antihypertensive effect (Hayakawa, Kimura & Kamata, 2002) and proline, by improving nitric oxide bioavailability, (Leal et al., 2019) may account for PSS vasodilation. Of note, also the presence of gallic acid, which is a well-known, endothelium-dependent and -independent vasodilator agent (de Oliveira et al., 2016). At concentrations  $\leq 30$   $\mu\text{g/ml}$  both endothelium-dependent and -independent vasorelaxant mechanisms concur to the relaxation observed with SS. In this case, Pecorello seemed to be more effective. An endothelium-dependent relaxation occurred also when vascular preparations were challenged with both types of BS (Figure 1B): potency resulted one order of magnitude lower while efficacy higher than those observed with SS (Table 2). In endothelium-denuded arteries, both BS caused a concentration-dependent vasodilation with similar efficacy but a significant lower potency as compared to endothelium-intact rings. Contrary to SS, BS appeared to stimulate the release from the endothelium of only vasorelaxant factors, but similarly to SS, BS possessed also an endothelium-independent vasorelaxant activity, evident at higher concentrations. When compared to other grape skin extracts (e.g. *Vitis labrusca*;) possessing only an endothelium-dependent capacity to relax in vitro vascular specimens, (Madeira et al., 2009); this phenomenon is worth of note. In fact, it is reasonable to think that this endothelium-independent vasorelaxant activity can counteract the reduced NO synthesis and/or the augmented production of superoxide radicals and various contracting factors released from a dysfunctional endothelium, occurring in cardiovascular diseases, such as hypertension (Vanhoutte et al., 2017).

### *3.3 Effects of SS and BS on high K<sup>+</sup>-induced contraction*

In a second series of experiments, the effects of SS and BS from both cultivar were assessed on rings depolarised with high extracellular K<sup>+</sup> concentrations. In this experimental protocol, contraction is essentially due to the influx of Ca<sup>2+</sup> through the open Cav1.2 channels. Both SS and BS were partially effective on this contraction, the relaxant effect starting at quite high concentrations and maximal effect never exceeding 50% of the active muscle tone (Fig 2 A-B). These results suggest that the extracts are scarcely efficacious as Cav1.2 channel blockers. Furthermore, as the vasorelaxant activity was more pronounced on phenylephrine-induced contraction, it can be speculated that they affect either the IP<sub>3</sub>-induced Ca<sup>2+</sup> release from the intracellular stores or the Ca<sup>2+</sup> influx through store- and receptor-operated Ca<sup>2+</sup> channels, or both, mechanisms that play a key role in this active muscle tone (Fransen *et al.*, 2015). Taken together, these data do not indicate any marked difference in the vasoactivity of the extracts except for the seed extracts. In particular PSS contains the larger chemical variety of aminoacids, like GABA, tyrosin and tryptophan, but not ascorbic acid, that is the main component in MSS, together with catechin and malic acid, which are not present in the skin extracts, capable to release contracting factors, such as prostanoids and/or endothelin-1, from the endothelium (Detremmerie *et al.*, 2016).

### *3.4 Effects of pectin-embedded polymers on either phenylephrine-or high K<sup>+</sup>-induced contraction*

In this series of experiments, extracts conjugated to pectin, as well as pectin alone, were assessed for their vasoactivity either on endothelium-intact or -denuded aorta rings. Pectin per se did not affect phenylephrine-induced tone (data not shown). Only at the highest concentration (3 mg/ml) tested, either a modest increase (about 18% of control) or a modest decrease (about 22% of control) of active muscle tone was observed in the presence or absence of the endothelium, respectively. In endothelium-intact rings, the vasoactivity of MSS pec was partly reduced by conjugation while that of PSS pec was markedly reduced and shifted to the right. Their main features (i.e. vasorelaxation followed by the return to basal values), however, were similar to those observed in the absence of the

polymer (Figure 3A; compare to Figure 1A). Pectin conjugation did not modify PSS activity in rings deprived of endothelium. Under the same experimental conditions, MSS lost its vasorelaxant activity, rather showing a constricting effect, probably due to the formation of poly-catechin during grafting reaction (*Liu, Wang & Song, 2018*) (see Supporting Information for characterization). In endothelium-intact rings, MBS was at least one order of magnitude less active when conjugated to pectin (Figure 3B). PBS lost its vasorelaxant activity and rather potentiated the constricting effect of pectin. In the absence of a functional endothelium, both MBS and PBS vasoactivity disappeared. Similar results were obtained in rings deprived of endothelium and depolarised with 60 mM K<sup>+</sup>. The already weak efficacy of both SS and BS extracts, in fact, was abolished in pectin conjugates (Figure 4 A,B). Under these experimental conditions, the polymer per se was ineffective. Taken together, these results indicate a general loss of vasoactivity when pectin polymer was used as a delivery system for Mantonico and Pecorello extracts.

### *3.5 Investigation of the mechanism of action underlying PSS-induced vasodilation*

This series of experiments was performed to explore the mechanism(s) involved in the vasoactivity of the most interesting pomace, i.e. PSS.

First, we investigated a possible K<sup>+</sup> channel opening activity of the extract. Agents that open these channels enhance K<sup>+</sup> efflux from the cell and produce membrane hyperpolarization that, in turn, will cause Cav1.2 channels to close and muscle to relax. A characteristic property of these drugs is that they effectively inhibit vascular smooth muscle contraction caused by a moderate increase in the extracellular K<sup>+</sup> concentration, being ineffective when the K<sup>+</sup> concentration is raised to higher levels (Gurney, 1994). Endothelium-denuded rings were, therefore, contracted with low extracellular K<sup>+</sup> concentrations (20-30 mM). Cumulative concentrations of PSS (up to 300 µg/mL) did not affect muscle active tone (Fig 5 A). Conversely, the subsequent addition of 100 µM pinacidil, a well-known K<sub>ATP</sub> channel opener (*Gollasch et al., 1995*), almost completely reverted K<sup>+</sup>-induced contraction to 6.5±1.8% of control (n=6), thus demonstrating that PSS is not capable to open K<sup>+</sup> channels.

In a second series of experiments, the endothelium-derived factor involved in PSS-induced relaxation was investigated. As already shown in Figure 1A, the cumulative addition of PSS relaxed intact aorta rings precontracted with phenylephrine (Figure 5B). This effect was suppressed by preincubation of tissues with 100  $\mu$ M L-NAME, a well-known inhibitor of eNOS (*Moncada et al., 1991*). The subsequent addition of sodium nitroprusside, a well-known NO-donor, completely reverted phenylephrine-induced contraction ( $0.0\pm 0.0\%$  of control, n=5), thus confirming that PSS relaxing effect is endothelium-dependent and mediated by eNOS. The production of an endothelium-dependent hyperpolarizing factor can be excluded, because its relaxing effect is not modified by NO synthase inhibitors (Fukao et al., 1995). Furthermore, as PSS-induced relaxation is abolished by the presence of L-NAME, also the production of prostanoids can be ruled out.

#### **4. Conclusions**

Waste SS and BS indeed represent, as previously demonstrated by others laboratories, a worth of potentially vasoactive compounds particularly useful in disease states accompanied by endothelial dysfunction along with vasoconstriction. The conjugation with pectin to improve their delivery, however, seems not an avenue worth to be paved.

#### **Supplementary Material**

<sup>1</sup>H-NMR original spectra of all the extracts, IR spectra of the pectin polymers, vasoactivity results of pectin non-enriched polymer.

#### **Conflicts of interest**

There are no conflicts to declare

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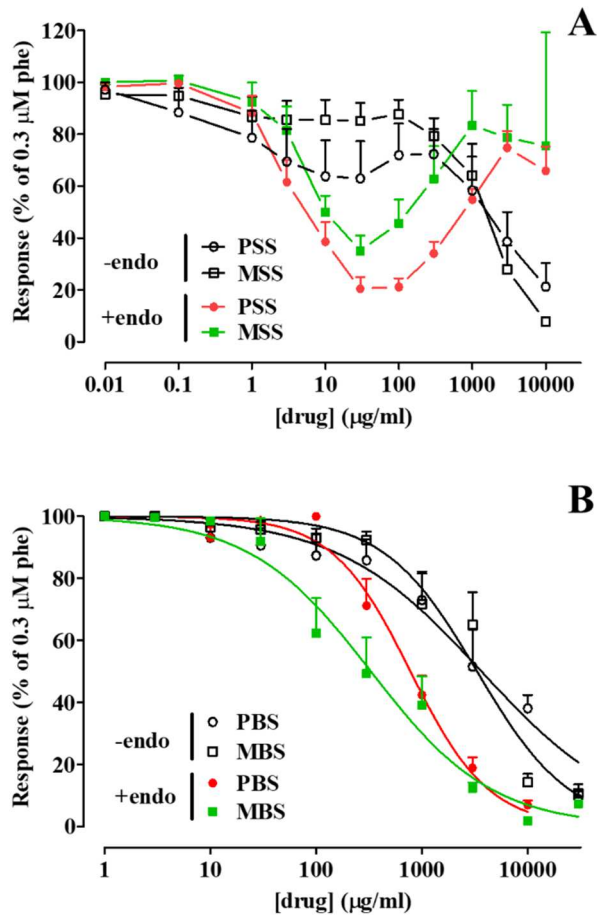
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	Molecule	Amount in PSS(mg/g)	Amount in PBS (mg/g)	Amount in MSS (mg/g)
<b>Aminoacids</b>	Valine	0.74	1.47	0.85
	Isoleucine	0.59	0.37	0.59
	Leucine	0.34	0.37	1.28
	Threonine	1.82	0.58	1.34
	Alanine	1.01	0.51	1.15
	Proline	5.78	6.14	-
	GABA	2.77	-	-
	Tyrosine	0.53	-	-
	Phenylalanine	0.53	0.40	-
	Tryptophan	0.69	-	-
<b>Organic Acids</b>	Lactic acid	2.46	0.52	0.75
	Butyric acid	-	0.71	
	Acetic acid	0.57	0.31	
	Quinic acid	8.85	12.34	
	Tartaric acid	3.87	4.97	
	Sorbic acid	-	2.40	2.50
	Succinic acid	3.17	0.75	2.05
	Ascorbic acid	-	8.15	74.89
	Malic acid	12.91	2.87	36.83
	p-Coumaric acid	3.10	1.04	
	Gallic acid	2.97	0.24	
	Shikimic acid	-	0.50	
	Formic acid	0.05	0.04	0.14
<b>Carbohydrates</b>	Glucose	254.62	616.67	490.27
	Rhamnose	3.46	8.24	8.11
	Sucrose	8.67	5.70	4.29
	Raffinose	7.90	5.47	4.02
	2,3-Butanediol	0.10	0.40	0.47
<b>Miscellaneous</b>	Glycosidated flavonoids	16.94	2.22	23.70
	Ethanol	-	45.92	-
	Acetamide	0.29	0.33	-
	Choline	0.63	-	-
	Catechin	1.77	1.28	55.72

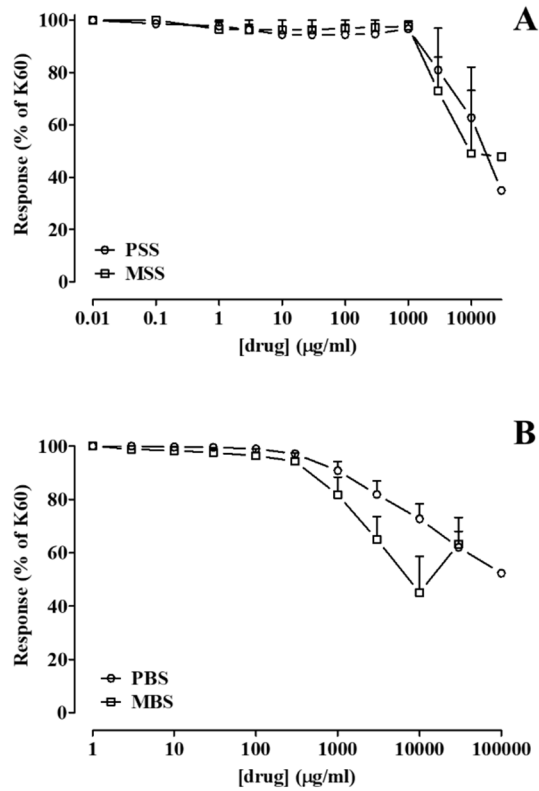
**Table 1. Amounts of compounds contained in the extracts determined by <sup>1</sup>H-NMR**

	<b>IC<sub>50</sub> (mg/ml)</b>		
	<b>Phe+endo</b>	<b>Phe-endo</b>	<b>K60-endo</b>
<b>MSS</b>	0.015±0.004 (11)	1.486±0.438 (6)*	□10 (2)
<b>PSS</b>	0.012±0.006 (11)	1.230±0.455 (5)*	□17 (2)
<b>MBS</b>	0.560±0.204 (10)	3.374±0.888 (10)*	□10 (6)
<b>PBS</b>	0.795±0.122 (10)	4.246±1.231 (10)*	□100 (1)

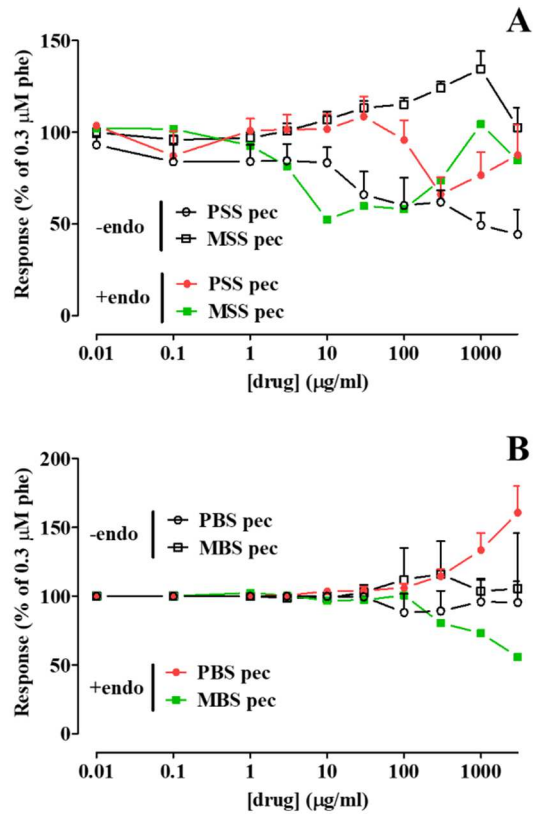
**Table 2. Effect of the extracts on either phenylephrine- or 60 mM KCl-induced contraction of rat aorta rings. Rings, endothelium-denuded (-endo) or -intact (+endo), were contracted either by 0.3 μM phenylephrine (phe) or by depolarization with 60 mM KCl (K60). Potency (IC<sub>50</sub>) is reported as mean ± SEM. \*P<0.05 vs. phe+endo, Student's t test for unpaired samples.**



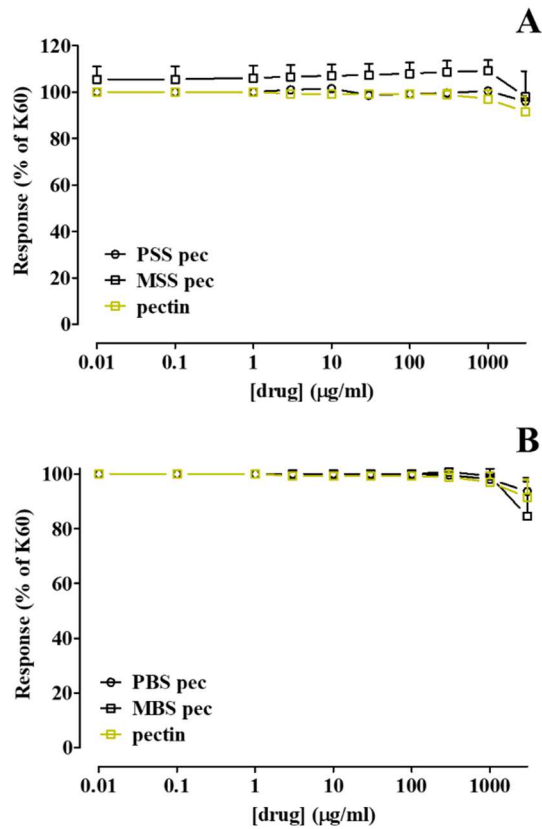
**Figure 1.** Effect of the extracts on phenylephrine-induced contraction of rat aorta rings. Concentration-response curves of (A) PSS and MSS or (B) PBS and MBS on endothelium-denuded (-endo) or endothelium-intact preparations (+endo) pre-contracted by 0.3  $\mu$ M phenylephrine (phe). In the ordinate scale, relaxation is reported as percentage of the initial tension induced by phenylephrine, taken as 100%. Data are mean  $\pm$  S.E.M. (n=6-11, last concentration being 2-6).



**Figure 2. Effect of the extracts on high KCl-induced contraction of rat aorta rings. Concentration-response curves of (A) PSS and MSS or (B) PBS and MBS on endothelium-denuded preparations pre-contracted by 60 mM KCl (K60). In the ordinate scale, relaxation is reported as percentage of the initial tension induced by KCl, taken as 100%. Data are mean  $\pm$  S.E.M. (n=3-10, last concentration being 1-4).**



**Figure 3. Effect of pectin-embedded polymers on phenylephrine-induced contraction of rat aorta rings. Concentration-response curves of (A) PSS pec and MSS pec or (B) PBS pec and MBS pec on endothelium-denuded (-endo) or endothelium-intact preparations (+endo) pre-contracted by 0.3  $\mu$ M phenylephrine (phe). In the ordinate scale, relaxation is reported as percentage of the initial tension induced by phenylephrine, taken as 100%. Data are mean  $\pm$  S.E.M. (n=4-8).**



**Figure 4. Effect of pectin and pectin-embedded polymers on high KCl-induced contraction of rat aorta rings. Concentration-response curves of (A) PSS pec and MSS pec or (B) PBS pec and MBS pec on endothelium-denuded preparations pre-contracted by 60 mM KCl (K60). The effect of pectin alone are represented in both panels for a better comparison. In the ordinate scale, relaxation is reported as percentage of the initial tension induced by KCl, taken as 100%. Data are mean  $\pm$  S.E.M. (n=3-5).**



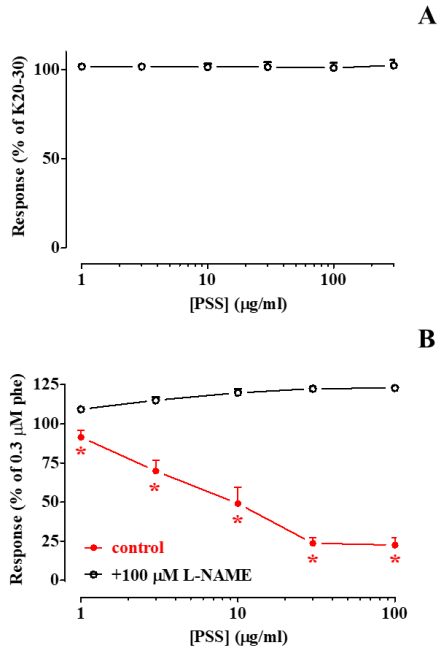


Figure 5. Effects of PSS on rat aorta rings. Concentration-response curves for PSS recorded either (A) in endothelium-denuded, 20-30 mM K<sup>+</sup> or (B) in endothelium-intact, 0.3 μM phenylephrine precontracted rings, the latter in the absence or presence of 100 μM L-NAME preincubated for 30 min. On the ordinate scale, relaxation is reported as a percentage of the initial tension induced by K<sup>+</sup> or phenylephrine (taken as 100%). Data points are means ± S.E.M. (*n*=5-8). \**P*<0.05, Student's *t* test for unpaired samples.