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# ENDOTHELIUM-MEDIATED VASORELAXANT EFFECTS OF CHOKEBERRY (*ARONIA MELANOCARPA*) EXTRACT, JUICE, AND WASTE: AN EXPERIMENTAL STUDY IN ISOLATED RAT AORTA

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*Aronia melanocarpa* (chokeberry, aronia) is rich in polyphenols and associated with vascular protective effects; however, comparative data on different aronia-derived products and their mechanisms remain limited. This study aimed to compare the vasorelaxant effects of aronia berry extract (AE), aronia waste extract (AWE), and aronia juice (AJ), and to investigate their underlying mechanisms. Preparations from organically grown berries (western Serbia) were chemically characterized using spectrophotometric methods and HPLC. Vasorelaxant activity was assessed in isolated thoracic aorta rings from Wistar rats precontracted with potassium chloride (KCl) or phenylephrine (PE). All preparations induced concentration-dependent relaxation. In KCl-precontracted rings, AE showed the greatest potency ($EC_{50}$ = 0.97 ± 0.02 mg/mL), followed by AJ (1.74 ± 0.08 mg/mL) and AWE (1.95 ± 0.12 mg/mL). In contrast, under PE-induced contraction, AWE exhibited the highest activity ($EC_{50}$ = 0.58 ± 0.07 mg/mL), followed by AJ (0.96 ± 0.06 mg/mL) and AE (1.27 ± 0.11 mg/mL). Pre-incubation with the nitric oxide synthase inhibitor L-NAME significantly attenuated relaxation responses, increasing $EC_{50}$ values for AWE (0.59 ± 0.03 to 2.37 ± 0.01 mg/mL), AE (0.92 ± 0.01 to 4.38 ± 0.05 mg/mL), and AJ (0.95 ± 0.02 to 2.78 ± 0.03 mg/mL) ($p < 0.01$), indicating a key role of endothelium-derived nitric oxide. These findings demonstrate that aronia-derived products, including processing waste, exert potent NO-mediated vasorelaxant effects and may represent promising functional ingredients for improving vascular function. Further clinical studies are warranted to confirm these effects in humans.

Keywords: *Aronia melanocarpa,* waste extract, vasorelaxation, nitric-oxide pathway, rat aorta

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## INTRODUCTION

Cardiovascular diseases (CVD) remain a leading cause of morbidity and mortality worldwide, with endothelial dysfunction and impaired vasorelaxation representing critical early events in their pathogenesis (1,2). Endothelium-derived nitric oxide (NO) is a key regulator of vascular tone, and reduced NO bioavailability is closely associated with hypertension, atherosclerosis, and other vascular disorders. Recent reviews have highlighted that impaired endothelium-dependent vasodilation and chronic inflammation are central to the initiation and progression of CVD, and that interventions targeting endothelial function are a promising strategy for prevention and management (2). Increasing evidence suggests that dietary strategies rich in bioactive phytochemicals may improve endothelial function and promote vasorelaxation. A recent narrative review concluded that polyphenol-rich foods, including berries, consistently improve flow-mediated dilation, a clinical measure of endothelial function, and are associated with enhanced NO bioavailability and reduced cardiovascular risk (4).

Berries are recognized as an important dietary source of polyphenolic compounds, including anthocyanins, flavonols, and phenolic acids, which have been consistently associated with cardiovascular protection (5,6). Anthocyanin-rich products and other polyphenol-rich foods have been shown to enhance the expression and phosphorylation of endothelial nitric oxide synthase (eNOS), increase NO production, and improve vasodilatory responses, thereby mitigating endothelial dysfunction and oxidative stress (4,7). Experimental and clinical studies indicate that regular consumption of berry fruits and berry-derived products improves endothelial function, enhances NO-mediated vasodilation, and reduces oxidative stress and vascular inflammation (4,8). These vascular effects are attributed to both direct modulation of endothelial signaling pathways, including eNOS activation, and indirect antioxidant mechanisms (9).

Among berry species, *Aronia melanocarpa* [Michx.] Elliott (aronia, chokeberry) has attracted considerable scientific interest due to its exceptionally high content of polyphenols, particularly anthocyanins and proanthocyanidins (10,11). Aronia preparations, including juices and standardized extracts, have demonstrated strong antioxidant, anti-inflammatory, and vasoprotective properties in various experimental models (12,13). In isolated vascular tissues, aronia juice induces potent endothelium-dependent vasorelaxation, primarily mediated by enhanced NO release following redox-sensitive activation of endothelial nitric oxide synthase (eNOS) through Src/PI3K/Akt signaling pathways (14). More recent evidence further confirms that polyphenol-rich *Aronia melanocarpa* juice sustains eNOS activation via phosphorylation and increased expression in endothelial cells, supporting its potential role in vascular protection (15).

*In vitro* studies further support these observations, showing that polyphenol-rich aronia extracts significantly increase NO production and eNOS phosphorylation in cultured endothelial cells in a concentration-dependent manner (16). Moreover, human intervention studies have reported improvements in endothelial function, assessed by flow-mediated dilation, as well as reductions in blood pressure following consumption of aronia-based products or anthocyanin-rich berry extracts (17,18). Collectively, these findings highlight the potential of aronia polyphenols to modulate vascular tone and endothelial homeostasis.

In recent years, attention has increasingly focused on valorizing berry processing by-products, particularly chokeberry waste materials, as sustainable sources of bioactive compounds with potential vascular benefits. Pomace and other residues remaining after juice production retain substantial amounts of polyphenols, including anthocyanins, flavan-3-ols, and phenolic acids, often at concentrations comparable to or even exceeding those found in the corresponding juices (11,19,20). Recent studies further emphasize that chokeberry pomace represents a rich and underutilized source of antioxidant phytochemicals suitable for functional food and nutraceutical applications (21,22). Utilizing chokeberry waste extracts not only supports circular economy and sustainable food production but also represents a promising approach for the development of functional ingredients and nutraceuticals.

Despite growing evidence supporting the vasorelaxant properties of berry-derived polyphenols, the relative contribution of different aronia preparations—including juices, whole-fruit extracts, and waste-derived extracts—and the precise molecular mechanisms underlying their vascular effects remain incompletely understood. Further investigation into these mechanisms may provide valuable insights for the development of novel dietary strategies and functional products that aim to improve vascular function and cardiovascular health.

The novelty of the present study lies in the direct comparative evaluation of vasorelaxant effects of three different aronia-derived preparations (berry extract, juice, and processing waste extract) under identical experimental conditions in isolated rat aorta. In particular, this study

provides new insight into the vascular activity of chokeberry processing by-products (pomace-derived extract), which remain insufficiently investigated despite their high polyphenolic content. In addition, the study contributes to understanding the relative contribution of NO-mediated mechanisms in these preparations under both receptor-mediated (phenylephrine) and depolarization-induced (KCl) contraction models.

## METHODS

### Plant material, extracts, and juice preparation and chemical characterization

Aronia (chokeberry) juice and extracts were prepared from organically cultivated berries harvested in western Serbia. Juice was first obtained from frozen–thawed fruits using a Hydropress 90 L (Speidel, Germany), while the remaining pressed pomace was collected as by-product material for waste extract production. Both whole berries and pomace were dried at 40°C for 48 h (Instrumentaria ST 01/02, Croatia), milled, and sieved (0.75 mm).

Dried samples were macerated with 50% ethanol (1:20, w/v) under previously optimized conditions for polyphenol recovery (23–25). Ethanol was removed under reduced pressure at 60°C (Buchi rotavapor R-114). The juice, whole berry extract, and waste extract were then freeze-dried after pre-freezing at −80°C, using a lyophilizer (Beta 1-8 Freeze Dryer, Martin Christ, GmbH, Osterode am Harz, Germany) at −60°C and low pressure.

The final lyophilized preparations, aronia berry extract (AE), aronia waste extract (AWE), and aronia juice (AJ), were stored in sealed glass containers at −4°C until further analysis.

The chemical composition of the preparations, including total polyphenols, flavonoids, anthocyanins, proanthocyanidins, and individual compounds, was determined using spectrophotometric methods and high-performance liquid chromatography (HPLC), following the protocols reported by Milutinović et al., 2024 (25).

### Vasorelaxant activity of the AE, AWE, and AJ

The vasorelaxant activity of chokeberry fruit extracts and juice was evaluated using isolated thoracic aorta rings from Wistar rats. The effects of the chokeberry extract (AE), the extract obtained from the fruit residue after juice pressing (AWE), and chokeberry juice (AJ) were assessed on induced contractions of isolated aortic segments. The influence of these preparations on vascular contractility was examined in the presence of KCl, the nitric oxide synthase inhibitor L-NAME, and phenylephrine (PE). Verapamil, a calcium channel blocker, was used as a positive control. All experiments were performed using thoracic aortic rings isolated from six independent male Wistar rats (n = 6). Throughout the manuscript, n refers to the number of biological replicates (individual animals).

### Experimental animals

All animal experimental procedures were approved by the Ethics Committee and conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes (approval issued by the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate, decision No. 323-07-04034/2018-05/2).

Male Wistar albino rats (180–220 g) were obtained from the animal facility of the Scientific Research Center for Biomedicine, University of Niš Faculty of Medicine. Animals were housed under standard laboratory conditions (20–24°C, 12 h light/dark cycle) with free access to food and water. Food was withdrawn 24 h prior to the experiment. The study involved a total of six male Wistar rats.

### Isolation and preparation of thoracic aorta rings

The isolation of thoracic aorta rings was performed as previously described (26). Briefly, rats were sacrificed, the thoracic cavity was opened, and the thoracic aorta was carefully excised. The tissue was immediately placed in ice-cold, oxygenated Krebs solution, cleaned of surrounding connective tissue, and cut into rings 3–5 mm in length.

Aortic rings were mounted in a 20 mL organ bath containing Krebs solution under a resting tension of 1 g and allowed to equilibrate for at least 30 min at 37°C before the experiments. The Krebs solution consisted of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2{\cdot}2H_2O$, 2.6 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, and 5.5 mM glucose (pH 7.4), continuously aerated with 95% $O_2$ and 5% $CO_2$.

Changes in isometric tension were recorded using a force transducer (TSZ-04-E, Experimetria Ltd., Budapest, Hungary) and analyzed with the SPEL Advanced ISOSIS data acquisition system (Experimetria Ltd., Budapest, Hungary).

### Assessment of vascular contractility and vasorelaxation

To evaluate the effects of chokeberry preparations on vascular reactivity, aortic rings were precontracted with potassium chloride ($K^+$ 80 mM) or phenylephrine (PE, 1 µM). Vasorelaxant responses to AE, AWE, and AJ were examined in endothelium-intact rings.

To investigate the involvement of nitric oxide (NO), aortic

rings were preincubated with L-NAME (0.1 mM) for 20 min before contraction with phenylephrine (PE, 1 µM). Vasorelaxant responses to the tested preparations were subsequently evaluated under the same experimental conditions.

To verify endothelial integrity, aortic rings were precontracted with phenylephrine (1 µM), followed by the addition of acetylcholine. Only rings exhibiting more than 70% relaxation in response to acetylcholine were considered endothelium-intact and included in subsequent experiments, whereas rings not meeting this criterion were excluded.

Chokeberry extracts and juice were applied cumulatively at concentrations ranging from 0.001 to 1 mg/mL. Each concentration was allowed to act until the vascular response reached a stable plateau before adding the subsequent concentration. The next concentration was applied only after no further change in isometric tension was observed, ensuring that cumulative concentration–response curves were generated under steady-state conditions.

Vasorelaxation was expressed as the percentage reduction of the initial contraction induced by the respective vasoconstrictor, which was taken as 100%. Accordingly, in KCl-precontracted rings, relaxation was calculated relative to the KCl-induced contraction, whereas in phenylephrine (PE)-precontracted rings, relaxation was calculated relative to the PE-induced contraction. Verapamil was used as a positive vasorelaxant control and was applied cumulatively at concentrations ranging from 0.01 to 1 mg/mL.

The inhibitory effects of the tested preparations were calculated from the area under the contraction curve relative to baseline and expressed as percentage inhibition (27,28).

The concentration range (0.001–1 mg/mL) was selected based on preliminary experiments as it was considered suitable for assessing concentration-dependent vasorelaxant responses while minimizing potential nonspecific physicochemical effects associated with higher concentrations of crude herbal preparations, including reduced solubility, precipitation of constituents, increased viscosity, and alterations of the organ bath medium.

### Statistical analysis

Statistical evaluation was performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). Data are presented in tables and figures as mean ± standard deviation. The normality of data distribution was assessed with the Shapiro–Wilk test. Differences between two groups were analyzed using the independent Student's t-test for normally distributed data or the Mann–Whitney test for non-normal data. Comparisons between multiple groups were conducted using either one-way ANOVA or the Kruskal–Wallis test, depending on the data suitability. When ANOVA was applied, post-hoc tests were conducted for pairwise group comparisons. Statistical significance was set at $p < 0.05$.

## RESULTS

### Chemical characterization of the AE, AWE, and AJ

The phytochemical composition of AE, AWE, and AJ was comprehensively characterized in our previous study using spectrophotometric assays and HPLC analysis (25). A concise summary of the major bioactive constituents is presented in Table 1. All preparations contained high levels of polyphenolic compounds, including phenolic acids, flavonoids, anthocyanins, and proanthocyanidins. Among the investigated preparations, AWE exhibited the highest content of most quantified polyphenolic constituents, whereas differences in the relative abundance of individual compounds were observed among AE, AWE, and AJ. These compositional differences may contribute to the differences in their vasorelaxant activities observed in the present study.

### Vasorelaxant activity of the AE, AWE, and AJ

The vasorelaxant effects of aronia extracts and juice on isolated rat thoracic aorta were evaluated in the presence of elevated $K^+$ concentration (80 mM) in the first experimental series (Figure 1). Contraction of aortic smooth muscle was induced by adding KCl to the organ bath, and the response progressively decreased following the cumulative application of aronia preparations at concentrations of 0.001 to 1 mg/mL.

Among the tested samples, AE demonstrated the most pronounced activity, with an $EC_{50}$ value of 0.97 ± 0.02 mg/mL, producing the highest and statistically significant relaxation of aortic smooth muscle ($p < 0.01$). Conversely, AWE exhibited the weakest vasorelaxant potential against KCl-induced contractions ($EC_{50}$ = 1.95 ± 0.12 mg/mL). Aronia juice (AJ) showed slightly greater efficacy compared to AWE, with an $EC_{50}$ value of 1.74 ± 0.08 mg/mL.

Verapamil, used as a positive vasorelaxant control, produced a concentration-dependent relaxation response, with an $EC_{50}$ of 0.00077 ± 0.00001 mg/mL ($p < 0.01$). Differences among $EC_{50}$ values obtained for AE, AWE, AJ, and verapamil were assessed using one-way ANOVA, which

**Table 1.** *Total phenolics, anthocyanins, flavonoids, proanthocyanidins, and HPLC quantification of individual compounds in AE, AWE, and AJ (adapted from Milutinović et al.,2024 (25)).*

| | AE | AWE | AJ | *P* |
|---|---|---|---|---|
| Compounds | Amounts | | | |
| Total phenolics content* | 261.71 ± 35.26[a] | 605.67 ± 64.58[b] | 495.91 ± 25.85[c] | 0 |
| Total anthocyanins** | 95.24 ± 15.33[a] | 423.81 ± 23.17[b] | 111.32 ± 10.72[a] | 0 |
| Total flavonoids*** | 39.25 ± 6.57[a] | 29.36 ± 1.83[a] | 29.21 ± 1.89[a] | 0.038 |
| Total proanthocyanidins**** | 97.1 ± 5.56[a] | 129.28 ± 21.9[a] | 105.32 ± 5.51[a] | >0.05 |
| Chlorogenic acid# | 2.29 ± 0.35[a] | 3.72 ± 0.47[b] | 2.77 ± 0.36[a] | 0.012 |
| Cyanidin-3-*O*-galactoside# | 0.88 ± 0.07[a] | 5.21 ± 0.4[b] | 1.32 ± 0.07[a] | 0 |
| Cyanidin-3-*O*-glucoside# | 0.12 ± 0.02[a] | 0.77 ± 0.1[b] | 0.15 ± 0.01[a] | 0 |
| Cyanidin-3-*O*-arabinoside# | 0.37 ± 0.06[a] | 2.31 ± 0.14[b] | 0.41 ± 0.05[a] | 0 |
| Quercetin-3-*O*-rutinoside# | 0.21 ± 0.03[a] | 0.04 ± 0.00[b] | 0.12 ± 0.02[c] | 0 |
| Quercetin-3-*O*-galactoside# | 0.13 ± 0.01[a] | Traces | 0.01 ± 0.00[b] | 0 |
| Quercetin-3-*O*-glucoside# | 0.14 ± 0.01[a] | Traces | 0.02 ± 0.00[b] | 0 |

Each value represents the mean of n = 3 ± standard deviation.
* mg GAE/g of AE, AWE or AJ.
** mg cyanidin-3-O-glucoside equivalents/g of AE, AWE or AJ.
*** mg catechin/g of AE, AWE or AJ.
**** mg catechins equivalent/g of AE, AWE or AJ.
# mg/g of AE, AWE or AJ.
[a,b,c] Different letters in the rows indicate a statistically significant difference in the content of active compounds between AE, AWE or AJ, based on the ANOVA test (Tukey post-hoc, $p < 0.05$).

**Table 2.** *$EC_{50}$ values for KCl-induced and PE-induced contractions obtained with AE, AWE, AJ, and verapamil (positive control)*

| | $EC_{50}$ values for KCl-induced contractions (mg/ml) | $EC_{50}$ values for FE-induced contractions (mg/ml) |
|---|---|---|
| **AE** | 0,97 ± 0,02 [a] | 1,27 ± 0,11 [a] |
| **AWE** | 1,95 ± 0,12 [b] | 0,58 ± 0,07 [b] |
| **AJ** | 1,74 ± 0,08 [b] | 0,96 ± 0,06 [c] |
| **Verapamil** | 0,00077 ± 0,00001[c] | |

Statistical significance was assessed by one-way ANOVA followed by Tukey's post-hoc test, and data are expressed as mean ± SEM. Different superscript letters within the same column denote statistically significant differences in $EC_{50}$ values (Tukey's post-hoc test; $p < 0.01$). Specifically, groups sharing different letters differ significantly, while groups sharing the same letter do not differ significantly.
*Abbreviations*: AE, aronia fruit extract; AWE, aronia waste extract; AJ, aronia juice; PE, phenylephrine

confirmed statistically significant variation between treatments (Table 2).

In the second experimental series, aortic rings were precontracted by the addition of phenylephrine (PE; 1 µM) to the organ bath. Verapamil, used as a positive vasorelaxant control, demonstrated a concentration-dependent relaxation response.

The strongest vasorelaxant effect against PE-induced contractions of rat aorta was observed for AWE, which exhibited the lowest $EC_{50}$ value (0.58 ± 0.07 mg/mL, $p < 0.01$). The chokeberry extract (AE) produced a weaker relaxation response ($EC_{50}$ = 1.27 ± 0.11 mg/mL), whereas the juice demonstrated greater efficacy ($EC_{50}$ = 0.96 ± 0.06 mg/mL) (Figure 2).

A comparison of $EC_{50}$ values for the extracts, juice, and verapamil in the PE-induced contraction model is shown in Table 2.

The vasorelaxant activity of AE, AWE, and AJ was further evaluated in the presence of the nitric oxide synthase inhibitor L-NAME (0.1 mM), applied after phenylephrine-induced precontraction, to clarify the involvement of NO-mediated mechanisms. Since endogenous NO contributes to vascular relaxation, inhibition of its synthesis markedly attenuated the effects of all tested preparations.

These results suggest that the vasorelaxant responses of AE, AWE, and AJ are predominantly NO-dependent (Figure 3). Under PE stimulation, AWE showed the highest potency ($EC_{50}$ = 0.59 ± 0.03 mg/mL), whereas L-NAME significantly shifted its $EC_{50}$ to 2.37 ± 0.01 mg/mL ($p < 0.01$).

Similarly, blockade of NO production increased $EC_{50}$ values for AE and AJ to 4.38 ± 0.05 mg/mL and 2.78 ± 0.03 mg/mL, respectively, compared with significantly lower values obtained under control conditions (0.92 ± 0.01 mg/mL and 0.95 ± 0.02 mg/mL; $p < 0.01$).

## DISCUSSION

Elevated blood pressure is a major risk factor for cardiovascular diseases and stroke. Even a modest reduction in systolic blood pressure by 3 mmHg has been estimated to lower stroke mortality by 8% and coronary heart disease mortality by 5% (29). A direct relationship between blood pressure and cardiovascular mortality has been observed in adults aged 40–89 years (30). Maintaining lower blood pressure throughout life is therefore crucial, highlighting the importance of identifying natural compounds and preparations that can help reduce blood pressure. Early dietary interventions in mild hypertension may also delay the need for pharmacological treatment (31).

Consequently, research into the effects of bioactive natural products on vascular health is of considerable interest (32). Several studies have shown that fruits and fruit juices, particularly berries such as blackcurrants and cranberries, can contribute to blood pressure reduction (33,34). Blackcurrants have also been reported to exhibit strong vasorelaxant properties (33).

Chokeberry juice and extracts are highly rich in polyphenolic compounds and exhibit strong antioxidant activity, as previously reported (25), indicating their potential utility in conditions linked to oxidative stress. Since oxidative stress plays a key role in the development of hypertension, suppression of reactive oxygen species may contribute to lowering blood pressure (35,36). Epidemiological and clinical evidence suggests that regular consumption of anthocyanin-rich foods is associated with a lower risk of cardiovascular diseases, including coronary heart disease (5,37,38). A recent meta-analysis of prospective cohort studies reported that higher dietary anthocyanin intake correlates with reduced incidence of coronary heart disease and total cardiovascular events (39).

Clinical trials further support these findings, showing that anthocyanin-rich interventions, such as blackcurrant extracts, improve endothelial function and modestly reduce blood pressure and inflammatory markers (40,41). These data indicate that dietary anthocyanins may contribute to cardiovascular protection and could be considered as part of preventive or adjunctive strategies. Nonetheless, long-term, large-scale studies are needed to confirm their effects on specific cardiovascular outcomes such as myocardial infarction.

Chokeberry preparations have been shown to reduce oxidative stress and vascular inflammation, major contributors to cardiovascular dysfunction, suggesting significant cardioprotective potential through their antioxidant and anti-inflammatory properties. Both in vitro and *in vivo* studies indicate that polyphenol-rich chokeberry extracts and juices improve endothelial function, inhibit pro-inflammatory pathways, and decrease oxidative damage in vascular tissues (42–44). These findings support the potential use of chokeberry products as functional dietary interventions for cardiovascular health.

Emerging evidence suggests that chokeberry preparations exhibit significant vasorelaxant activity, predominantly through endothelium-dependent mechanisms involving increased NO bioavailability, activation of endothelial nitric oxide synthase (eNOS), and attenuation of oxidative stress in vascular tissues (15,45). These effects are primarily attributed to enhanced endothelial nitric oxide (NO) release (46,47), although $K^+$ channel activation has also been implicated in mediating polyphenol-induced relaxation (33,48,49). Given the central role of $K^+$ channels in vascular smooth muscle contraction (50), it is likely that part of the vascular effects of chokeberry preparations is mediated via $K^+$ channel activation. Polyphenols can modulate vascular tone by inhibiting $Ca^{2+}$ channels and/or activating $K^+$ channels in vascular cells (51).

Rhythmic contractions of vascular smooth muscle can occur spontaneously or in response to agonists (52,53). These contractions are preceded by membrane hyperpolarization through $Ca^{2+}$-activated $K^+$ channels (54,55). For example, phenylephrine (PE) induces rhythmic contractions via membrane depolarization, leading to $Ca^{2+}$ influx through voltage-dependent channels (53,56). PE stimulation of α-adrenergic receptors causes smooth muscle depolarization and vasoconstriction; conversely, activation of endothelial NO synthase (eNOS) by intracellular $Ca^{2+}$ triggers NO release, which promotes $K^+$ efflux via protein kinase G and subsequently repolarizes vascular smooth muscle through $K^+$ channel opening (57).

Our results demonstrate that chokeberry preparations exhibit significant vasorelaxant activity in the isolated rat aorta model. The observed relaxation was predominantly mediated by nitric oxide (NO), as evidenced by the marked attenuation of vasorelaxation following administration of L-NAME, a nitric oxide synthase inhibitor. This finding indicates that NO release from the endothelium plays a central role in the mechanisms underlying the vascular effects of chokeberry extracts and juice. Furthermore, relaxation responses were more pronounced in phenylephrine-induced contractions compared to those induced by KCl, although AE, AWE, and AJ effectively relaxed aortic smooth muscle in both conditions.

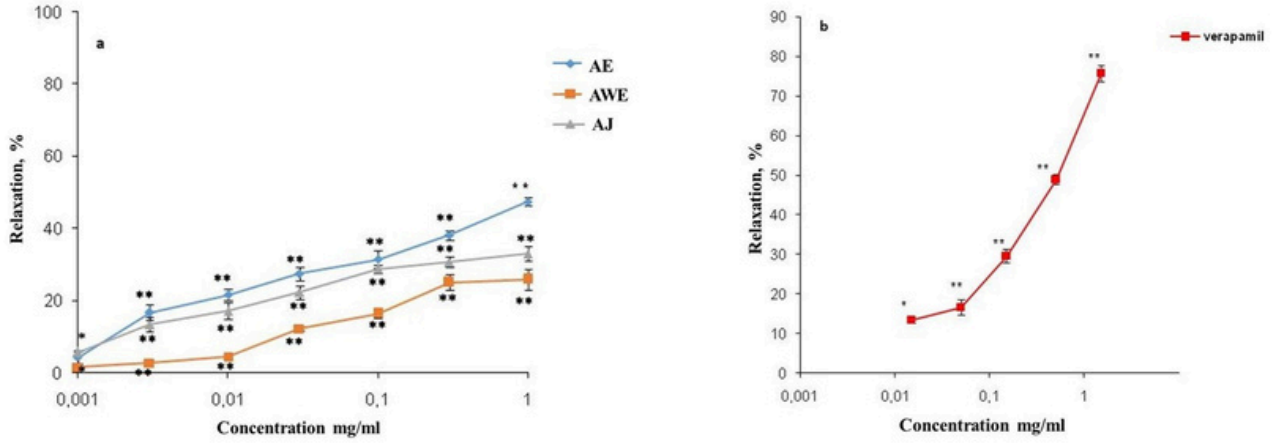


**Figure 1.** *Relaxant effects of AE, AWE, AJ (a) on KCl-induced contractions of isolated rat aorta. The concentration–response curve of verapamil, included as the positive control, is presented separately (b) to facilitate direct comparison among the three chokeberry preparations. Each point represents the mean ± SD of responses obtained from thoracic aortic rings isolated from six independent rats (n = 6) (Student's t-test, *p < 0.05, **p < 0.01).*

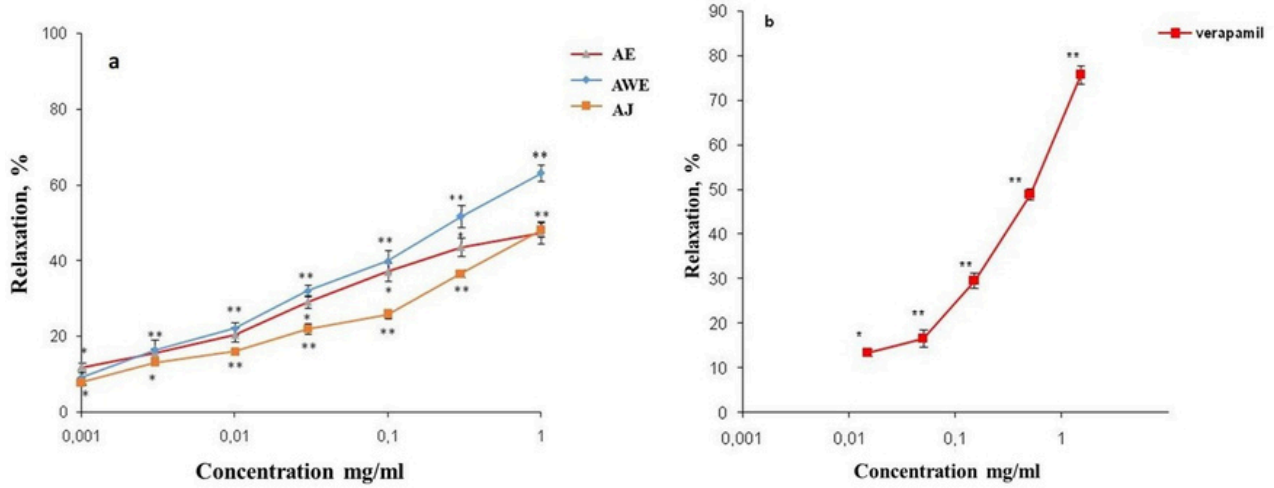


**Figure 2.** *Relaxant effects of AE, AWE, and AJ (a) on PE-induced contractions of isolated rat aorta. The concentration–response curve of verapamil, included as the positive control, is presented separately (b) to facilitate direct comparison among the three chokeberry preparations. Each point represents the mean ± SD of responses obtained from thoracic aortic rings isolated from six independent rats (n = 6) (Student's t-test, *p < 0.05, **p < 0.01).*

These results are consistent with previous studies demonstrating that polyphenol-rich berry extracts induce endothelium-dependent vasorelaxation through enhanced NO bioavailability. For example, flavonoid-rich extracts from berries such as blackcurrant and blueberry have been shown to increase endothelial nitric oxide synthase (eNOS) activity and improve nitric oxide-mediated relaxation in isolated vascular tissues (47,58,59). Similar mechanisms have been proposed for anthocyanins, which can stimulate NO release and improve endothelial function in both experimental and clinical settings (7,39).

The greater vasorelaxation observed in phenylephrine-induced contractions compared to KCl-induced contractions is also supported by the literature. Phenylephrine triggers contraction via α-adrenergic receptor activation and subsequent increases in intracellular $Ca^{2+}$, whereas KCl depolarizes the membrane more directly through the opening of voltage-gated calcium channels (53,56). Endothelium-dependent mechanisms, particularly NO release, tend to have a stronger modulatory effect in agonist-induced contractions than in depolarization models, which aligns with our findings.

Moreover, polyphenols have been shown to exert vascular effects not only through NO-dependent pathways but also via modulation of ion channels involved in smooth muscle tone. Specifically, activation of potassium ($K^+$) channels and inhibition of calcium ($Ca^{2+}$) influx have been implicated in the vasorelaxant actions of berry polyphenols (48,49). These

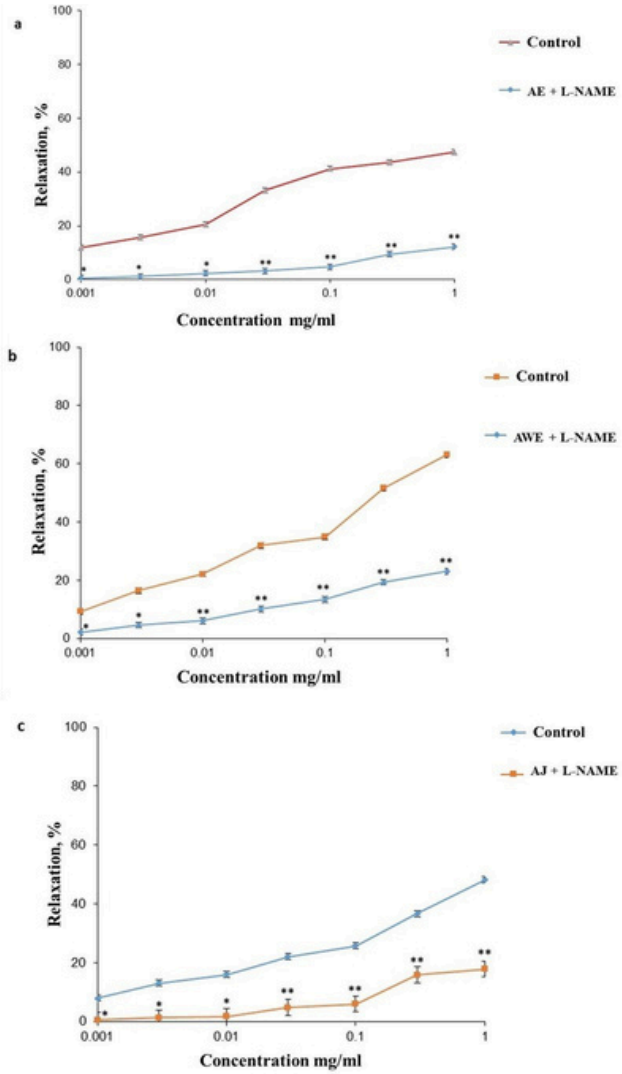


**Figure 3.** *Relaxant effects of AE (a), AWE (b), and AJ (c) on contractions of isolated rat aorta in the presence of L-NAME. Each point represents the mean ± SD of responses obtained from thoracic aortic rings isolated from six independent rats (n = 6) (Student's t-test, *p < 0.05, **p < 0.01).*

mechanisms likely contribute to the robust relaxation responses seen in our phenylephrine model and further support the cardiovascular relevance of chokeberry preparations.

Taken together, our findings reinforce the concept that chokeberry extracts and juice can positively influence vascular function through multiple converging pathways, primarily related to NO bioavailability and endothelial modulation. This aligns with growing evidence that polyphenol-rich diets, especially those containing anthocyanins, are beneficial for vascular health and may help mitigate risk factors for hypertension and other cardiovascular diseases.

Although concentration-dependent vasorelaxation was observed for all tested preparations, the concentration–response curves did not reach a clear plateau within the investigated concentration range, indicating that maximal vasorelaxation may not have been achieved. Previous studies have evaluated higher concentrations of crude plant extracts, including 0.01–10 mg/mL for Alchemilla vulgaris extracts (60) and up to 16.91 mg/mL for a polyphenol-rich extract of Steganotaenia araliacea (61), demonstrating that broader concentration ranges can be employed in studies of vascular reactivity. However, because the present study investigated complex crude aronia-derived preparations, concentrations above 1 mg/mL were not evaluated in order to minimize nonspecific physicochemical effects, such as increased viscosity, reduced solubility, precipitation of constituents, and alterations of the organ bath medium, which could interfere with the interpretation of vascular responses. Therefore, the absence of a clear plateau should be considered a limitation of the present study. Future investigations should evaluate a broader concentration range and/or purified fractions to better define the maximal vasorelaxant efficacy of these preparations.

Despite the valuable mechanistic insights provided in this study, several additional limitations should be acknowledged. Although the involvement of nitric oxide was investigated using L-NAME, other potential mechanisms, including endothelium removal, prostaglandin-mediated pathways, and potassium or calcium channel modulation, were not evaluated. Therefore, while our findings strongly suggest a predominant role of NO-mediated signaling, the contribution of other endothelial- and smooth muscle-dependent mechanisms cannot be excluded. Further studies investigating these pathways are warranted to provide a more comprehensive understanding of the vasorelaxant mechanisms of aronia-derived preparations.

The results demonstrated significant vasorelaxant activity of chokeberry preparations in the isolated rat aorta model. Relaxation was largely mediated by nitric oxide (NO), as evidenced by the reduction in aortic relaxation following L-NAME, an NO synthase inhibitor. Relaxation responses were more pronounced in phenylephrine-induced contractions compared to KCl-induced contractions, yet extract, waste extract, and juice effectively relaxed the aortic smooth muscle in both models. These findings suggest that aronia berry extract, waste by-product, and juice may be beneficial for blood pressure regulation or as an adjunct to antihypertensive therapy, although confirmation in clinical studies is required.

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## Author Contributions

Conceptualization, M.M., S.B., and D.K.; Investigation, M.M., N.Ć.N., M.R., and B.M.; Methodology, M.M., N.Ć.N., and S.B.; Data Curation, M.M.; Formal analysis, M.M.; Software, M.M., N.Ć.N., M.R., and B.M.; Visualization, M.M.; Writing—original draft: M.M.; Writing—review and editing, M.M., S.B. and D.K.; Supervision, K.Š.; and S.B.; Validation, M.M., N.Ć.N., K.Š., S.B., and D.K.; Funding acquisition, D.K. All authors have read and approved the published version of the manuscript.

## Statement of Ethics

All experimental procedures were approved by the Ethics Committee and conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes (approval issued by the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate, decision No. 323-07-04034/2018-05/2).

## Statement of Competing Interest

The authors declare no relevant conflicts of interest.

## Statement of Data Availability

The data supporting the findings of this study are included in the article.

## Statement of Generative AI Use

No generative AI was used.

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