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VASORELAXANT ACTIVITY AND FLAVONOID PROFILE OF JUICE, PEEL, AND SEED EXTRACTS IN LOCAL POMEGRANATE (*PUNICA GRANATUM* L.)

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SUMMARY

Local pomegranate (*Punica granatum* L.) cultivars are valuable plant genetic resources rich in bioactive compounds. In different fruit parts, the quantitative and functional variations of flavonoids are crucial for plant science, breeding, and phytopharmacology. Understanding the organ-specific accumulation of bioactive compounds supports the effective use of plant resources and identification of promising raw materials. The presented study aimed to investigate the biological activity of fruit juice, peel, and seed extracts of local pomegranate cultivars and determine their relationship with the flavonoid composition. In this study, the preparation of ethanol extracts proceeded from biomaterials of five local pomegranate cultivars grown under field conditions. The flavonoid composition analysis used high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection. Biological activity assessment was in vitro under isometric conditions. Extracts tested had concentrations ranging from 100 to 300 µg/mL. The results showed peel and seed extracts accumulated higher flavonoid levels, particularly gallic acid, rutin, and quercetin, than the fruit juice. These fruit parts exhibited greater biological activity, whereas juice samples showed lower activity. In conclusion, the peel and seed of local *P. granatum* cultivars proved promising resources for future breeding and functional product development.

Keywords: Pomegranate (*P. granatum* L.), local cultivars, flavonoid composition, peel and seed extracts, plant bioactive compounds, HPLC-PDA

Key findings: Among the local pomegranate (*P. granatum* L.) genotypes, the cultivar Qizil anor exhibited robust functional potential, particularly in peel extracts, highlighting its value as a promising source of bioactive compounds for future breeding and functional plant-based applications.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the most ancient fruit crops, representing an important component of plant genetic resources, with a wide cultivation in arid and semi-arid regions worldwide. Owing to its extremely high ecological plasticity, tolerance to drought and salinity, and stable productivity under diverse agroclimatic conditions, pomegranate is a widely cultivated fruit crop across Central Asia, the Mediterranean basin, the Middle East, and South Asia (Holland *et al.,* 2009; Ergasheva *et al.,* 2021). In Uzbekistan, the local pomegranate cultivars have reached traditional planting for centuries, which represents a unique pool of genetic diversity adapted to regional soil-climatic conditions, making them valuable materials for breeding and sustainable horticulture.

From a plant science perspective, *P. granatum* has attracted considerable attention as a source of biologically active secondary metabolites. Based on biochemical analysis, its different fruit parts, including peel, seeds, and arils, accumulate diverse phenolic compounds, such as flavonoids, ellagitannins, phenolic acids, and anthocyanins (Gil *et al.,* 2000; Teixeira-da-Silva *et al*., 2013). These bioactive compounds play pivotal physiological roles in plants, contributing to protection against oxidative stress, ultraviolet radiation, pathogens, and herbivores, as well as influencing the fruit color, taste, and overall fruit quality (Kader, 2006).

In pomegranates, flavonoids represent one of the most significant classes of secondary metabolites. In plant systems, flavonoids function as antioxidants and signaling molecules and regulate the growth and stress responses under diverse climatic conditions (Fischer *et al*., 2011). Their accumulation is significantly organ-specific and genetically controlled, while also attaining influences from environmental factors and developmental stages. In the flavonoid composition, such variations among the plant organs could serve as biochemical markers for varietal differentiation and selection of promising genotypes (Hasnaoui *et al.,* 2011).

Previous studies have consistently shown that pomegranate peel contains particularly higher concentrations of phenolic compounds, whereas seeds and juice possess distinct and comparatively lower flavonoid profiles (Li *et al.,* 2006; Viuda-Martos *et al.,* 2010). However, most existing data were reliant on commercial and widely distributed cultivars, while information on locally adapted cultivars remains limited. Local pomegranate genotypes may exhibit unique phytochemical characteristics shaped by long-term selection under specific environmental pressures, which is highly relevant for the conservation and utilization of plant genetic resources (Holland *et al.,* 2009).

Understanding organ-specific distribution of flavonoids is also an important perspective from an applied plant science viewpoint, as pomegranate processing generates large amounts of by-products, particularly the peel and seeds. These plant materials often entail underutilization despite the abundance of valuable bioactive compounds (Fawole and Opara, 2013). Their rational use aligns with sustainable agriculture and circular bioeconomy concepts, enabling the development of functional plant-based products from local raw materials.

The integration of in vitro approaches and phytochemical analysis provides effective tools for comparative evaluation of biological activity among the different cultivars and their fruit parts. Such integrated studies allow linking flavonoid composition with functional properties and facilitate the identification of plant organs with the highest bioactive potential (Gil *et al.,* 2000; Fischer *et al.,* 2011).

Despite growing interest in pomegranate bioactivity, comprehensive studies integrating flavonoid profiling with comparative phytochemical evaluation of biological activity across different fruit organs of local pomegranate cultivars remain scarce, particularly for the Central Asian germplasm. Addressing this gap is essential for expanding the scientific basis for breeding, functional agriculture, and sustainable utilization of local pomegranate genetic resources.

Therefore, the following study aimed to evaluate the biological activity of fruit juice, peel, and seed extracts obtained from selected local pomegranate (*P. granatum* L.) cultivars and analyze their flavonoid composition using high-performance liquid chromatography. By comparing organ-specific flavonoid accumulation and functional activity, this research seeks to identify valuable plant parts and provide scientific justification for using local pomegranate cultivars in plant science research, breeding programs, and the development of value-added functional products.

MATERIALS AND METHODS

Plant materials and procedure

The study commenced using five local pomegranate (*P. granatum* L.) cultivars: Qizil anor, Qora qayim, Oq shirin, Oq dona (Tuyatish), and Achchiq dona. The procurement of plant materials was from a long-established household orchard located in Halkobod MFY, Mirzaobod District, Syrdarya Region, Uzbekistan (40°32′39″ N, 68°41′58.9″ E). The orchard, established in 1960, remained under traditional cultivation practices with vegetative propagation by cuttings. Trees of similar age (8–10 years) and uniform agronomic conditions were selected specimens for sampling.

Fruit sample collection occurred during the physiological ripening stage under open-field conditions. Harvesting took place in the morning (08:00–10:00) to minimize diurnal variations in metabolite content. Fruits free from visible mechanical damage and disease symptoms were the choice selections. Fruit juice, peel, and seed separation continued manually before immediate processing and storage under controlled conditions until analysis.

Preparation of plant extracts

Peel and seed samples received washing with distilled water, air-drying, and further drying at 60 °C until constant weight. The dried material underwent grinding into fine powder, followed by extraction using 70% ethanol, which is the common treatment for efficient recovery of flavonoids from pomegranate tissues (Li *et al.,* 2006; Viuda-Martos *et al.,* 2010). Extracting approximately 10 g of powdered material was successful at a solid-to-solvent ratio of 1:5 (w/v) for 48 h with periodic agitation. Extracts' filtration and concentration ensued under reduced pressure. Juice samples came from fresh arils and sustained freeze-drying before analysis. All extracts reached storage at 4 °C until further use.

HPLC-PDA analysis of flavonoid composition

Flavonoid profiling proceeded using high-performance liquid chromatography coupled with photodiode array detection (HPLC–PDA), following previously described protocols with minor modifications (Gil *et al.,* 2000; Fischer *et al.,* 2011). Fruit extract samples underwent dissolving in ethanol, filtering through membrane filters, and injection into the HPLC system. Separation succeeded in a reversed-phase C18 column under gradient elution. Performing detection was at 254 nm.

Identification of gallic acid, rutin, quercetin, apigenin, and kaempferol was according to retention times and UV spectra comparison with authentic standards. Quantification, as carried out, used external calibration curves, with results expressed on a dry weight basis, as recommended for comparative phytochemical studies of fruit crops (Alighourchi *et al.,* 2008).

Assessment of biological activity (in vitro)

Biological activity of fruit juice, peel, and seed extracts bore evaluation in vitro using an isometric experimental model. Such bioassay-based approaches were widely applicable for comparative assessment of functional properties of plant extracts and their relation to phytochemical composition (Gil *et al.,* 2000; Choi *et al.,* 2007). The extracts reached testing at concentrations ranging from 100 to 300 µg mL^{-1}, allowing evaluation of concentration-dependent responses.

Statistical analysis

All the experiments proceeded in independent replicates. The expression of data was the mean ± standard error (SE). Statistical analyses performed used standard statistical software. Differences among the treatment means entailed evaluation using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT), with statistical significance accepted at $P \leq 0.05$, as commonly applied in plant biochemical studies (Fawole and Opara, 2013).

RESULTS

Flavonoid composition

The HPLC-PDA analysis revealed obvious varietal and organ-specific differences in the flavonoid composition of local pomegranate (*P. granatum* L.) cultivars. Quantitative variations were evident among the peel, seed, and juice samples, indicating distinct patterns of secondary metabolite accumulation in different fruit tissues.

Overall, the results indicated that the evaluation of local pomegranate genetic resources requires the consideration of both varietal differences and organ-specific characteristics. The findings further disclosed that peel tissues exhibited the highest flavonoid accumulation and biological activity, followed by seeds and fruit juice (Table 1). Among the evaluated pomegranate genotypes, the cultivar Qizil anor showed consistently superior performance, particularly in peel extracts, by showing the topmost levels of major flavonoids with robust vasorelaxant activity. Seed tissues appeared mainly enriched in flavonols, whereas juice samples displayed a comparatively lower activity. The results clearly divulged that both genetic background and fruit organ type play a decisive role in determining the functional potential of local pomegranate cultivars.

By analyzing the fruit's different organ samples, the gallic acid was the predominant compound detected with the highest accumulation in peel tissues. The concentration of gallic acid in peel extracts was several-fold higher than in the seed and juice fractions, confirming the prominent role of peel as the main storage tissue for hydrolyzable phenolic compounds. Rutin also exhibited robust tissue specificity, with maximum levels recorded in peel samples, while seed and juice extracts contained comparatively lower concentrations.

In contrast, quercetin had a predominant accumulation in seed tissues. Seed extracts consistently showed higher quercetin levels than in peels and juices, revealing that seeds represent an important source of flavonols. The detection of apigenin and kaempferol was at relatively low concentrations and demonstrated a main association with juice samples, suggesting that these bioactive compounds represent minor flavonoid components in pomegranate arils.

Table 1. Quantitative profile of major flavonoids (gallic acid, rutin, quercetin, apigenin, and kaempferol) identified in different plant parts and juices of different fruits by HPLC analysis. The expression of flavonoid contents was mg/kg of dry weight.

Cultivar / Fruit organ	Gallic acid (mg/kg)	Rutin (mg/kg)	Quercetin (mg/kg)	Apigenin (mg/kg)	Kaempferol (mg/kg)
Qora qayin seed	68.124	22369.366		23.960	
Qora qayin seed	454.130	127.242	874.057		
Oq shirin seed	9.043	115.725	28.215		12.377
Oq shirin peel	19515.028	4687.694	28.225		
Qizil anor peel	19331.963	3386.384	365.208		12.231
Qizil anor seeds	7.255	146.651	29.463		12.523
Oq shirin juice	163.323	82.501	27.945	13.222	12.764
Qora qayin juice	166.773	35.166	12.899		
Qizil anor juice	629.906	109.514	54.887		12.764

Overall, flavonoid accumulation followed a distinct organ-dependent pattern: peel > seed > juice for total flavonoid content, while individual compounds exhibited tissue-specific distribution profiles.

Vasorelaxant activity under KCl-induced contraction

Under basal conditions, none of the tested extracts affected the resting tone of isolated rat aortic rings, enunciating the absence of nonspecific vasoactive effects. However, all extracts significantly reduced KCl-induced contraction in a concentration-dependent manner.

Peel extracts exhibited the robust vasorelaxant activity across all tested concentrations. At a higher concentration, the peel extracts markedly suppressed KCl-induced contraction, and seed extracts produced moderate relaxation, whereas juice extracts showed comparatively weaker effects. However, the magnitude of inhibition varied among the pomegranate cultivars, and some genotypes showed a consistently higher responsiveness.

The remarkable vasorelaxant activity observed in peel extracts corresponds well with their higher flavonoid content, suggesting a close relationship between organ-specific phytochemical composition and functional activity.

Effects on phenylephrine-induced contraction

Phenylephrine-induced contraction revealed a significant attenuation in juice, peel, and seed extracts in a concentration-dependent manner (Figure 1). Peel extracts again showed the highest inhibitory effect, indicating robust suppression of receptor-mediated smooth muscle contraction.

Juice and seed extracts also reduced phenylephrine-induced contraction, although their effects were less prominent compared with peel extracts. The response pattern was consistent across the pomegranate cultivars; however, the degree of inhibition differed among the genotypes, reflecting genetic variations in their biological activity. The results confirmed that pomegranate extracts effectively interfere with receptor-dependent contractile mechanisms, with peel tissues contributing the most robustly to the observed effects.

Influence of endothelium on vasorelaxant responses

Evaluating the contribution of the vascular endothelium had the conducted experiments used endothelium-intact and endothelium-denuded aortic rings. Removal of the endothelium partially reduced the vasorelaxant effects of the extracts; however, a significant level of relaxation was still noticeable (Figure 2).

These results revealed the vasorelaxant activity of pomegranate cultivars extract achieved mediation through both endothelium-dependent and endothelium-independent mechanisms. Direct effects on vascular smooth muscle cells appear to play a primary role, with additional modulation through endothelial signaling pathways.

Overall, the results recognized the pronounced organ-specific differences in flavonoid accumulation and vasorelaxant activity among local pomegranate cultivars. Peel tissues consistently exhibited the highest flavonoid content and biological activity, followed by seeds and juice. Among the pomegranate cultivars, considerable variations were noteworthy, highlighting the importance of both plant organ selection and genetic background when evaluating the functional potential of local *P. granatum* genetic resources.

Vasorelaxant responses under endothelial removal and pathway inhibition

Figure 3 illustrated the vasorelaxant effects of juice, peel, and seed extracts (500 µg/mL) in local pomegranate cultivars under the conditions of endothelial removal, nitric oxide synthase inhibition (L-NAME), and cyclooxygenase inhibition (indomethacin). In all the evaluated genotypes, endothelial removal caused a partial, but not complete,

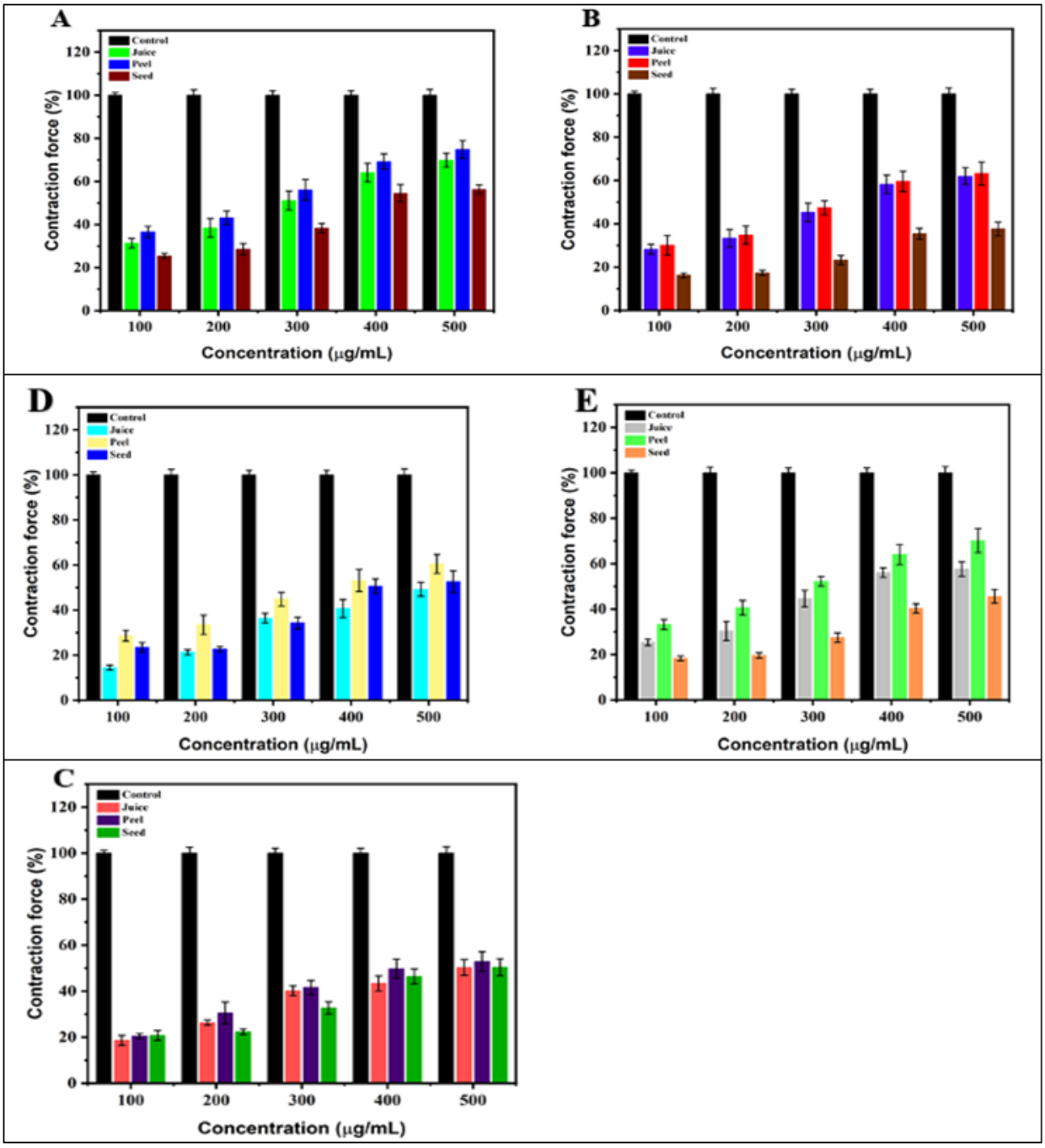

Figure 1. Vasorelaxant effect of juice, peel, and seed extracts (100–500 μg/mL) of local pomegranate (*Punica granatum* L.) cultivars on KCl (60 mM)-induced contractions of isolated aortic rings (M ± m). A) Qizil anor, B) Qora qayin, C) Oq dona, D) Oq shirin, and E) Achchiq dona.

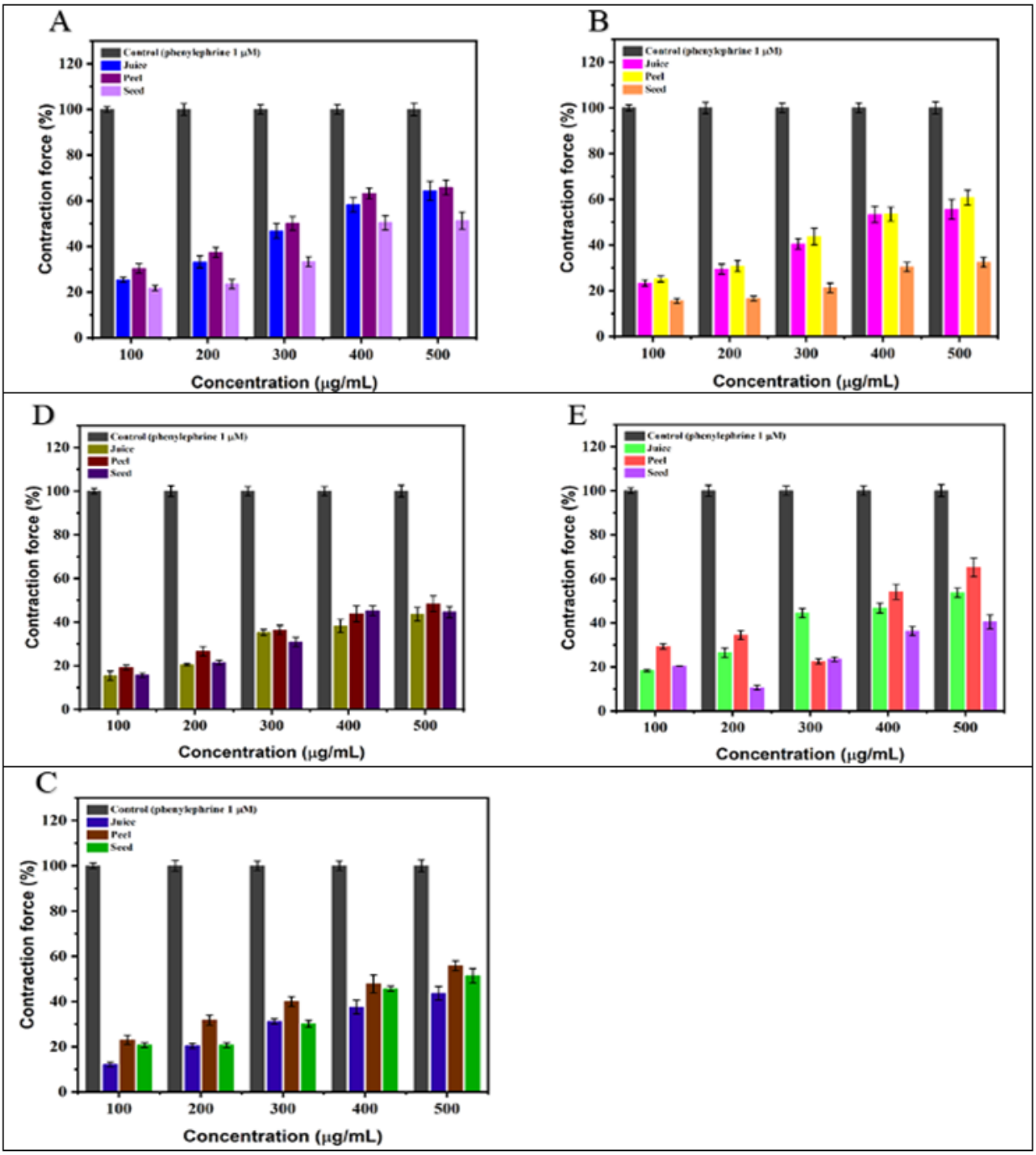

Figure 2. Vasorelaxant effects of juice, peel, and seed extracts (100–500 μg/mL) of local pomegranate (*Punica granatum* L.) cultivars on phenylephrine (PE)-induced (1 μM) contraction of isolated rat aortic rings (Mean ± SEM). A) Qizil anor, B) Qora qayin, C) Oq dona, D) Oq shirin, and E) Achchiq dona.

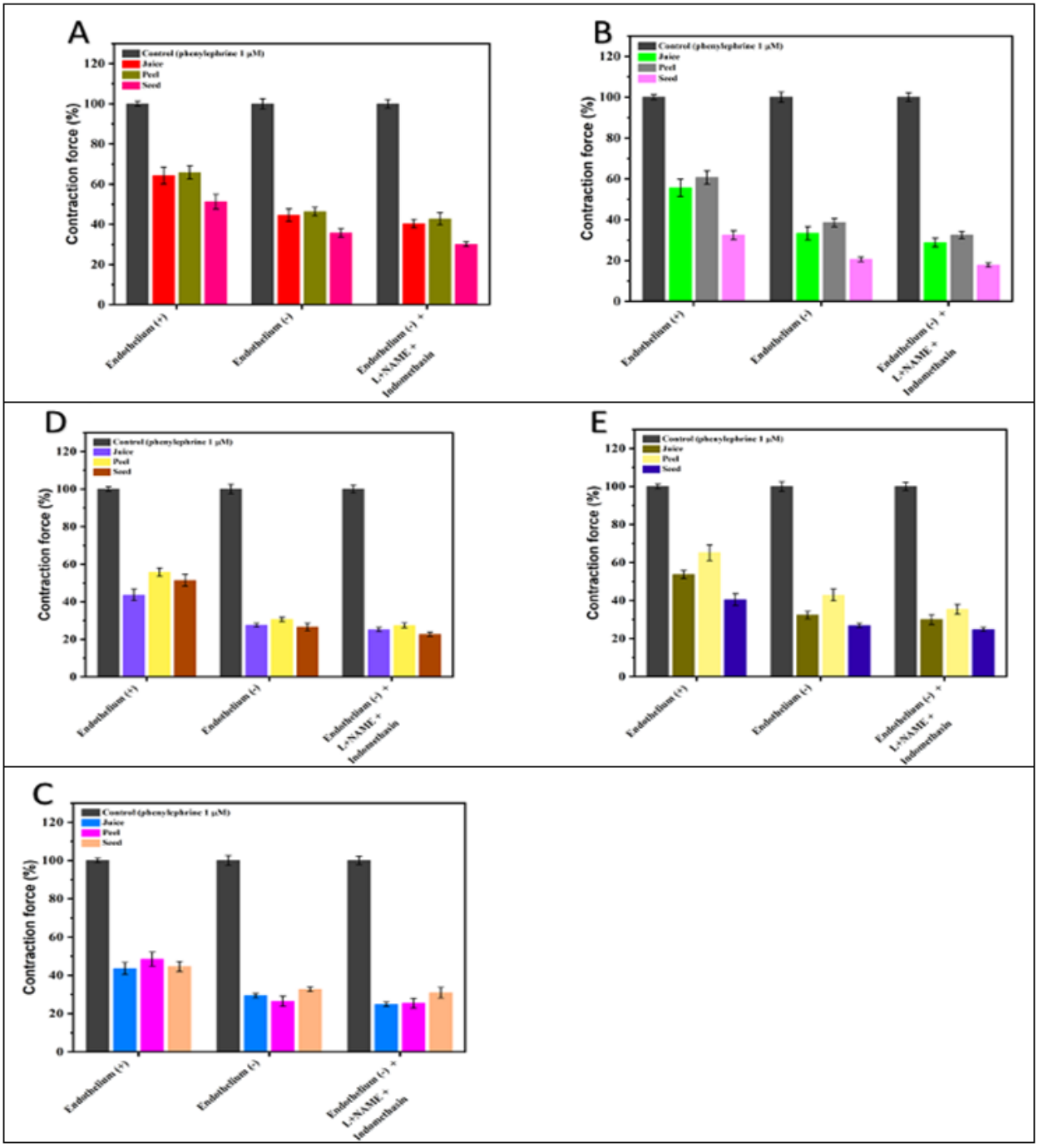

Figure 3. Vasorelaxant effects (Mean ± SEM) of juice, peel, and seed extracts (500 µg/mL) of local pomegranate (*Punica granatum* L.) cultivars under conditions of endothelial removal, e-NOS inhibition (L-NAME 100 µM), and cyclooxygenase inhibition (indomethacin 100 µM) on phenylephrine-induced (1 µM) contraction of isolated aortic rings. A) Qizil anor, B) Qora qayin, C) Oq dona, D) Oq shirin, and E) Achchiq dona.

reduction in vasorelaxant responses, revealing that the juice, peel, and seed extracts exert both endothelium-dependent and endothelium-independent effects.

In the presence of L-NAME, vasorelaxant activity had a significant weakening compared with control conditions, particularly in peel extracts, suggesting a substantial involvement of the eNOS-NO signaling pathway in mediating vascular relaxation. Nevertheless, a residual relaxant response persisted despite eNOS inhibition, implying a direct action of bioactive compounds on vascular smooth muscle cells.

Treatment with indomethacin resulted in only minor variations in vasorelaxant responses, enunciating that cyclooxygenase-derived prostanoids play a limited role in the impacts induced by pomegranate extracts. Among the tested pomegranate fruit's different organs, peel extracts consistently exhibited the robust vasorelaxant activity across all inhibitory conditions, with the most pronounced effects observed in the cultivar Qizil anor.

Overall, the results demonstrated that vasorelaxation induced by local pomegranate extracts gained mediation through multiple mechanisms. These include endothelial nitric oxide signaling and direct smooth muscle relaxation, further emphasizing the superior functional potential of pomegranate peel tissues (Figure 3).

DISCUSSION

The results confirmed that flavonoid accumulation and associated vasorelaxant activity in local pomegranate (*P. granatum* L.) cultivars appeared to be considerably dependent on both the genetic background and fruit organ differentiation. Recent studies have emphasized that tissue-specific regulation of phenolic biosynthesis was a common feature in fruit crops and reflects adaptive strategies against environmental stress factors (Afaq *et al.,* 2018; Zhao *et al.,* 2020). In pomegranates, peel tissues play a central protective role and therefore represent a major site of secondary metabolite accumulation.

In consideration of recent phytochemical investigations, in this study, peel tissues exhibited the highest concentrations of major flavonoids, including gallic acid and rutin, as well as robust biological activity. Similar findings have had reports for pomegranate genotypes grown in different agroecological regions, where peel tissues consistently showed the higher phenolic content than fruit arils and seeds (Fawole *et al.,* 2020; Russo *et al.,* 2021). Enhanced flavonoid accumulation in pomegranate peel tissues contributes to antioxidant capacity and resistance to biotic and abiotic stress factors, which are critical traits from plant physiological and postharvest perspectives (Kader, 2021).

Pomegranate cultivars' seed tissues showed a distinct flavonoid profile characterized by enrichment in flavonols, particularly quercetin. Recent metabolomic studies revealed that seed-associated phenolics emerged as closely associated with reproductive protection and regulation of seed development, supporting the tissue-specific metabolic specialization observed in this study (Liu *et al.,* 2019; Hernández *et al.,* 2022). Although total flavonoid content in seed tissues was lower than in the peel, their specific composition may contribute to moderate biological activity.

Pomegranate cultivars' juice samples exhibited comparatively lower flavonoid levels and reduced biological activity. This pattern has been a consistent report in recent literature, revealing that fruit aril tissues prioritized the carbohydrate accumulation and seed dispersal functions rather than the chemical defense (Shaygannia *et al.,* 2020; Tohidi *et al.,* 2021). These findings highlighted the importance of valorizing underutilized fruit fractions, particularly peel and seed tissues, in pomegranate research and utilization strategies.

Pronounced varietal differences observed in the presented study further disclosed the importance of genetic factors in regulating flavonoid biosynthesis. Recent genetic and transcriptomic analyses enunciated that differential expression of phenylpropanoid and flavonoid pathway genes contributes to genotype-dependent variations in the

pomegranate phenolic composition (Zhang *et al.,* 2019; Yuan *et al.,* 2022). Among the evaluated pomegranate genotypes, the cultivar Qizil anor consistently showed superior flavonoid accumulation and biological activity, suggesting favorable genetic regulation of secondary metabolism.

Between the flavonoid content and vasorelaxant activity, the observed close association supported the recent evidence that phenolic-rich plant tissues displayed an enhanced functional bioactivity (Russo *et al.,* 2021; Hernández *et al.,* 2022). As mechanistic aspects extend beyond the primary scope of plant science, these functional responses reinforce the relevance of phytochemical profiling for assessing the vital biological values of plant genetic resources.

From the breeding and genetic resource perspectives, recent studies emphasized the integration of biochemical and functional traits in the selection criteria of fruit crops (Fawole *et al.,* 2020; Kader, 2021). The latest results considerably supported this approach and indicate that the selection of pomegranate genotypes should consider both yield and fruit quality traits and organ-specific phytochemical profiles. In this context, the peel tissues of the pomegranate cultivar Qizil anor represent a particularly valuable resource for breeding, functional product development, and sustainable utilization of local pomegranate (*P. granatum* L.) genetic resources. Overall, the study demonstrated that the evaluation of local pomegranate (*P. granatum* L.) genetic resources must consider both varietal and organ-specific differences, with peel tissues showing the highest flavonoid content and biological activity compared with seeds and juice.

CONCLUSIONS

The results confirmed flavonoid accumulation and vasorelaxant activity in local pomegranate (*P. granatum* L.) cultivars had considerable influences from the genotypes and fruit organs. An apparent organ-specific pattern was evident, with peel tissues exhibiting robust biological activity, while seeds and juice showed comparatively lower functional effects. Vasorelaxation mediation occurred through both endothelium-dependent and smooth muscle-related pathways, with nitric oxide signaling contributing significantly to the response. Among evaluated genotypes, the cultivar Qizil anor displayed the highest flavonoid accumulation and the strongest vasorelaxant activity, particularly in peel extracts. From breeding perspectives, the results emphasized the importance of integrating organ-specific phytochemical evaluation into germplasm assessment. Overall, these results provided a robust scientific foundation for the conservation, targeted improvement, and sustainable utilization of local pomegranate genetic resources.

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