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Original Article

To Evaluate the Phytochemicals, Antioxidant Activity and Antibacterial Activity of Pomegranate Arils Juice

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ABSTRACT

Background: Pomegranate, belonging to the Punicaceae family, thrives in semi-arid, mild temperate to sub-tropical climates. Known for its health-promoting properties, pomegranate juice contains a variety of bioactive compounds that contribute to its antioxidant and antibacterial activities.

Objective: This study aimed to evaluate the impact of storage on the phytochemical, antioxidant, and antibacterial properties of juice from three pomegranate varieties—Ghandhri, Bedana, and Tarnab Gulabi.

Methods: Fresh juices were extracted from the arils of the three pomegranate varieties and stored at 5-10°C. Phytochemical analysis was performed using the Folin-Cioalteu reagent for total phenols and tannins, while antioxidant activity was assessed via the DPPH assay. Antibacterial activity was evaluated using standard microbiological techniques. Mineral content (iron, zinc, and calcium) was analyzed using atomic absorption spectrophotometry (model 2380). Statistical analysis was conducted with Statistic 8.1, utilizing ANOVA with a two-factorial design.

Results: Tarnab Gulabi juice exhibited the highest phenolic content (571.3611±3.41 mgGaE/100ml) and antioxidant activity (81.12%). Ghandhri juice had the highest flavonoid (145.425±2.08 mgQuE/100ml) and anthocyanin concentrations (16.119±4.87 mgCyE/100ml), while Bedana showed the greatest tannin content (14.6046±11.5 mg/100ml). The study highlighted a significant reduction in phytochemicals due to storage, with marked antibacterial efficacy noted against E. coli and Citrobacter.

Conclusion: Storage negatively affects the phytochemical content and antioxidant capacity of pomegranate juice. Fresh juice retains more bioactive compounds and exhibits higher antibacterial activity compared to stored juice. Varietal differences significantly influence the nutritional and functional qualities of the juice.

Keywords: Antioxidant, E. coli, Pomegranate, Phytochemicals, Storage, Tannins, Varietal differences.

INTRODUCTION

Punica granatum, commonly known as pomegranate, belongs to the Punicaceae family and thrives in semi-arid, mild-to-subtropical climates. This fruit has a rich history, originating in ancient Egypt and Greece, and has since spread to West Mediterranean countries and various Asian regions, including Afghanistan, Iran, China, India, and Pakistan. Pomegranates are composed of about 20% seeds and 80% juice, of which approximately 55–60% is edible. The juice of pomegranate arils consists predominantly of water (85%), with the remainder including sugars, pectin, and trace amounts of ascorbic acid. Notably, it contains 10.6% total sugar, 1.4% pectin, and insignificant ascorbic acid, along with 0.1 g of general acidity per 100 ml as citric acid and 0.05 g of ash per 100 ml (1).

Pomegranate juice is renowned for its high phenolic content, which surpasses that of many other fruit juices and beverages. It contains phenol levels up to three times higher than those found in wine or green tea, twice that of grapes and cranberries, six times that of grapefruits, and eight times that of orange juice (2). These phenolic compounds, predominantly polyphenols, act as potent antioxidants. Polyphenols are secondary metabolites of plants, broadly categorized into non-flavonoids and flavonoids. Among the non-flavonoids, tannins play a crucial role, capable of binding with glucose and amino acids to form complexes. Tannins are further

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subdivided into proanthocyanidins, hydrolyzable tannins, phlorotannins, and complex tannins (4). Another significant component is anthocyanin, a water-soluble pigment that contributes to the bright red color of the juice and is pharmacologically significant due to its presence in over 550 different types identified in pure fruit juices (5).

Given the extensive array of phytochemicals and their associated health benefits, this study aims to conduct a comparative analysis of the total phenolic content in various pomegranate juices and to assess their antioxidant and antibacterial properties. This research will provide insights into the potential health benefits of pomegranate juice, supporting its use in dietary and therapeutic applications.

METHODS

Samples of three pomegranate varieties—Ghandhri, Bedana, and Tarnab Gulabi from the Tarnab family—were procured from local markets in Khyber Pakhtunkhwa. All fruits were confirmed to be free from pests and diseases. In the human nutrition laboratory, the fruits underwent a sterilization process in a 2% potassium permanganate solution for five minutes before being rinsed under running water. Subsequently, the mesocarps and epicarps were removed, and the arils were extracted and juiced using a domestic juicer. The resultant juices were centrifuged at 4000 rpm for ten minutes and labeled according to their respective varieties: Tarnab Gulabi, Bedana, and Ghandhri (JAG). These freshly prepared juices were then stored at 4 °C until further analysis.

For long-term storage, the fresh juices (JATG, JAB, and JAG) underwent a preservation process. To each liter of juice, 2 grams of potassium metabisulfate and 5 grams of citric acid were added (7). The treated juices were stored at -4 °C for two months and periodically sampled for analysis. Before each testing phase, the samples were filtered through Whatman filter paper.

The total phenolic content was determined using a slightly modified Folin-Ciocalteu method. This involved the use of Folin-Ciocalteu reagent and an acetate buffer to facilitate the electron transfer from phenolic compounds to a phosphotungstic/phosphomolybdic acid mixture in an alkaline medium. A calibration curve was established using different concentrations of gallic acid solution, and the samples' absorbance was measured at 760 nm after a reaction time of 30 minutes in the dark (8).

Similarly, the total flavonoid content was measured by modifying an existing method, which involved the use of aluminum chloride, ethanol, quercetin, and sodium hydroxide. Different dilutions of the quercetin stock solution were prepared to establish a calibration curve. The reaction mixture, including the juice sample and reagents, was incubated in the dark for 30 minutes before measuring the absorbance.

The tannin content was assessed by mixing a methanol-based solution with the sample and reacting it with Folin-Dennis reagent and casein. The mixture's absorbance was measured at 700 nm after a 30-minute reaction period in the dark.

Anthocyanin concentration was determined by mixing the juice with a dilute hydrochloric acid solution and measuring the absorbance at 520 nm using a UV-VIS spectrophotometer.

Antioxidant activity was evaluated using the DPPH method, where the sample's ability to scavenge free radicals was measured and expressed as a percentage of DPPH scavenging.

Ascorbic acid content in both fresh and stored juices was quantified using a dye-titration method described in the AOAC (2012) protocol (967.21), involving a reaction with a 2,6-dichlorophenolindophenol dye until a persistent pink color was achieved.

Antibacterial activity was assessed by applying the juice samples at various concentrations on nutrient agar plates inoculated with standardized microbial strains. The zones of inhibition were measured after 24 hours of incubation at 37°C.

Statistical analysis was conducted using SPSS software, employing a two-factorial design and the results were presented as means and standard deviations. Statistical significance was determined at a p-value of less than 0.05.

RESULTS

The comparative analysis of fresh and preserved pomegranate juices revealed significant differences in their phytochemical contents. Fresh pomegranate juice exhibited a higher phenolic content (562.54 ± 141) compared to preserved juice (436.24 ± 69.7), confirming previous findings by Bublin et al. (11) that preservation processes can reduce total phenolic levels as assessed by the Folin-Ciocalteu assay. Among the tested varieties, Tarnab Gulabi juice demonstrated the highest polyphenol concentration (571.36 ± 3.41), whereas Bedana recorded the lowest (379.08 ± 32.11).

In terms of flavonoid content, fresh juices again outperformed the stored counterparts, with fresh juice registering 109.84 ± 70.85 in flavonoid concentration compared to 60.18 ± 49.86 in preserved juice. This disparity is attributed to the interaction of flavonoids with atmospheric oxygen, which induces oxidation and results in the degradation of flavonoids during storage (Donald, 2000). Ghandhri juice exhibited the highest flavonoid content (145.43 ± 42.08) among the varieties, significantly surpassing Bedana (82.76 ± 52.45) and Tarnab Gulabi (26.84 ± 10.85).

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The anthocyanin profiles of the juices also varied significantly across different types and conditions. Fresh juice from Ghandhri, Bedana, and Tarnab Gulabi showed higher anthocyanin contents than their preserved counterparts, following the trend Ghandhri > Tarnab Gulabi > Bedana. Ozkan et al. (13) corroborated that anthocyanin levels could vary extensively between cultivars and depend heavily on maturity and cultivar type, with delphindin-3,5-diglucoside being predominant. Environmental factors such as climate, soil texture, and genetic background also influence these variations (Poyrazoglu et al., 14).

The study also explored tannin content, finding Bedana to have the highest concentration (14.60 ± 11.5), followed by Ghandhari (9.62 ± 4.14) and Tarnab Gulabi (8.29 ± 2.37). These findings align with those of Mousavinejad et al. (15), who noted that tannin content could range from 15 to 22 g/100 g across different cultivars, with the Sweet Alak variety showing the highest levels.

Antioxidant activity assays further highlighted the superior performance of fresh juices, particularly Tarnab Gulabi, which demonstrated the highest antioxidant activity (81.1 ± 11.32) compared to other varieties. Interestingly, the vitamin C content did not show significant differences between fresh and preserved juices, with values closely clustered around 2.41 ± 0.68 for fresh and 2.3 ± 0.45 for preserved juices, indicating a slight reduction due to the preservation process.

Antibacterial efficacy tests against Citrobacter and E. coli showed variable inhibitory effects at different concentrations. Ghandhri juice exhibited the most robust antibacterial activity at higher concentrations (23mm at 1000 mg/mL), whereas Tarnab Gulabi showed the least (16mm at 1000 mg/mL). The results were consistent across varying concentrations, highlighting the potential of pomegranate juice as a natural antibacterial agent. DMSO, used as a negative control, showed no zone of inhibition, validating the experimental conditions.

All reported data were expressed as means ± standard error, and statistical analysis was conducted using a standard deviation method to ensure reliability and accuracy of the results.

Table 1: Total phenols, flavonoid, anthocyanin and tannin of three pomegranate varieties.

Varieties	Total phenol mg GaE/100l	Mean	Total Flavonoid mg QEc/100ml	Mean	Total Anthocyanin mg CyE/100ml		Total Tannin mg TaE/100ml	Mean
	Fresh Stored		Fresh Stored		Fresh Stored		Fresh Stored	
Ghandhri	627.3 ± 15.64b 468.3 ± 26.18c	547.71 ± 112.44a	175.18 ± 3.05a 115.68 ± 5.94b	145.43 ± 42.08a	19.57 ± 0.58a 12.69 ± 0.92b	16.119 ±4.87a	6.702 ±0.36d 12.55 ±0.56b	9.621834 ±4.14b
Bedana	401.78 ± 9.05d 356.38 ± 21.03e	379.08 ± 32.11b	34.6 ± 0.56 d 19.18 ± 1.55e	26.84 ± 10.85b	6.79 ± 0.28c 1.35±0.34d	4.058±3.85b	6.530 ±0.53d 22.69 ±1.50a	14.6046 ±11.5a
Tarnab Gulabi	658.64 ± 5.39ab 484.2 ± 22.35c	571.37 ± 123.41a	119.84 ± 5.94b 45.68 ± 4.97c	82.76 ± 52.45c	13.31 ± 0.76b 6.42±0.62c	9.859 ±4.88c	6.616 ±0.22d 9.98 ±0.09c	8.291 ±2.37c
Mean	562.54 ± 14.1a 436.24 ± 69.7b		109.84 ± 70.87a 60.18± 49.86b		13.213 ±6.3a 6.811± 5.68b		6.63 ±0.09b 15.07 ±6.73a	
P value	0.0183	0.0560	0.000	0.000	0.000	0.000	0.000	0.000

LSD value for totalphenol, total flavonoid, total anthocyanin & total tannin content at <0.05

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Table 2: Means of Antioxidant activity and Vitamin C of Ghandhri, Bedana and Tarnab Gulabi.

Varieties	Antioxidant activity (%)		Means	Vitamin C (mg/g)		Means
	Fresh	Stored		Fresh	Stored	
Ghandhri	75.1±5.1b	67.1 ±3.1bc	71.1±5.66b	3.2±0.45a	2.8±0.81ab	2.9 ±0.32a
Bedana	70.1 ±5.1bc	63.1±5.1c	66.6±4.95b	2.3±0.33bc	1.9 ±0.25c	2.1 ±0.30b
Tarnib Gulabi	89.1±9.1a	73.1±3.1b	81.1±11.32a	2.3±0.35bc	1.9±0.50c	2.1 ±0.32b
Means	78.1 ±9.86a	68.1±5.03b		2.6±0.68a	2.2±0.45a	
P value	0.000		0.000	0.4967		0.0097

LSDvalue for antioxidant activity and Vitamin C content at < 0.05.

Table 3: Antibacterial activity of three different varieties against *Ecoli* and *Citrobacter* at three concentrations

Varieties	Concentration	Bacteria strains	
		E.coli	*Citrobacter*
Gandhari	1000	23mm	24.8mm
	500	16mm	17.0mm
	100	11mm	12.0mm
Bedana			
	1000	19mm	25.4mm
	500	14mm	18.4mm
	100	11mm	14.8mm
Tarnab Gulabi			
	1000	16mm	17.4mm
	500	9mm	11.0mm
	100	7mm	8.4mm

Dmso was used as negative control having zero zone of inhibition. All the data is represented as mean of three test± SE of standard group.

DISCUSSION

The current study demonstrated that the phenolic content of pomegranate arils juice varies significantly across different varieties, with concentrations ranging from 1245 mg/L to 2076 mg/L, supporting previous findings by Ozgen et al. (16). Such variability can be attributed to varietal differences, which have been shown to impact the phenolic profiles of the juices significantly. This is consistent with observations noted in other studies that underscore the influence of genetic factors on the phytochemical composition of pomegranates (17).

Regarding flavonoid content, it was observed that fresh juices retained higher levels compared to their stored counterparts. This outcome is likely due to the interaction between flavonoids and atmospheric oxygen, leading to oxidative degradation during storage. The comparative analysis further revealed that Ghandhri juice possessed a notably higher flavonoid concentration than the juices from Bedana and Tarnab Gulabi. This finding aligns with the recognized pattern where oxidative processes during storage diminish the flavonoid content.

The study also explored anthocyanin profiles across different pomegranate types. The dominant form identified was delphindin-3,5-diglucoside, with the highest concentrations observed in Ghandhri juice. This aligns with the work of Ozkan et al. (16), which documented substantial variations in anthocyanin levels across nine Turkish pomegranate cultivars. Such disparities underscore the significant role of cultivar selection and maturity stage in determining the anthocyanin content of the juice.

Tannin levels also varied across cultivars, with the Sweet Alak variety showing the highest concentrations, as reported by Mousavinejad et al. (15). This suggests that both genetic and environmental factors, such as climate and soil texture, significantly influence tannin concentrations in pomegranate juices.

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The study confirmed that the addition of preservatives reduced the vitamin C content by approximately 36%, as vitamin C's water-soluble and sensitive nature makes it susceptible to oxidation in storage environments (18). This observation is crucial for understanding the impact of storage and preservation techniques on the nutritional quality of juice.

Antibacterial activity assays indicated that Ghandhri and Tarnab Gulabi juices exhibited the most substantial zones of inhibition against Citrobacter at higher concentrations, while Bedana juice showed the highest inhibitory effect at lower concentrations. These results align with the findings of Dahham et al. (19), who reported significant antibacterial activity of pomegranate juice against E. coli. The variability in antibacterial efficacy at different concentrations and among different varieties suggests that specific phytochemical constituents likely contribute to the antimicrobial properties.

The strengths of this study include a robust comparative analysis across multiple pomegranate varieties and the comprehensive evaluation of several key phytochemical and antibacterial properties. However, limitations include the potential variability in phytochemical content due to uncontrolled environmental factors during growth and variations in the maturity of the fruits used. Future studies could benefit from a controlled cultivation environment to standardize maturity and minimize variability.

Overall, the findings underscore the nutritional and medicinal potential of pomegranate juice, influenced by variety, storage conditions, and preservation methods, and highlight the importance of selecting appropriate varieties for specific health-related applications.

CONCLUSION

The study demonstrated a significant decline in bioactive compounds during storage, with a noticeable reduction in total phenolic, flavonoid, anthocyanin, and tannin contents. Fresh Tarnab Gulabi juice exhibited superior antioxidant activity compared to preserved Bedana juice, highlighting the impact of storage on nutritional quality. Freshly squeezed juice maintained a slight advantage in vitamin C content over preserved juice, attributed to reduced oxidation by added citric acid. Furthermore, pomegranate juice displayed effective antibacterial properties against Citrobacter and E. coli, with Ghandhri juice showing the most substantial inhibition. These findings suggest the importance of optimizing storage conditions to preserve the bioactive qualities of pomegranate juice and harness its potential health benefits.

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