

Original Research Article

DEVELOPMENT AND CHARACTERIZATION OF ANTIOXIDANT-RICH POMEGRANATE AND BEETROOT JUICE

ABSTRACT

The demand for functional beverages that contain natural antioxidants has been steadily increasing due to their potential health benefits. Pomegranate and beetroot are renowned for their rich antioxidant content and diverse phytochemical profiles. This research paper presents a comprehensive development process of an antioxidant-rich juice blend derived from pomegranate and beetroot. The study began with selecting high-quality pomegranate and beetroot varieties known for their antioxidant potency. These raw materials were carefully sourced from reputable suppliers to ensure consistent quality throughout the production process. Upon procurement, the fruits underwent rigorous cleaning, sorting, and preparation to remove any impurities and ensure optimal flavor extraction. Formulation studies were conducted to determine the optimal ratio of pomegranate to beetroot juice to enhance the palatability and consumer acceptance of the juice blend. Sensory evaluation panels were employed to assess different formulations' flavor profiles, color, aroma, and overall acceptability. Through iterative testing and feedback analysis, a balanced blend with harmonious flavor characteristics was identified. On the basis of organoleptic evaluation, the blended juice containing 30% Beetroot, 60 % Pomegranate 5 % Amla, and 5 % Ginger was found to have the best acceptability, freshly and also after storage.

Keywords: Antioxidant, Beetroot, Health benefits, Juice, Pomegranate,

Comentado [EHyl1]: The title should be more extensive. Not only the antioxidant content was evaluated

Comentado [EHyl2]: Suggested title: "DEVELOPMENT AND PHYSICAL, CHEMICAL AND SENSORIAL CHARACTERIZATION OF POMEGRANATE AND BEETROOT JUICE"

Comentado [EHyl3]: ¿what is Amla?

Comentado [EHyl4]: The abstract could be improved by starting with the purpose of the work and weighing into account the results in each of the sections or methods used. The authors never specify what amla is?

1. INTRODUCTION

Antioxidants can stop the chain reaction caused by free radicals, which can damage biological molecules like lipids, proteins, and nucleic acids. Fruit and vegetable juices contain polyphenols and vitamins, affecting cardiovascular risk factors and improving blood lipid profiles. Drinking juices may improve cardiovascular health due to antioxidant effects, anti-inflammatory effects, and prevention of hyperhomocysteinemia (Islam & Kabir, 2019).

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Beetroot is a taproot vegetable belonging to the Amaranthaceae and the subfamily Chenopodiaceae. Beetroot is a native of the Mediterranean region. Germany, France, and various European nations, Africa, and South America cultivated it (Abera, n.d.). The world production of beetroot in 2018 was 274 million tonnes with France, the USA, Russia, Germany, and Ukraine being the top five producers (Nirmal et al., 2021). Beetroot is a popular and simple-to-grow vegetable in India, consistently ranking among the top 10 (Chauhan et al., 2020). Beetroot is mostly grown in the Indian states of Himachal Pradesh, Haryana, Maharashtra, West Bengal, and Uttar Pradesh (Pandita et al., 2020). The beetroot productivity in India is 20-25 tons per hectare per year (Neha P et al., 2018). Beetroot is a popular short-duration crop in Pune, Maharashtra (Sv et al., 2020).

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Pomegranates are a fruit from the Punicaceae family, cultivated since ancient times for their delicious taste (Benedetti et al., 2023). Pomegranates originate from central Asia and are cultivated in various Mediterranean countries including Iran, Spain, Egypt, India, China, and the United States. They are also grown in both Near and Far East countries (Kahramanoglu & Usanmaz, 2016); (Ahed J Alkhatib 2021). Currently, there are several countries around the world where pomegranates are grown. These countries include Afghanistan, Bangladesh, Chile, China, Cyprus, Egypt, France, Georgia, India, Iran, Iraq, Israel, Italy, Lebanon, Mexico, Morocco, Myanmar, Portugal, Spain, Syria, Tajikistan, Thailand, Tunisia, Turkey, Turkmenistan, the USA, and Vietnam (Jain & Desai, 2018). Pomegranates are cultivated on a global scale across approximately 300,000 hectares of land, resulting in a total production of around 3.0 million metric tons. Globally India holds the top position in worldwide pomegranate production, contributing 0.81 million metric tons with a productivity rate of 7.40 tons per hectare (Venkitasamy et al., 2019). Cultivation of pomegranate expanded to more than 300,000 ha worldwide with an estimated production of over 4,500,000 t/year (Esposito et al., 2021). India is the world's leading country in the production of pomegranate around 3 lakh ha and production is 3.0 million tonnes (Sharma et al., 2014). Maharashtra is the leading State with 82

thousand ha area under pomegranate cultivation, Karnataka 13.6 thousand ha and Gujarat with 5.8 thousand ha, Andhra Pradesh and Tamil Nadu stood 2.8 thousand, 0.5 thousand ha of pomegranate cultivation in India (Kulkarni & Sanap, 2019). India is the world's top producer of fruits and vegetables, but sadly, a significant amount is wasted due to inappropriate handling and poor postharvest management. Fruits and vegetables are naturally nutritious, and refreshing, and have a variety of delicious flavors (Panghal et al., 2017). Pomegranates are an ancient fruit that can be cultivated in semi-arid, arid, and poor soil and produce high yields. Because they can flourish in low moisture and endure saltwater water, they are widely distributed and cultivated worldwide (Habib et al., 2023).

Pomegranate and beetroot juice have been shown to have cardiovascular protective effects. Ellagitannins and anthocyanins in pomegranate juice have antioxidant and anti-atherogenic effects, while beetroot juice consumption can help lower blood pressure due to its high nitrate content (Zheng et al., 2017).

The present work aimed to prepare a nutritious drink by blending beetroot juice, pomegranate juice, and a small amount of amla, and ginger extract in an appropriate ratio, such that the blending is accepted to increase the efficiency in terms of health benefits and desirability.

2. MATERIALS AND METHODS

Materials

1. Raw materials

Pomegranate, beetroot, amla, and ginger were purchased from the local market of Phagwara district, Punjab, India.

2. Chemicals

Chemicals that are used to perform this experiment are provided by the food science and technology laboratory.

Preparation of beetroot juice, Beetroot was peeled out and sliced, crushed in a grinder, then the juice was extracted by using a mixer the extracted juice was again filtered by using a four-layer muslin cloth to remove the remaining pomace.

After thoroughly washing the Pomegranate fruits they were cut into quarters with the help of a stainless steel knife. Arils were separated, and juice was extracted. Extracted juice was again filtered by using a four-layer muslin cloth to remove the remaining pomace.

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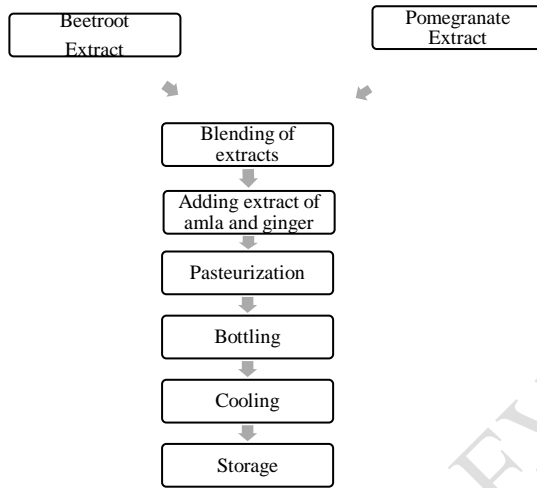
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Fig .1 Extraction and storage process of juice



(J T et al., 2016);(Mena et al., 2014)

After that, the juice of beetroot, pomegranate, ginger, and amla juices should be blended in different ratios respectively. Then sugar (5%) was added to the juice properly and then the mixture was filtered through muslin cloth.

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The blended juice was prepared by following the below combinations of juice of each fruit

Comentado [EHyl13]: Treatments should correspond to Table No. 1. The numbering of the following tables must have a shift.

TREATMENT
T1 -70% Beetroot + 20% Pomegranate + 5% Amla + 5%Ginger
T2 - 65% Beetroot + 25% Pomegranate + 5%Amla+ 5% Ginger
T3 - 60%Beetroot + 30%Pomegranate + 5%Amla +5%Ginger
T4 - 55%Beetroot + 35% Pomegranate +5%Amla +5 %Ginger
T5 - 50 %Beetroot + 40% Pomegranate + 5% Amla +5% Ginger
T6 - 45% Beetroot + 45% Pomegranate +5% Amla + 5%Ginger
T7 - 40% Beetroot + 50% Pomegranate +5 %Amla + 5 %Ginger
T8- 35 %Beetroot + 55% Pomegranate +5 %Amla + 5%Ginger
T9- 30% Beetroot + 60 %Pomegranate +5 %Amla + 5 %Ginger

T10 -25% Beetroot + 65 %Pomegranate +5 %Amla +5 %Ginger

Sensory Evaluation

A panel of ten members conducted the sensory evaluation of the beetroot and pomegranate juice blends after preparation following organoleptic qualities were assessed: taste, odor, color, mouthfeel, and overall acceptability. The presentation order of the samples to the panel was randomized, and potable water was provided to rinse the mouth between evaluations to prevent the transfer of sensory attributes from one sample to the other. According to the method, each sensory attribute was scored on a 9-point Hedonic Scale, ranging from 9 to 1, respectively (liked extremely and disliked extremely).

Comentado [EHyl14]: The term organoleptic (obsolete) should be replaced by sensory.

Physical and chemical analyses

Total soluble solids (TSS), pH, total acidity, and ascorbic acid content were determined as quality indexes. General parameters were measured following the official methods AOAC (2005): The total soluble solids are measured using a digital refractometer and expressed in °Brix, followed by Ranganna (1986). The pH of the juice, blended juice, was recorded with the help of a digital pH meter (Deluxe pH meter model 101). Total acidity was titrated with 0.1 N NaOH solutions and calculated as g citric acid /100 ml juice. Ascorbic acid was determined by visual titration, using 2, indophenol 6-dichlorophenol method and expressed as mg/100ml juice. Total and reducing sugars were determined according to Ranganna (1986).

Comentado [EHyl15]: Only use acronyms once they have been declared.

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Determination of Total Phenolic Content of Beetroot and Pomegranate Blended Juice

The total phenolic content of expressed blended juice was measured with the Folin-Ciocalteu reagent. Firstly, 20 µL of expressed juice was taken in a test tube. It was made up of 1.6 mL of distilled water. 100 µL of Folin-Ciocalteu reagent was mixed, then 300 µL of saturated Na₂CO₃ (20%) was added. The mixture was heated in a water bath at 40°C for 30 minutes and then cooled in an ice bath. The absorbance of this prepared sample solution was measured at 765 nm by using a UV/ Visible spectrophotometer. The total phenolic content of fresh juice was expressed as mg gallic acid equivalent (GAE) /L (Win et al., 2019).

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Determination of Total Flavonoid Content of Beetroot and Pomegranate Blended Juice

The total flavonoid content of blended juice was measured by aluminum chloride (AlCl₃) according to the spectrophotometric method using quercetin as a standard. Firstly, 0.5 mL of fresh juice was taken in a test tube and 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride,

0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water were added into a tube. This tube was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with a UV/ Visible spectrophotometer. The total flavonoid content of fresh juice was expressed as mg quercetin equivalent (QE) /L. Quercetin was used as a reference compound to produce the standard curve (Win et al., 2019).

Antioxidant activity assay

DPPH Test: The total antioxidant activity of blended juice was determined using a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The antioxidant activity of the beetroot pomegranate blended juice was achieved by the method of (Brand-Williams et al., 1995), Briefly, 100 µL of juice diluted at 1:100 with methanol: water (6:4) and 2 mL of 0.1 mM DPPH in methanol were mixed. After 30 min of reaction, the absorbance was determined at 517 nm. For the background correction, the mixture was prepared without DPPH. The results were expressed as efficient concentration (EC50) and antioxidant activity (AA).

The antioxidant activity was determined according to the equation mentioned below:

$$\text{Inhibition of DPPH (\%)} = \frac{(\text{Absorbance Control} - \text{Absorbance sample})}{(\text{Absorbance control})}$$

Determination of Total anthocyanin content

Total anthocyanins present in all samples were determined by the spectrophotometric method as per Ranganna (1986). 50 ml of 95% ethanolic HCL was added to the 10 ml sample and transferred into the volumetric flask. The sample was stored at 4°C overnight in an airtight volumetric flask. The sample was kept in the dark for 2 hrs measuring its optical density at 535 nm.

Anthocyanins were calculated and expressed as mg/100 mL using the formula given below

$$\text{Total optical density} = \frac{\text{OD value at 535nm} \times \text{volume made up for colour estimation}}{\text{weight of sample}} \times 100$$

$$\text{Total Anthocyanin Content (mg/100g)} = \frac{\text{Total optical density}}{98.2}$$

*98.20 = Extraction coefficient

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Statistical analysis

Beetroot pomegranate beverages are evaluated for various physicochemicals using the mean values obtained from the analyses of variance (ANOVA).

The SPSS statistical programme (Version 23.0, IBM Corporation, Somers, NY, USA) was used to analyze variance (ANOVA) and Tukey multiple range tests. The data are shown as mean values with standard deviations and are considered substantially different when $p < 0.05$, $n = 3$ is used.

3. RESULTS AND DISCUSSION

Table 1 Sensory analysis of a different combination of beetroot and pomegranate juice

Treatments	Appearance and Colour	Taste	Flavor	Mouth Feel	Overall Acceptability
T ₁	2.9± 1.20 ^d	3.9±1.20 ^f	2.9±1.20 ^f	3.9± 1.20 ^f	2.9± 1.20 ^g
T ₂	3.9± 1.20 ^{cd}	4.9±1.20 ^e	3.9± 1.20 ^e	4.9± 1.20 ^e	3.9± 1.20 ^f
T ₃	3.9± 1.20 ^{cd}	5.9±1.20 ^d	4.9± 1.20 ^d	5.9± 1.20 ^d	4.9± 1.20 ^{de}
T ₄	4.9± 1.20 ^{bc}	5.9±1.20 ^d	4.9± 1.20	5.9± 1.20 ^d	5.9± 1.20 ^c
T ₅	4.9± 1.20 ^{bc}	6.9±1.20 ^c	5.9± 1.20 ^c	6.9± 1.20 ^c	5.9± 1.20 ^c
T ₆	5.9± 1.20 ^b	6.9±1.20 ^c	5.9± 1.20 ^c	6.9± 1.20 ^c	6.9± 1.20 ^b
T ₇	5.9± 1.20 ^b	7.8±1.03 ^{ab}	6.8± 1.03 ^b	7.6± 1.03 ^{ab}	6.9± 1.20 ^b
T ₈	4.9± 1.20 ^{bc}	5.9±1.20 ^d	4.9± 1.20 ^d	5.9± 1.20 ^d	5.9±1.20 ^c
T ₉	7.8± 1.03 ^a	7.9±0.99 ^a	7.4± 0.84 ^a	7.7± 0.84 ^a	7.8± 1.03 ^a
T ₁₀	3.9± 1.20 ^{cd}	4.9± 1.20 ^e	3.9± 1.20 ^e	4.9± 1.20 ^e	4.8± 1.03 ^{de}

Data are expressed as mean ± SD (Standard deviation)

Table 1 presents the sensory evaluation results for ten treatments (T₁ to T₁₀) based on five parameters: Appearance and Colour, Taste, Flavor, Mouth Feel, and Overall Acceptability. The scores for Appearance and Colour range from 2.9±1.20 for T₁ to 7.8±1.03 for T₉, with T₉ having the highest score and T₁ the lowest. Taste scores range from 3.9±1.20 for T₁ and T₁₀

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to 7.9±0.99 for T9, again with T9 rated highest and T1 lowest. For Flavor, scores range from 2.9±1.20 for T1 to 7.4±0.84 for T9, with T9 being the most preferred and T1 the least. Mouth Feel scores range from 3.9±1.20 for T1 to 7.7±0.84 for T9, with T9 receiving the highest rating and T1 the lowest. Finally, Overall Acceptability scores range from 2.9±1.20 for T1 to 7.8±1.03 for T9, making T9 the most preferred treatment overall and T1 the least preferred.

TABLE 2: Physicochemical Analysis of Blended Juice of Beetroot Pomegranate

Treatments	pH	TSS	Vitamin c	Titration acidity
T1	3.48±0.60 ^a	14.12±0.54 ^a	10.03±0.21 ^f	0.26±0.12 ^e
T2	3.70±0.57 ^a	13.81±0.58 ^a	10.45±0.12 ^{ef}	0.29±0.23 ^{de}
T3	3.14±0.55 ^a	14.80±0.63 ^a	10.87±0.30 ^e	0.32±0.19 ^d
T4	3.80±0.57 ^a	14.44±0.58 ^a	11.08±0.42 ^{de}	0.35±0.22 ^d
T5	3.81±0.55 ^a	14.24±0.54 ^a	11.57±0.38 ^d	0.41±0.16 ^{cd}
T6	4.03±0.56 ^a	14.01±0.62 ^a	11.98±0.24 ^{cd}	0.44±0.22 ^c
T7	4.20±0.58 ^a	14.34±0.54 ^a	12.29±0.41 ^b	0.47±0.14 ^{bc}
T8	4.29±0.58 ^a	14.89±0.57 ^a	12.68±0.23 ^b	0.48±0.10 ^{bc}
T9	4.32±0.56 ^a	14.91±0.59 ^a	12.98±0.34 ^a	0.50±0.22 ^{ab}
T10	4.35±0.57 ^a	14.97±0.62 ^a	13.06±0.11 ^a	0.54±0.16 ^a

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Comentado [EHyl33]: Standardize titles in all the text. Table 2. Capitalize or lowercase? Titles only at the beginning with capital letters or only significant words with capital letters.

Comentado [EHyl34]: "c" is capitalized.

Comentado [EHyl35]: Specify in the table for each physicochemical property the units in which it is measured. Do not repeat them in the discussion.

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Table 2 presents the physicochemical analysis of blended juice from beetroot and pomegranate across ten different treatments (T1 to T10), measuring pH, Total Soluble Solids (TSS) in °Brix, Vitamin C content (mg/100ml), and Titrable Acidity (%). The pH values range from 3.14±0.55 (T3) to 4.35±0.57 (T10), with no significant differences among the treatments. Similar results were reported by Kumar V.(2016). TSS values are relatively consistent across treatments, ranging from 13.81±0.58 (T2) to 14.97±0.62 (T10). Similar results have been reported by Bhardwaj & Mukherjee (2011) kinnow juice blended with pomegranate and ginger juice. Vitamin C content varies more significantly, from 10.03±0.21 (T1) to 13.06±0.11 (T10), with T10 having the highest content and T1 the lowest. Mohamed et al., (2023) support this observation. Titrable acidity ranges from 0.26±0.12 (T1) to 0.54±0.16 (T10), indicating a

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gradual increase in acidity from T1 to T10. The results were in line with the results reported by Vidhya & Sri (2018)

TABLE 3; Biochemical composition of blended juice of beetroot and pomegranate

Sample	Total phenolic content (mgGAE/L)	Total flavonoid content (mgQE/L)	Antioxidant activity	Anthocyanin mg/100ml
T1	1048.03±7.22 ^f	558.83±4.80 ^{ab}	44.64±0.26 ^f	30.01±0.31 ^a
T2	1065.37±5.29 ^f	572.91±6.51 ^a	51.36±0.31 ^e	29.59±0.47 ^a
T3	1147.27±5.51 ^e	348.43±4.27 ^c	51.36±0.39 ^e	28.72±0.55 ^b
T4	1200.50±7.75 ^{de}	601.04±8.02 ^a	59.25±0.60 ^d	27.94±0.16 ^b
T5	1229.90±7.16 ^{bc}	559.89±6.1 ^{ab}	59.27±0.51 ^d	26.91±0.22 ^{de}
T6	1261.95±7.22 ^c	500.51±9.55 ^b	67.99±0.81 ^c	26.35±0.29 ^{de}
T7	1269.63±7.22 ^c	506.94±2.36 ^b	78.09±0.15 ^b	26.16±0.25 ^{de}
T8	1376.16±8.53 ^b	582.29±4.07 ^a	80.60±0.38 ^b	25.84±0.23 ^{de}
T9	1400.06±6.93 ^{ab}	595.88±4.04 ^a	85.69±0.34 ^a	25.54±0.35 ^{ef}
T10	1432.28±3.35 ^a	611.61±6.30 ^a	87.53±0.44 ^a	25.13±0.18 ^f

Table 3 presents the biochemical composition of blended juice from beetroot and pomegranate for ten different samples (T1 to T10), including Total Phenolic Content (mgGAE/L), Total Flavonoid Content (mgQE/L), Antioxidant Activity, and Anthocyanin (mg/100ml). The Total Phenolic Content ranges from 1048.03±7.22 (T1) to 1432.28±3.35 (T10), with T10 having the highest content and T1 the lowest. Total Flavonoid Content varies from 348.43±4.27 (T3) to 611.61±6.30 (T10), with T10 again having the highest content. Similar results were recorded (El Kar et al., 2011) and Win et al., (2019) in pomegranate juice. Antioxidant Activity increases from 44.64±0.26 (T1) to 87.53±0.44 (T10), indicating T10 has the highest antioxidant capacity. Similar results were reported by Kathiravan et al. (2015) in beetroot-passion blended juice and Sree, K. B., & Vanajalata, K. (2015) Studies on the blending of sweet orange and pomegranate juice for RTS beverage. Anthocyanin content ranges from 25.13±0.18 (T10) to 30.01±0.31

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Comentado [EHyl42]: Improve the way of the references...

(T1), with T1 having the highest anthocyanin content and T10 the lowest. Results agreed with (El Kar et al., 2011); (Bafna & Bafna, 2014).

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4.6 EFFECT ON STORAGE ON PHYSICAL-CHEMICAL PARAMETERS

Table 4 presents the effect of storage on the physicochemical parameters of a blended juice sample (T9: 30% Beetroot + 60% Pomegranate + 5% Amla + 5% Ginger) over 21 days. The pH decreased from 4.35±0.56 on the 0th day to 3.12±0.27 on the 21st day. Total Soluble Solids (TSS) slightly decreased from 14.97±0.59 °Brix on the 0th day to 14.56±0.59 °Brix on the 21st day. Titrable acidity increased from 0.50±0.22% on the 0th day to 0.73±0.67% on the 21st day. Vitamin C content decreased from 12.98±0.34 mg/100ml on the 0th day to 11.50±0.15 mg/100ml on the 21st day. Antioxidant activity also showed a decrease from 85.69±0.34 on the 0th day to 76.35±0.13 on the 21st day.

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Table 4. EFFECT ON STORAGE ON PHYSICO CHEMICAL PARAMETERS

T9- 30 % Beetroot + 60% Pomegranate + 5%Amla + 5% Ginger				
PARAMETER	0 th day	7 th day	14 th day	21 st day
pH	4.35±0.56 ^a	4.12±0.33 ^a	3.78±0.45 ^a	3.12±0.27 ^a
TSS	14.97±0.59 ^a	14.89±0.29 ^a	14.72±0.34 ^a	14.56±0.59 ^a
Titrable acidity	0.50±0.22 ^a	0.67±0.34 ^a	0.69±0.32 ^a	0.73±0.67 ^a
Vitamin C	12.98±0.34 ^a	12.02±0.22 ^a	11.85±0.12 ^a	11.50±0.15 ^a
Antioxidant activity	85.69±0.34 ^a	82.57±0.66 ^a	79.22±0.67 ^a	76±35±0.13 ^a

CONCLUSION

The present investigation was undertaken to evaluate the development and quality of beetroot juice and pomegranate juice and their blends. Among the ten blended juice treatments evaluated, treatment nine (T9) 30% Beetroot, 60 %Pomegranate,5 %Amla, and 5 %Ginger of blended juice has greater acceptability. It shows **respectable** antioxidant activity (DPPH 85.69%). Beetroot and Pomegranate have the benefit of reducing health issues like heart

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disease, cancer, high blood pressure, etc. Hence it has been suggested that it is better to take the blend of three juices together as both taste and benefits are enhanced with the blending due to the synergistic effect of blended juices. This study suggest that beetroot and pomegranate blended drink has high amount of nutritive chemical constituents that can be beneficial to human being.

UNDER PEER REVIEW

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