

Nutritional, Antioxidant and Sensory Properties of a Functional Beverage Developed from Guava Leaves and Barley Seeds

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Abstract: The growing demand for functional beverages has led to the exploration of plant-based ingredients with health-promoting properties. This study aimed to formulate and evaluate a natural functional beverage using guava leaves (*Psidium guajava*), barley seeds (*Hordeum vulgare*), ginger (*Zingiber officinale*) and cinnamon (*Cinnamomum* spp.). Three variations of the beverage were prepared by altering the quantities of guava leaves and barley seeds, while maintaining constant levels of cinnamon, ginger, and water. Sensory evaluation was conducted using a 7-point hedonic scale, and the best-performing variation was selected based on statistical analysis. Nutritional composition, vitamin, mineral content, antioxidant potential and microbial safety of the optimized beverage were assessed using standard AOAC methods and in vitro antioxidant assays (DPPH, ABTS, and FRAP). The results showed that the detox drink was low in calories (12 kcal/100 g) and contained beneficial nutrients including dietary fiber (1.14 g/100 g), potassium (24 mg/100 g), vitamin E (1.7 mg/100 mg) and vitamin K (4.8 mg/100 mg). The beverage also exhibited strong antioxidant activity particularly in the FRAP (2700.2 mg/100 g) and ABTS (2500.7 mg/100 g) assays. One-way ANOVA revealed a significant difference in sensory attributes among the formulations ($F = 7.81$, $p = 0.0093$), indicating the influence of ingredient concentration on consumer acceptability. Microbiological evaluation confirmed the product's safety, with negligible counts of aerobic bacteria, yeast, and mold, and the absence of pathogens. Overall, the formulated functional beverage demonstrated promising potential as a safe, palatable, and health-enhancing functional beverage.

Keywords – Functional beverage, guava leaves, barley seeds, ginger, sensory evaluation, ANOVA analysis, product consistency, consumer acceptance

I. Introduction

The increasing global demand for functional foods and beverages has led to a growing interest in natural detoxifying agents that promote general health and wellness. Functional beverages formulated from plant-based sources are gaining popularity due to their rich content of bioactive compounds particularly antioxidants. Antioxidants play a key role in combating oxidative stress and preventing chronic diseases (Zhao et al., 2020). Guava leaves (*Psidium guajava*), barley seeds (*Hordeum vulgare*), ginger (*Zingiber officinale*) and cinnamon (*Cinnamomum* spp.) has each of which a long history of traditional medicinal use.

Guava leaves of the Myrtaceae family are traditionally utilized for their therapeutic benefits. These leaves contain phytochemicals which includes flavonoids such as quercetin and guaijaverin, as well as phenolic acids and carotenoids. Their bioactive profile revealed numerous pharmacological effects including antioxidant, antimicrobial, anti-inflammatory, antidiabetic and antihypertensive properties (Naseer et al., 2018; Prabu & Gnanamani, 2007). Their high antioxidant potential makes them an ideal component in detox formulations aimed at reducing oxidative damage and supporting metabolic health.

Barley (*Hordeum vulgare*) a cereal grain from the Poaceae family, is widely cultivated and recognized for its nutritional and functional benefits. It ranks fourth in global cereal production and is particularly valued for its high β -glucan content and contributes to its cholesterol-lowering, glycemic-regulating and anti-inflammatory effects (Saisho et al., 2011; Geng et al., 2021). Regular consumption of barley-based products has been linked to reduced risks of metabolic disorders such as type 2 diabetes, cardiovascular disease and colorectal cancer (Zeng et al., 2020).

Ginger (*Zingiber officinale*) a rhizomatous plant native to Southeast Asia, has long been used in culinary and medicinal applications. Its therapeutic efficacy is primarily attributed to its rich phytochemical composition, including gingerols, shogaols and zingerone, which exhibit potent antioxidant, anti-inflammatory and gastrointestinal-protective effects (Shaukat et al., 2023). These properties make ginger a valuable addition to detoxifying formulations to improve digestion and systemic detoxification.

Cinnamon, derived from the bark of *Cinnamomum* species such as *C. verum* and *C. cassia*, is a well-known spice with strong medicinal attributes. It is rich in cinnamaldehyde, cinnamic acid, coumarin and polyphenolic compounds that contribute to its antioxidant, antimicrobial, anti-inflammatory and antidiabetic effects (Sharifi-Rad et al., 2021; Bandara et al., 2011). Its inclusion in detox beverages may enhance flavor while providing additional health benefits.

Although the advantages of each of these substances have been well-established but limited research has examined their combined use in a functional beverage. Therefore, this study aims to develop a natural functional beverage drink utilizing the potential of guava leaf and barley seed extracts, enhanced with ginger and cinnamon. The objective is to evaluate the new beverage's potential as a functional drink that promotes health.

II. Materials and Methods

Formulation of the Functional beverage

The raw materials for this study which includes guava leaves (*Psidium guajava*), barley seeds (*Hordeum vulgare*), fresh ginger (*Zingiber officinale*), cinnamon bark (*Cinnamomum* spp.), and lemon—were sourced from local markets in Toopran, Medak district, Telangana, India. All ingredients were selected based on freshness, absence of physical damage and lack of contaminants.

Ingredient Preparation and Processing

Barley seeds were thoroughly rinsed with potable water and soaked for 2–3 hours. After soaking, 15–25 g of barley seeds (depending on the formulation variation) were boiled in 200 ml of water for 10–15 minutes. Once the liquid turned milky white and the seeds softened, washed guava leaves (30–50 g) were added, along with 10 g of cinnamon bark, 5 g of freshly grated ginger, and a 5 ml of lemon juice. The mixture was simmered for an additional 10–15 minutes. After boiling, the infusion was filtered using a sterile fine-mesh sieve to remove solid residues. The filtrate was cooled to room temperature and stored at 4°C until further use (Fig 1,2,3,4,5).

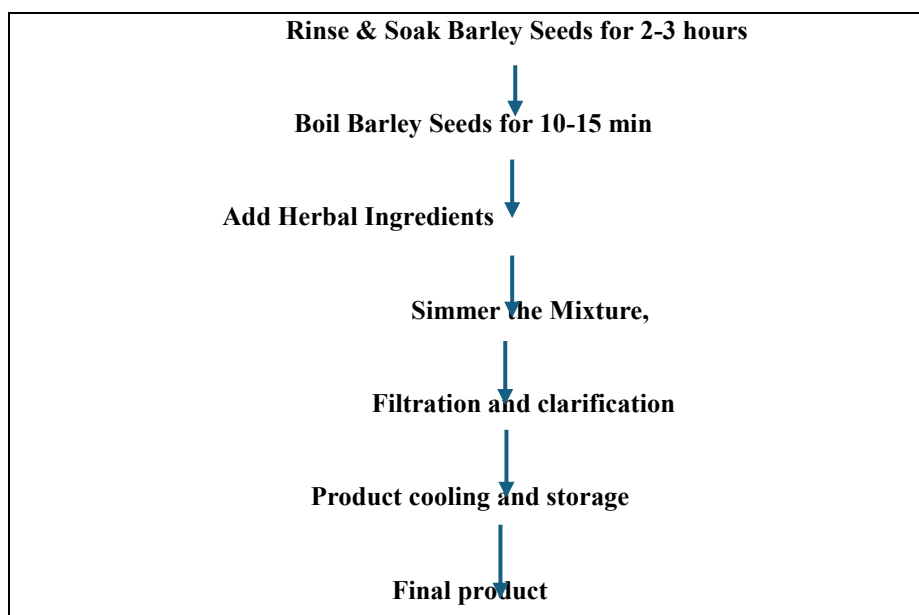


Fig 1: Preparation of The Functional Beverage from Guava Leaves and Barley Seeds



Fig 2: soaked barley seeds



Fig 3 : soaked guava leaves



Fig 4: added cinnamon, Lemon and salt



Fig 5: filtration of mixture

Table 1- Standardization of Functional beverage with Variation

S. No	Ingredients	Variation 1	Variation 2	Variation 3
1	Guava leaves	30gm	40gm	50gm
2	Barley seeds	15gm	20gm	25gm
3	Cinnamon	10gm	10gm	10gm
4	Ginger	5gm	5gm	5gm
5	Water	200ml	200ml	200ml

Sensory Evaluation

The organoleptic properties of the functional beverage were evaluated using a 7-point hedonic scale, assessing five sensory attributes: color, aroma, texture, taste, and overall acceptability. A panel of 15 semi-trained evaluators participated in the sensory evaluation under controlled conditions (Meilgaard, Civille, & Carr, 2006). Each panelist received coded samples of all three variations and rated them independently (Fig 6). The results were statistically analyzed using one-way ANOVA to determine significant differences among variations ($p < 0.05$).

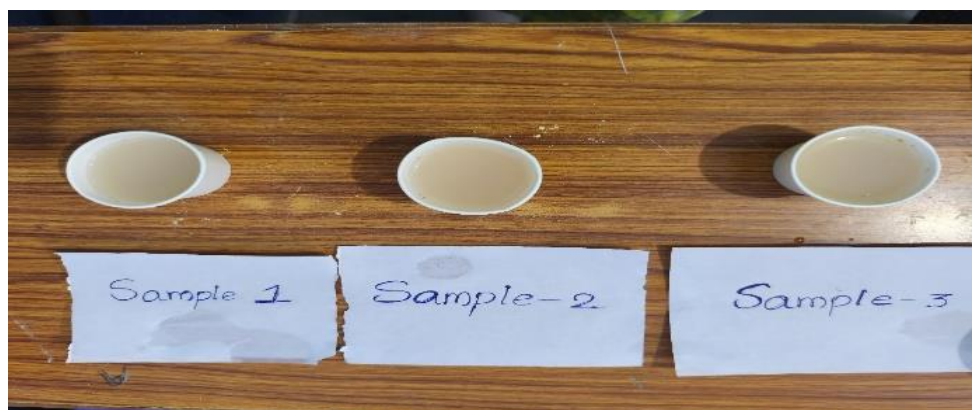


Fig 6: Variation of the Samples T1, T2 and T3

Proximate Composition Analysis

The proximate analysis of the detox drink included determination of energy, protein, carbohydrate, fat, dietary fiber, and moisture content. These were analyzed using standard AOAC International methods. Protein: Determined by the Kjeldahl method (AOAC 2001.11), Carbohydrates: Calculated by difference (AOAC 945.66), Total Fat: Determined using Soxhlet extraction (AOAC 996.06), Dietary Fiber: Determined using enzymatic-gravimetric method (AOAC 2009.01), Energy Value: Estimated using Atwater factors based on protein, fat, and carbohydrate contents (IS 7874:1975).

Vitamin Content Analysis

Vitamin content was determined by AOAC official protocols: Vitamin C: AOAC 2015.14; Vitamin E: AOAC 2015.14; Vitamin K: AOAC 2015.14; Vitamin B1 (Thiamine) and B2 (Riboflavin): AOAC 2015.14.

Mineral Analysis

Mineral elements such as iron, calcium, and potassium determined by AOAC method 985.35. Samples were digested using a microwave digestion system with nitric acid prior to analysis. Calibration was performed using certified reference standards.

Antioxidant Activity Assays

The antioxidant potential of the functional beverage was evaluated using the following in vitro methods: DPPH Radical Scavenging Activity: The method of Brand-Williams et al. (1995) was used, wherein sample absorbance was measured at 517 nm. ABTS Radical Cation Decolorization Assay: Performed according to Re et al. (1999), involving generation of ABTS⁺ and measuring reduction in absorbance at 734 nm. Ferric Reducing Antioxidant Power (FRAP) Assay: Conducted as per Benzie and Strain (1996), where absorbance at 593 nm was used to determine ferric ion reduction. All antioxidant assays were conducted and results expressed in mg Trolox equivalent per 100 ml.

Microbial Analysis

Microbiological safety of the final product was assessed using standard AOAC methods: Aerobic Plate Count (APC): AOAC 990.12; Yeast and Mold Count: AOAC 997.02; Enterobacteriaceae and Staphylococcus aureus: AOAC 991.14. The Samples were serially diluted and plated on appropriate selective agar media. Results were expressed as colony-forming units per gram (CFU/g). Pathogen presence was evaluated based on absence in 25 g as per food safety standards.

III. Results and Discussion

Sensory Evaluation of Project

The sensory evaluation was carried out at Shapur Nagar, Jeedimetla. Sensory evaluation was conducted to assess the organoleptic quality of the functional beverage samples (T1, T2, and T3), focusing on five key parameters: colour, texture, aroma, flavour, and overall acceptability. The results, presented in Table 2, reflect the mean scores $\pm$ standard deviation as rated by a semi-trained panel using a 7-point hedonic scale.

Sample T1 received the highest scores across most sensory attributes, with particularly favorable ratings for colour (6.65), aroma (6.6), and flavour (6.7). Its overall acceptability was also the highest (6.65 ± 0.489), suggesting that the formulation containing 30 g guava leaves and 15 g barley seeds offered a balanced and pleasant sensory experience. The slightly lower barley and guava concentration may have contributed to a milder, more palatable flavor and aroma, aligning with consumer preferences for naturally infused beverages (Sharma et al., 2017).

T2 and T3 showed comparable performance in texture and overall acceptability, but slightly lower scores for aroma and flavour. T3, which contained the highest concentrations of guava leaves (50 g) and barley seeds (25 g), received the lowest aroma (6.25) and flavour (6.25) ratings. This could be attributed to the stronger vegetal or earthy notes imparted by higher concentrations of guava and barley extracts, which may not be preferred by all consumers (Ghosh et al., 2014). While higher levels of bioactive compounds can enhance functional benefits, they may negatively influence organoleptic qualities. No statistically significant differences ($p > 0.05$) were observed between the treatments in terms of texture, suggesting consistent mouthfeel across all formulations. However, slight variations in aroma and flavour ratings indicate a need for careful optimization of herbal concentrations to balance health benefits and consumer acceptability. Overall, T1 showed as the most favorable formulation based on sensory evaluation. This supports the hypothesis that lower-to-moderate concentrations of active ingredients may enhance acceptability while maintaining functional potential. These findings are consistent with previous research indicating that consumer preference for herbal beverages often correlates with moderate flavor intensities and well-balanced aroma profiles (Kaur & Singh, 2019).

Table 2: Sensory parameters of treated samples (T1, T2 and T3) of the functional beverage

Sample	Colour	Texture	Aroma	Flavour	Over all acceptability
T1	6.65 $\pm$ 0.587	6.3 $\pm$ 0.656	6.6 $\pm$ 0.680	6.7 $\pm$ 0.571	6.65 $\pm$ 0.489
T2	6.35 $\pm$ 0.587	6.45 $\pm$ 0.759	6.225 $\pm$ 0.617	6.45 $\pm$ 0.759	6.5 $\pm$ 0.606

T3	6.35±0.812	6.3±0.656	6.25±0.716	6.25±0.716	6.5± 0.512
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Mean value

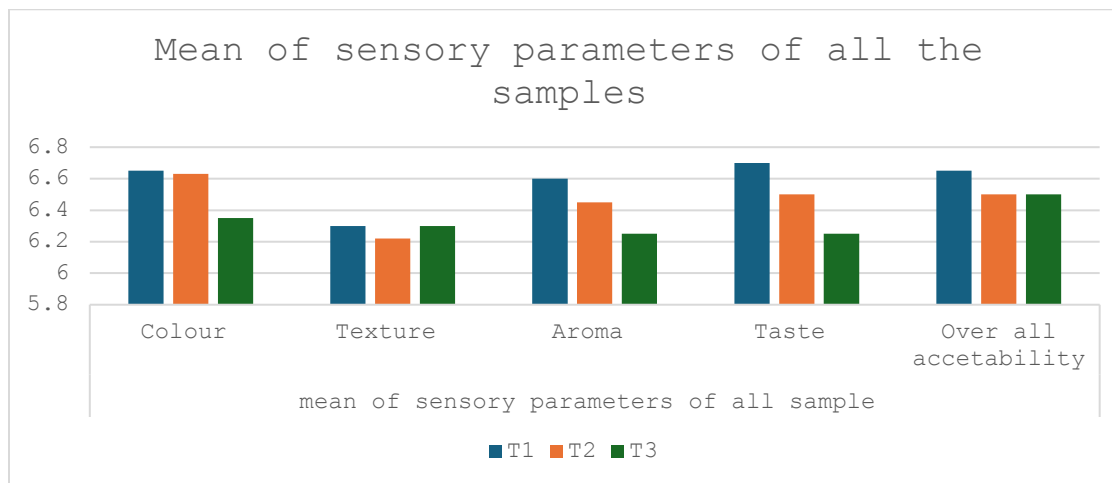


Fig 6: shows the mean of sensory parameters of T1, T2 and T3 samples

Nutritional analysis of functional beverage

The Nutritional composition of the formulated functional beverage is presented in Table 3. The beverage yielded a modest energy content of 12 kcal/100 ml with a low-calorie functional drink. This energy contribution is primarily derived from its carbohydrate content (8.6 g/100 ml), which can offer a quick source of energy without excessive caloric intake, making the beverage suitable for health-conscious consumers or individuals managing weight.

The Protein content (1.3 g/100 ml), although low, indicates the presence of amino acids that can contribute to basic physiological functions and repair mechanisms. This protein level is attributed largely to the guava leaves and barley seeds, both of which contain modest protein fractions (Naseer et al., 2018; Saisho et al., 2011).

The total fat content was minimal at 0.5 g/100 ml, consistent with expectations for plant-based infusions. This low-fat level is favorable for a CVD person and complies with dietary recommendations for low-fat beverages (Zeng et al., 2020). The Dietary fiber content, measured at 1.14 g/100 ml. Dietary fiber, particularly β -glucans from barley, plays a significant role in improving gut health, modulating glycemic responses and supporting cholesterol regulation (Geng et al., 2021). Collectively, the formulation represents a well-balanced, functional beverage suitable for detoxification and general wellness. It provides essential nutrients with minimal calories and fat. it is enriched with plant-derived bioactives that may contribute to antioxidative and metabolic benefits. These results are consistent with previous literature supporting the health-promoting properties of the individual ingredients (Sharifi-Rad et al., 2021; Shaukat et al., 2023).

Table no-03: Nutritional analysis of functional beverage

Test parameters	Units	Results
Energy	kcal	12
Protein	g/100gm	1.3
Carbohydrates	g/100gm	8.6
Total fat	g/100gm	0.5
Dietary fibre	g/100gm	1.14

Mineral analysis of the functional beverage

The mineral composition of the formulated functional beverage is showed in Table 4. The beverage exhibited a moderate potassium content of 24 mg/100 g, along with calcium at 9 mg/100 g and iron at 0.2 mg/100 g. These values, while not high compared to concentrated mineral sources, which are notable for a plant-based infusion and contribute to the functional quality of the drink.

Potassium plays a crucial role in maintaining electrolyte balance, nerve transmission and muscle function. Its presence is primarily attributed to guava leaves and ginger, both of which are known sources of dietary potassium (Naseer et al., 2018; Shaukat et al.,

2023). The inclusion of potassium supports cardiovascular health by aiding in the regulation of blood pressure and reducing the risk of stroke (He & MacGregor, 2008). Calcium, present at 9 mg/100 g, contributes to bone mineralization and neuromuscular function. Though not a significant source when compared to dairy or fortified products, its presence adds nutritional diversity, particularly for populations relying on plant-based beverages (Geng et al., 2021). Guava leaves have been traditionally cited for their calcium content, complementing the mineral profile of barley and cinnamon (Prabu & Gnanamani, 2007). Iron content, measured at 0.2 mg/100 g, offers trace-level contributions to daily iron intake. Iron is essential for hemoglobin synthesis and oxygen transport and when adding in small amounts it is valuable in formulations to support general wellness and detoxification (Sharifi-Rad et al., 2021). While plant-based sources generally provide non-heme iron with lower bioavailability, the presence of vitamin C from lemon in the formulation may enhance iron absorption (Hallberg et al., 1989). Although the drink cannot be considered a primary source of these minerals, its contribution to daily micronutrient intake is beneficial, especially when consumed regularly as part of a balanced diet. The combination of multiple botanical ingredients has produced a beverage with a favorable mineral profile and potential health-promoting effects.

Table 4: The mineral analysis of the functional beverage

Test parameters	Units	Results
Iron	mg/100gm	0.2
Calcium	mg/100gm	9
Potassium	mg/100gm	24

Vitamin analysis of functional beverage

The Vitamin composition of the functional beverage, as shown in Table 5, representing a diverse profile of essential micronutrients. The drink exhibited a particularly high level of Vitamin K (4.8 mg/100 mg), followed by Vitamin E (1.7 mg/100 mg). These fat-soluble vitamins contribute significantly to the beverage's functional benefits. Vitamin K plays a critical role in blood clotting, bone metabolism, and cardiovascular health (Booth, 2012). Its abundance may be attributed to guava leaves and barley, both known to contain substantial levels of this nutrient (Naseer et al., 2018; Geng et al., 2021). Similarly, Vitamin E, a potent antioxidant, helps combat oxidative stress by neutralizing free radicals, thus enhancing the beverage's health-promoting and detoxifying potential (Traber & Atkinson, 2007).

This drink have Vitamin C (0.5 mg/100 mg), Vitamin B1 (0.1 mg/100 mg) and Vitamin B12 (0.14 mg/100 mg). Vitamin C is a water-soluble antioxidant essential for immune function and iron absorption, likely derived from the lemon component of the formulation (Carr & Maggini, 2017). The presence of Vitamin B1 (thiamine) supports energy metabolism and nervous system health, while Vitamin B12, though typically limited in plant sources, may be present in trace amounts due to microbial activity during preparation or possible contamination from raw materials (Watanabe & Bito, 2018). Though these B vitamins are present in small quantities, their inclusion adds to the comprehensive nutritional profile of the drink, supporting its classification as a functional beverage for health maintenance.

Table 5: Vitamin analysis of the functional beverage

Test parameters	Units	Results
Vitamin E	mg/100mg	1.7
Vitamin K	mg/100mg	4.8
Vitamin C	mg/100mg	0.5
Vitamin B1	mg/100mg	0.1
Vitamin B12	mg/100mg	0.14

Antioxidant activity of functional beverage

The antioxidant potential of the formulated functional beverage was assessed using DPPH, ABTS, and FRAP assays presented in Table 6. The FRAP assay exhibited the highest antioxidant activity (2700.2 mg/100 g), followed closely by ABTS (2500.7 mg/100 g), while DPPH activity was 65.3 mg/100 g. The high values in the ABTS and FRAP assays indicate strong electron-donating and ferric-reducing capacities, which suggest the presence of potent hydrophilic and lipophilic antioxidant compounds in the formulation (Benzie & Strain, 1996; Re et al., 1999). The comparatively lower DPPH value is consistent with the fact that DPPH primarily measures radical scavenging of nonpolar compounds and may be less sensitive to certain water-soluble antioxidants (Brand-Williams et al., 1995).

The rich antioxidant profile can be attributed to the synergistic effect of phytochemicals present in guava leaves, barley, ginger, and cinnamon. Guava leaves are known for high levels of flavonoids like quercetin and guaijaverin (Naseer et al., 2018), while ginger

contains gingerols and shogaols, and cinnamon offers cinnamaldehyde and polyphenols these all of which exhibit significant antioxidant potential (Shaukat et al., 2023; Sharifi-Rad et al., 2021, Nandini and Anil, 2024). These findings suggest that the beverage is a promising source of natural antioxidants and may contribute to oxidative stress reduction, cellular protection, and overall health promotion when included as part of a balanced diet.

Table 6: Antioxidant activity of functional beverage

Test parameters	Units	Results
DPPH	mg/100gm	65.3
ABTS	mg/100gm	2500.7
FRAP	mg/100gm	2700.2

Microbial analysis of the functional beverage

The microbial quality assessment of the functional beverage, as presented in Table 7, indicates that the product meets acceptable microbiological safety standards. The aerobic plate count and yeast and mold counts were both <10 CFU/g, reflecting a low microbial load, while Enterobacteriaceae and *Staphylococcus aureus* were absent, confirming the absence of common pathogenic contaminants. These results suggest that the hygienic handling, proper thermal processing, and storage conditions (4°C) were effective in maintaining the microbiological safety of the beverage (ICMSF, 2011). The absence of *S. aureus* and Enterobacteriaceae is particularly important, as their presence would indicate potential post-processing contamination or inadequate sanitation (Jay et al., 2005). Overall, the product's microbial stability supports its suitability for consumption and enhances its shelf-life potential, provided appropriate refrigeration and handling are maintained.

Table7: Microbial analysis of functional beverage

Test parameters	Units	Results
Aerobic plate count	CFU/g	<10
Yeast and Molds	CFU/g	<10
Enterobacteriaceae	CFU/g	Absent
<i>S. aureus</i>	CFU/25g	Absent

Statistical analysis

The results of the one-way ANOVA analysis for the sensory evaluation of the functional beverage formulations (T1, T2, and T3) revealed a statistically significant difference among the treatment groups, with an **F-value of 7.81** and a **p-value of 0.0093**. The F-value indicates that the variability between the group means is considerably greater than the variability within the groups, suggesting the influence of the differing ingredient concentrations on sensory perception. The p-value, being less than the standard threshold of 0.05, confirms that the differences in sensory scores among the samples are not due to random variation. Hence, the null hypothesis is rejected, demonstrating that at least one formulation (most likely influenced by varying guava leaf and barley concentrations) significantly affected sensory acceptability (Meilgaard, Civille, & Carr, 2006). These findings underscore the importance of optimizing ingredient proportions in functional beverages to enhance consumer preferences, which is essential for product development and market success.

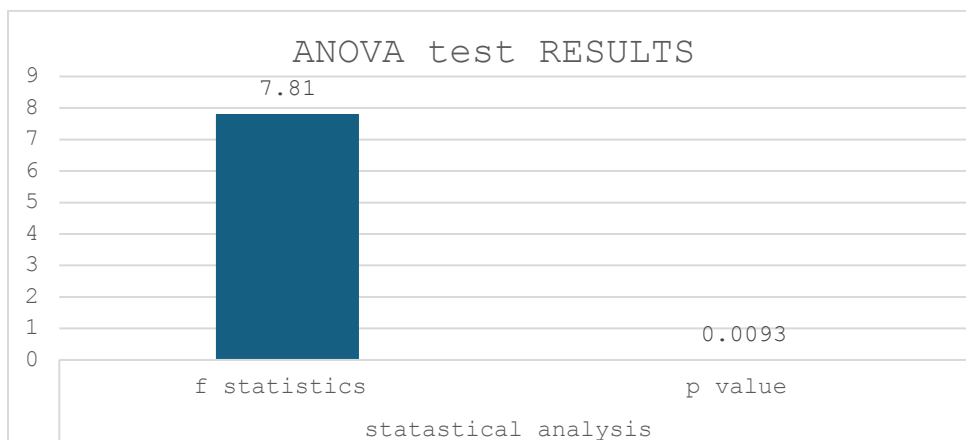


Fig 7: Analysis of the variance results

IV. Conclusion

The present study successfully developed a natural detoxifying beverage using guava leaves, barley seeds, ginger, and cinnamon. This drink demonstrated a favorable nutritional profile with low energy content, appreciable levels of dietary fiber, essential vitamins and minerals, having potent antioxidant activity as confirmed by DPPH, ABTS, and FRAP assays. Sensory evaluation indicated significant differences among formulations, with at least one variation showing enhanced acceptability as supported by one-way ANOVA results. Microbial analysis confirmed the safety of the product under hygienic preparation and refrigerated storage. These findings highlight the potential of the formulated beverage as a functional health drink with detoxifying properties, supporting its application in health-conscious and preventive nutrition markets.

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