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STUDY THE EFFECT OF BIOACTIVE COMPOUNDS OF GUAVA LEAVES EXTRACT INCORPORATED IN THE FORMATION OF BAKED GOODS USING COMPOSITE FLOUR

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Abstract

Globally, natural resources are increasingly used in dietary treatments for managing physiological issues. This study investigated the preventive potential of phytonutrients-rich bioactive compounds in guava leaves extract. Employing the maceration approach, extracts were prepared with aqueous ethanol, methanol and n-hexane. Results showed the aqueous ethanolic extract had the highest total flavonoid content (TFC) and DPPH antioxidant activity, while the methanolic extract had the highest total phenolic content (TPC).The HPLC quantification elucidated highest concentration of catechin in aqueous ethanol extract. HPLC analysis revealed the highest

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catechin concentration in the aqueous ethanolic extract, along with strong antibacterial efficacy towards *P. aeruginosa, B. subtilis, S. aureus, and E. coli*. Six muffin formulations (T_{A0}, T_{A1}, T_{A2}, T_{A3}, T_{A4}, and T_{A5}) with different concentrations of this extract were developed using wheat-barley composite flour and jaggery as a sugar substitute. The addition of guava leaf extract significantly affected the phytochemical, antioxidant and pasting properties of the muffins ($p<0.05$). TA_2 had superior physical and sensory attributes, while TA_5 showed the lowest PV and TBA values during storage, indicating high oxidative stability. Overall, guava leaves extract proved to be an effective functional ingredient for developing healthier baked goods as an alternative to high-calorie commercial products.

1. INTRODUCTION

Due to the presence of biologically active compounds that promote human health and manage various illnesses interest in plant-based research has grown. In functional foods and dietary supplements the use of bioactive compounds is a trending area of study(Mittal et al., 2024). *Psidium guajava* (guava) is one of them (Thiyagarajan et al., 2024). It is a tropical tree widely cultivated for its fruits and is a member of the Myrtaceae family. Guava has a long history of traditional medicine use across various cultures. In particular, the leaves have been used in infusions and decoctions for treating illnesses such as rheumatism, diabetes, cough, and diarrhea (Kalyani, 2024).

Phenolic compounds in guava leaves such as gallic acid, catechin, and quercetin contribute to their medicinal properties (Kumar et al., 2021). A significant rise in diabetes cases globally has predicted by the World Health Organization (WHO), increasing from 171 million people in 2000 to 366 million by 2030, and type 2 diabetes (T2DM) is the most prevalent(Rao & Tejomurtula, 2024). There is an urgent need to find alternative natural treatments because of the side effects of conventional hypoglycemic drugs, such as liver dysfunction and gastrointestinal distress. However, guava leaves are beneficial in regulating glucose levels, inhibiting enzymes that hydrolyze carbohydrates, and providing other health benefits (Huang et al., 2021).

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Guava leaves also possess anti-cancer properties beyond diabetes (Gunasekaran et al., 2024). Flavonoids and terpenoids present in guava leaves inhibit tumor growth and regulate immune function by preventing cell proliferation, angiogenesis, and signal transduction(Alhamdi, 2019). Furthermore, the antioxidants present in guava leaves reduces oxidative stress which causes cancer (Jamieson et al., 2022). Kaempferol and quercetin have demonstrated the capacity to stop the growth of malignant cells and cause them to undergo apoptosis. Additionally, quercetin can help treat diarrhea caused by bacterial toxins and has muscle-relaxing properties. The extract of guava leaves also exhibit strong antibacterial properties, inhibiting the growth of *Salmonella*, *Bascillus* species, and *Staphylococcus aureus*. Bark and methanolic extracts of guava leaves have shown antimicrobial activity (Melo et al., 2020). Guava has anti-inflammatory, anti-allergic, and wound-healing qualities due to the presence of terpenoids and flavonoids.

Guava leaves have antioxidant properties because they contain a significant amount of substances such protocatechuic acid, ferulic acid, gallic acid, catechin, and caffeic acid (Joshi et al., 2023). By neutralizing free radicals, these antioxidants can lower the chance of developing chronic diseases. These antioxidants help reducing the risk of chronic diseases and neutralize free radicals. Guava leaf extract can also regulate blood cholesterol levels by inhibiting enzymes like α-glucosidase and α-amylase (Shabbir et al., 2020). To extract phenolic constituents from guava leaves different solvents such as water, methanol, ethanol, and their combinations have been used. Consequently, conventional extraction techniques including infusion, maceration, soxhlet extraction, and percolation were employed. The consumption of guava in various formulations can help retain its nutraceutical properties and fortify food products.

One of the largest organized food industries, the bakery industry produces staple products like bread, crackers, cakes, muffins, and biscuits (Owusu-Apenten & Vieira, 2022). These products can be enriched with fiber, minerals, vitamins, and proteins to meet nutritional needs, particularly in malnourished population (Owusu-Apenten & Vieira, 2022). The primary ingredient in bakery goods, wheat flour can be partially replaced with more nutritious alternatives such as barley. Barley is rich

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in β-glucan which lowers blood sugar and cholesterol levels while improving immune function. Antioxidants like flavanols, tocopherols, and phenolic acids are also present in barley, making it an excellent choice for functional food development (Geng et al., 2022).

In baked goods, sugar plays a crucial role but reducing sugar content alters product texture, flavor, and shelf life significantly. Natural sweeteners like jaggery can be used as an alternative to formulate low-calorie and sugar free bakery products(Singh et al., 2020). Jaggery is widely consumed and is rich in vitamins, minerals, and amino acids, making it a nutraceutical ingredient with various health benefits. In addition, it contains phytonutrients that contribute to its bioactive properties and is used in a variety of value added and traditional food products (Sharifi-Rad et al., 2023).

Muffins are widely consumed as convenient, energy-rich product among baked goods (Sluková & Skřivan, 2020). Incorporating guava leaf extract into baked goods along with barley and jaggery can help develop functional food with therapeutic properties and various health benefits. Therefore, this study aimed to develop a functional baked product by incorporating therapeutically active guava leaf extract. Further, the study objective was to extract bioactive compounds from guava leaves using solvents and evaluate their phytochemical composition, to investigate the effects of substituting jaggery from sugar on the rheological properties of flour blend and storage features of muffins, and to evaluate prepared muffins for physical parameter, nutritional profiling and sensory attributes for consumer acceptability.

2. Materials and Methods

2.1. Procurement of raw material

Study was conducted at Jinnah University for Women in the department of Food Science & Technology. The Guava leaves were collected from guava farm located at Saakran, Las Bela, Balochistan in the month of February, 2023. The ingredients required for production of muffins flour, jaggery, fat and eggs were purchased from local market for study. All the chemicals used for

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conducting the research work mentioned were analytical grade, provided by Jinnah University for women and procured from LabsCo and Nawaid Scientific.

2.2. Preparation of sample

The identity of *Psidium guajava L.* was confirmed and authenticated by Botany department of Jinnah University for Women. The leaves were washed properly and dried through dehydrator at 40°C for 12 hours and then the leaves and ground to achieve fine and uniform powder by using hi-tech grinding machine. Powder was passed through 20 mesh sieve to obtain uniform sized particles. Dried powder was stored in air tight glass container box in refrigerator for other investigations (Sampath Kumar et al., 2021).

2.3. Proximate analysis of guava leaves powder

Guava leaves powder was evaluated for moisture, crude protein, fat, and ash using AACC (2010) Method Nos. 44-15, 46-30, 30-25, and 08-01, respectively. Crude fiber content of guava leaves powder was determined by (Dwiloka et al., 2019). The percentage of nitrogen-free extract was calculated using the following formula(Ogoloma et al., 2013). The resulting figure represents the sample's carbohydrate proportion.

$$\%\ of\ NFE = 100 - (\%\ of\ Moisture + \%\ of\ crude\ fat + protein + \%\ of\ ash\%\ of\ fiber)$$

Preparation of guava leaves (*Psidium guajava L.*) extract

N-hexane (>95%), methanol (>95%) and aqueous-ethanol (80%) were employed for the maceration extraction process. These three solvents were organized in increasing order of polarity. To get a concentrated extract, 20g of leaves powder were immersed in 200 mL of each solvent. In order to prevent evaporation and exposure to light, the mixes were prepared in sterile 250mL Erlenmeyer flask that was wrapped in aluminum foil. The samples were kept for approximately a month at room temperature. The sample was subsequently concentrated using a vacuum rotary evaporator (Yamato

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Scientific Co., Ltd RE 600, Japan) at 75°C after being filtered once more via filter paper and muslin cloth. Until a thick, viscous extract was visible, crude extracts were collected. The samples were stored at 20°C until they were assessed for bioactive chemical quantification. (GOJE et al., 2023).

Quantitative analysis of guava leaves extract

Total phenolic content (TPC)

Folin-Ciocalteu reagent was used to determine the total phenolic content with slight modification (Melo et al., 2020). 0.5 mL extract (1:5 dilutions) and 0.1 mL folin-ciocalteu reagent (0.5N) were combined and incubated for 15 minutes at room temperature. After that 2.5 mL saturated sodium carbonate was added and the absorbance was measured at 760 nm after 30 minutes at room temperature. The TPC content of the sample was quantified in terms of mg of gallic acid equivalents (GAE)/g fresh weight of guava leaf (mg/g sample) using a gallic acid calibration curve.

The concentration was calculated using following formula:

$$C = \frac{c \times v}{m}$$

Where;

C = total content of phenolic compounds, mg/g plant extract in GAE,

c = the concentration of gallic acid established from the calibration curve (mg/mL),

V = the volume of extract in mL,

m = the weight of crude plant extract in g.

2.3.1. Total flavonoids content

Using a technique based on the formation of a flavonoid-aluminum complex, total flavonoids were estimated by a method described by (Pandhi et al., 2022). Quercetin was used as a standard to measure the total amount of flavonoids in peel extracts. A volumetric flask (10 mL) was filled with

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1 mL of guava leaves extract. The capacity was then filled to 5 mL with distilled water and 0.3 mL of 5% sodium nitrite. After duration of 5 minutes, 0.6 mL of 10% w/v AlCl3 was taken, followed by 6 minutes, 2 mL of 1 M NaOH was added. Finally, 2.1 mL of distilled water was added. Absorbance was immediately measured using a UV/visible light spectrophotometer at 510 nm. The data gathered were expressed as mg of extract/100 g of quercetin equivalents (QE).

Antioxidant activity by DPPH assays (Radical scavenging method)

The free radical scavenging activity was evaluated using DPPH (2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picryl-hydrazyl) of guava leaves extract was measured (Amaral et al., 2021). Further, 1.0mL of DPPH in methanol (0.3 mM) and 1.0mL of the each extract was used in the experiment. The absorbance was measured at 517 nm after a 30-minute incubation period in the dark. The amount of ascorbic acid equivalent (mg/g) used to measure DPPH scavenging activity. Control sample was prepared containing the same volume without any extract.

The percent DPPH scavenging effect was calculated by using following equation:

$$AA\ (\%) = \frac{Ac - As}{As} \times 100$$

Where,

Ac = the absorbance of control reaction and

As = the absorbance in presence of test sample.

2.4. Antimicrobial assessment of guava leaves extract

Bacillus subtilis, E. coli, Staphylococcus aureus and *Pseudomonas aeruginosa* were chosen for antibacterial testing because of their clinical and pharmacological value. These bacterial strains were taken from Department of Microbiology, Jinnah University for Women.

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2.4.1. Culture media and inoculums preparation

The bacterial stock cultures were cultured on nutritional agar for 24 hours at 37°C for 24 hours at 27°C, before being stored at 4°C until further use. To see if the plant extracts had antibacterial activity, they were tested on Mueller-Hinton Agar plates (Bisht et al., 2016).

2.4.2. Culture media and inoculums preparation

This study examined at the antibacterial activity of four different plant extracts by trailing protocol (Azizan et al., 2020). The disc-diffusion method was used to assess antibacterial susceptibility. To see if the plant extracts had antibacterial activity, they were tested on Mueller-Hinton Agar plates. All plates were inoculated with the test bacteria and excess inoculum was removed by dipping a sterile cotton swab into the solution, rotating it several times and pressing hard on the inside wall of the tube above the fluid level. To ensure an even distribution of inoculums, the agar plate's surface was streaked across the whole sterile agar surface while rotating the plate

2.6.3 Assessment of antibacterial activity

The activity of tested microbes was analyzed according to the procedure described by Egga et al., (2014). Using a sterile borer, 5 mm diameter discs were punched into the medium after the plates had been distributed with bacteria. Allowed 3-5 minutes for the plates to dry off any excess moisture. Followed the inoculation of the plates with bacteria, 1mL aliquots of each test extract was poured into each disc. Controls were kept for each bacterial strain, where pure solvents were used instead of the extract. The plates are then parafilm-sealed, tagged and put in a 37°C incubator. Each plate was checked for inhibitory zones after 24 hours of incubation. The inhibitory zones were measured in millimeters using a ruler (Egga et al., 2014).

2.5. Quantification of catechin by HPLC

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The phenolic components in guava leaves were extracted using various ethanol/water combinations. HPLC was used to identify the phenolics (Díaz-de-Cerio et al., 2017).

2.5.1. Preparation of sample for HPLC analysis

For half an hour, mix 0.5 g of sample powder with 30 mL of methanol at room temperature. The extracts were prepared in triplicate utilizing 0.5 g of guava powder that was manufactured on a shaker table at 100 rpm and 25 °C for 30 minutes, along with 30 mL of methanol acidified with 100 mL of strong hydrochloric acid. Following filtering, the extracts were concentrated in an IKA RV ten rotary evaporator set to 40 °C and 40 mbar. The resulting sample was dissolved in 1.5 mL of methanol and stored at 20 °C prior to HPLC analysis. It was then filtered using a PTFE syringe filter (0.45 m) (Dos Anjos et al., 2017).

2.5.2. Preparation of standard for HPLC

Catechin was utilized as phenolic standards for quantification of phenolic components in guava leaves extracts according to the guidelines of (Díaz-de-Cerio et al., 2016). Except for ellagic acid, which was dissolved in water, the standard stock solutions were made at 250 mg/L in methanol. After then, each solution was diluted to a concentration of 0.01 mg/L.

2.5.3. HPLC analysis

Chromatographic investigations were carried out using an Agilent 1260 series HPLC equipped with a binary pump, an online degasser, an autos ampler and a thermostatically controlled column compartment as well as a UV–Vis Diode Array Detector (Agilent Technologies, Santa Clara, CA, USA) (DAD). The temperature of the column was kept constant at 25°C. At room temperature, phenolic components from *P. guajava L.* leaves were isolated. To separate the components, an Agile Technologies Poroshell 120 EC-C18 (4.6 mm 100 mm, particle size 2.7 m) was employed. The gradient elution was performed using water with 1% acetic acid as solvent system A and acetonitrile as solvent system B. The identified components were quantified by peak area and compared to

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calibration curves generated with the corresponding standards, and expressed as µg/g of extract. (Díaz-de-Cerio et al., 2016).

2.6. Proximate analysis of Wheat barley flour

Proximate analysis of flour blend including moisture, crude-protein, fat, fiber, ash and NFE was accomplished using method of AOAC (2003).

2.7. Pasting properties of flour blend by Viscoamylograph

A Rapid Visco Amylograph (Brabender, Duisburg, Germany) was used to test the pasting qualities of the flour blends with addition of extracts of concentration 3, 6, 9, 12 and 15% according to the method described by (Mitharwal & Chauhan, 2022). The flour blend suspension was made by combining 15g sample of flour was taken in a cup followed by addition of 100 ml water. The mixture was shaken vigorously until it forms slurry, then it is attached with Micro Visco Amylograph and the slurry was stirred at 50°C at 160 rpm for 10 sec. The slurry was heated at 95°C over a period of 7.3 minute and then heated again for 15.7 minutes at 95°C providing 5-minute holding time. When temperature was dropped down to 50°C, slurry was cooled during a time period of 7.7 minutes. Following are the pasting parameters hat were assessed:

- Peak viscosity (the maximal hot paste viscosity, PV),
- Holding strength (trough viscosity TV),
- Final viscosity FV
- Pasting temperature
- The setback viscosity
- Breakdown viscosity

2.8. Product development

For the development of muffins, six treatments of different concentration of extract (3%, 6%,9%, 12% and 15 %) designated as $T_{A1,}$ T_{A2}, $T_{A3,}$ T_{A4} and T_{A5} respectively whileT_{A0} served as control. Wheat

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and barley flour, jaggery, eggs, shortening, cocoa powder and baking powder was used in the formulation. The dry ingredients were sifted and combined in a mixing bowl. Eggs were beaten in a separate missing bowl of spar mixer with a flat beater (800B, Taiwan). Under constant stirring for about 5 minutes at 170 rpm, shortening butter and powdered jaggery were mixed into the beaten eggs. After that, the emulsion was combined with different concentration of extract and completely mixed at 120 rpm for 1 minute to get a homogenous mix. Each cup cake pan (65 g) was filled with batter and the batter-filled cups were cooked in an oven at 180°C for 30 minutes. After baking, the muffins were allowed to cool at room temperature and packed in air tight polyethene pouches and stored at ambient temperature for further analysis. The muffins were prepared according to the method described by (Jadhav et al., 2021).

Table 1. Formulation of muffins

Treatments	Guava leaves extract (ml)	Wheat flour	Barley flour	Jaggery powder	Eggs	Butter	Baking powder	Cocoa powder
T_0	0 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm
T_1	3 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm
T_2	6 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm
T_3	9 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm

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T_4	12 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm
T_5	15 ml	70 gm	30 gm	50 gm	84 gm	65 gm	1.7 gm	25 gm

2.9. Phytochemical screening of muffins

Phytochemical screening of extract incorporated muffins was performed by the method described by (Sagar & Pareek, 2021) with slight modification as described earlier. The aim for the current assessment was to determine how ethanolic extract of guava leaves enrichment affected total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (AA) of formulated muffins.

2.12. Physical characteristics of muffins

2.12.1. Muffins volume

Using rapeseed displacement method, the volume of muffins was calculated. An empty pan was filled with rapeseed and the capacity of the pan was measured using a graduated cylinder based on the rapeseed volume (A_1). Muffins were then put in the pan. Rapeseed was used to fill the remaining space, and the volume of rapeseed was estimated using a graduated cylinder (A_2).

A_1-A_2 determined the volume of muffins.

2.12.2. Specific volume

By dividing the volume of cakes by their weights, the specific volume was calculated where a analytical balance was used to weigh the samples (Kaur et al., 2017).

Using the following formula, the specific volume of GLE muffin was determined.

$$Specific\ volume\ \left(\frac{cm3}{g}\right) = \frac{volume\ of\ muffins}{weight\ of\ muffins}$$

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2.12.3. Textural measurement of muffins

Texture profile analyser (TPA), (Model TA-XT. plus, Stable Micro Systems, Godalming, UK) was used to examine the texture of designed muffins. A 50kg load cell and a cylindrical aluminium probe with a 36mm diameter were used to conduct the test. The maximum force (N) necessary to compress the muffins by 50% depth at 1 mm/sec speed test with duration between 30 seconds was measure by the protocol described by (Arslan et al., 2017).

2.12.4.Color measurement

The brightness (L) and color (+ a: red, - a: green, + b: yellow, - b: blue) of muffins were assessed using the Hunter Lab Color Measurement system (Color measuring Labscan XE system, USA). For establishing the instrument with the illuminant D65 light, a standard white board constructed of barium sulphate (100 percent reflectivity) was utilized as a flawlessly white object. Muffin samples were placed in the sample holder, and the reflectance for wavelengths ranging from 360 to 800 nm was automatically recorded. Each number is the average of three readings.

The total color difference (ΔE) between the extract-containing samples (x) and the extract-free control sample (0) was determined using following equation (Lamdande et al., 2018).

$$\Delta E = \sqrt{(L_x - L_0)^2 + (a_x - a_0)^2 + (b_x - b_0)^2}$$

2.13. Sensory evaluation

A nine-point hedonic scale was used to evaluate the overall acceptability of different treatment muffins sample. Sensory attributes includedtexture, appearance, color, flavor and overall quality of the muffin sample. By 20 semi-trained judges in the age group 20 to 50 years comprising of professionals, students and consumers sensory evaluation was conducted. The mean for every attribute was taken out by the method (Chicaiza, 2021).

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2.14. Storage and shelf life studies of muffins

All muffin samples were cooled, packaged individually in polypropylene pouches (150 gauge) and sealed for storage tests. Muffins were kept for 21 days at ambient temperature (27 ±2 ^{0}C) and relative humidity (65 ±5%). On the 0th, 7th, 14th and 21st days, the muffin samples were exposed to objective evaluations such as moisture content, peroxide value and thiobarbituric acid value (TBA).

2.14.1Moisture content

Moisture content of all treatment during storage was analyzed by a method described by Jadhav et al., (2021) with slight modification as described earlier.

2.14.2.Peroxide value (PV)

Peroxide value was determined by method described by(Zahidah et al., 2011). In accordance with the protocol, a 250 ml conical flask containing 5 g of sample was weighed before 30 ml of 3:2 ratios of acetic acid and chloroform was added. To dissolve the mixture, it was swirled. After adding and waiting a minute, 0.5 cc of saturated potassium iodide solution was added. Add 30mL of distilled water, then give it a stir. The yellow color was eliminated by gradually titrating the mixture with 0.01N sodium thiosulphate. To remove the blue tint, 1% starch indicator was applied and titrated. Concurrently, the blank sample was ascertained.

$$\text{Peroxide value} = \frac{(Vs - Vb) \times N \times 1000}{W}$$

Where,

Vs= Volume of titration in ml of sodium thiosulphate used for the sample

V_b= Volume of titration in ml of sodium thiosulphate used for the blank

N= Normality of sodium thiosulphate used

W= weight in gm of extracted lipids

2.14.3.Thiobarbituric acid value (TBA)

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In order to assess TBARS in oils and fats directly, without first isolating secondary oxidation products, the TBA value was calculated using the methodology outlined by AOAC (2006). Animal and vegetable fats and oils can both be used with it. A 20 ml test tube containing a 10–40 mg fat sample was inserted, with its weight precisely recorded, and dissolved in 4.95 ml of 1-butanol (containing 50 mg of BHT per 100 ml). Five milliliters of 1-butanol were used for the blank trial. 5mL of TBA reagent (made by carefully heating 0.2 grams of TBA to 75°Celsius in 1mL of butanol in a 100mL volumetric flask) were added. The tubes were sealed, blended using a vortex mixer, then heated for two hours in a water bath at 95 °C before being cooled to room temperature. In order to measure absorbance at 532 nm, polycarbonate cuvettes with route lengths of 1.00 ± 0.01 cm were used. A series of 0.2 to 2.0 ml aliquots of a 0.02 mM 1, 1, 3, 3-tetramethoxypropane (TMP) solution were used to create calibration curves.

Using polycarbonate cuvettes (path lengths of 1.00 ± 0.01 cm), absorbance values at 532 nm were obtained. Calibration curves were created by adding 1-butanol to each tube till a total of 5mL, then serially dividing 0.02 mM 1, 1, 3, 3-tetramethoxypropane (TMP) solution aliquots (0.2 to 2.0 ml). TBA was calculated by multiplying the absorbance value with factor 7.8 and expressed as mg MDA/Kg (Antone et al., 2010).

2.15. Statistical analysis

The acquired data in this study were statistically modeled using the Two-Factor Factorial under Completely Randomized Design (CRD) to ascertain the degree of significance. In addition, the methods were also explained. Analysis of variance (ANOVA) was performed using statistical software (Statistix-8.1) to ascertain the degree of significance ($P<0.01$ and $P>0.05$). Furthermore, Tukey's Honest Significant Difference was used to perform a post-hoc comparison for means (Kuehl, 2000).

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3. Results and Discussion

This section summarizes the results of proximate analysis, qualitative analysis of phytochemicals, qualitative analysis of phytochemical constituents, quantitative analysis of phytochemicals, total phenolic content, total flavonoid content, antioxidant activity, quantification of catechin by HPLC and microbial assessment of guava leaves extracts. Proximate analysis of composite flour (wheat-barley blend) and effect of guava leaves extract on pasting properties of flour is also discussed. A high carbohydrate content (70.08±0.24%), followed by fiber (12.92±0.11%), moisture (9.46±0.16%), ash (3.33±0.37%), protein (2.49±0.38%), and a low fat concentration (1.56±0.18%) was examined through proximate analysis of guava leaves. Alkaloids, flavonoids, tannins, phenols, glycosides, steroids, saponins, and terpenoids in aqueous methanol and ethanol extracts were identified by qualitative phytochemical screening. However, quantitative analysis revealed significant differences ($p<0.05$) in total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity across different solvent extracts. Aqueous ethanol exhibited (89.38±0.16 mg GAE/g) followed by n-hexane (61.21±0.17 mg GAE/g) and methanol exhibited highest TPC (162.44±0.41 mg GAE/g). Similarly, the highest flavonoid concentration was obtained through aqueous ethanol (39.95±0.28 mg/100g), while the antioxidant activity was greatest (62.50±1.01%) in the aqueous ethanol extract. Furthermore, hydroxyethanolic extraction was considered the most effective method as catechin quantification by HPLC confirmed its highest concentration in aqueous ethanol extract (10.95±0.03 mg/gm). These findings showed that bioactive potential of guava leaves are high for the utilization in functional foods and pharmaceuticals for their health benefits and antioxidant properties.

Moreover, significant antibacterial activity was exhibited by guava leaf extracts, with aqueous ethanol showing the highest inhibition against P. aeruginosa, S. aureus, E. coli, and B. subtilis. Phytochemicals like flavonoids, tannins, and saponins showed strong antimicrobial activity. The proximate composition of 80:20 wheat-barley composite flour indicated increased protein (11.40%) and fiber (5.73%) compared to wheat flour alone. Particularly due to high fiber content, including beta-glucan, barley enhanced the formulation. Similarly, the previous research indicated increased

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fat, protein, and ash content when incorporating barley into wheat flour blends. In addition, guava leaves extract impact on pasting properties of composite flour was also analyzed. Pasting temperature and peak viscosity at lower concentrations but reduced viscosity at higher concentrations due to reduced aeration was significantly increased by guava leaves extract. With increasing guava leaves extract the breakdown and setback viscosity values decreased, indicating reduced retrogradation and improved starch stability.

The formulation of muffins with 80:20 wheat-barley composite flour and guava leaves extract varying concentrations (3-15%) showed improved nutritional value. This addition influenced the textural, physical, and sensory attributes of the developed product. Table 2 indicates a significant ($p < 0.05$) increase in TFC, TPC, and antioxidant activity with increasing concentration of extract n muffins. The highest values for TPC, TFC, and DPPH were observed in TA5 (564.13±0.46 mg GAE/g, 28.94±0.16 mg QE/g, and 41.82±0.46%), respectively. Whereas, the control sample TA0 showed the lowest values for TPC (3.50±0.22 mg GAE/g), TFC (5.66±0.28 mg QE/g), and DPPH radical scavenging activity (4.860±0.10%).

Table 2. Phytochemical screening of developed muffins

Treatments	TPC	TFC	DPPH
T_{A0}	3.50 ± 0.22^{f}	5.66 ± 0.28^{f}	4.860 ± 0.10^{f}
T_{A1}	29.57 ± 1.01^{e}	9.88 ± 0.19^{e}	12.87 ± 0.28^{e}
T_{A2}	301.85 ± 1.34^{d}	12.89 ± 0.16^{d}	18.41 ± 0.78^{d}
T_{A3}	403.98 ± 0.17^{c}	17.27 ± 0.17^{c}	26.94 ± 0.14^{c}
T_{A4}	483.49 ± 0.14^{b}	21.54 ± 0.14^{b}	34.60 ± 0.08^{b}
T_{A5}	564.13 ± 0.46^{a}	28.94 ± 0.16^{a}	41.82 ± 0.46^{a}

A significant decline ($p<0.05$) in specific volume and increase in bulk density and hardness with increasing guava leaves extract was evaluated in muffins. The control sample (TA0) exhibited the

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highest specific volume (3.71±0.01 cm^3/g) and the lowest bulk density (0.269±0.01 g/cm^3) and hardness (28.5±0.23 N). As the concentration of GLE increased, specific volume progressively decreased, reaching the lowest value in TA5 (1.51±0.21 cm^3/g), while bulk density and hardness increased, with TA5 showing the highest values (0.61±0.18 g/cm^3 and 34.11±0.10 N, respectively), as shown in Table 3.

Table 3. Physical characteristics of muffins

Treatment	Specific volume (cm^3/gm)	Bulk density (g/cm^3)	Hardness (N)
T_{A0}	3.71±0.01[a]	0.269±0.01[e]	28.5±0.23[e]
T_{A1}	3.65±0.13[b]	0.273±0.11[e]	28.9±0.07[e]
T_{A2}	3.45±0.02[c]	0.29±0.07[d]	29.35±0.14[d]
T_{A3}	2.15±0.10[d]	0.48±0.02[c]	31.35±0.08[c]
T_{A4}	1.72±0.06[e]	0.56±0.14[b]	32.66±0.17[b]
T_{A5}	1.51±0.21[f]	0.61±0.18[a]	34.11±0.10[a]

The incorporation of guava leaves extract in muffin production significantly ($p<0.05$) altered their color aspects through changes in L* (lightness) value along with a* (red-green) value and b* (yellow-blue) value and total color difference (ΔE), as shown in Table 4. The control sample (TA0) contained the brightest L* value of 39.787±0.11 along with maximum a* value of 11.70±0.31 and highest b* value of 14.29±0.24 and demonstrated the lowest ΔE score of 0.20±0.04 because it displayed minimal color deviation. The addition of GLE gradually made the product darker along with exhibiting a greener tinge particularly visible in TA5 where L* equaled 25.35±0.18 while a* equaled -4.25±0.48. An investigation of the b* parameter showed this value decreased to demonstrate

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lowered yellowness in the sample. The color difference (ΔE) showed substantial growth whereby TA3 demonstrated the greatest alteration (ΔE = 17.50±0.23) because GLE's phenolic compounds prompted extensive pigment interaction.

Table 4. Color characteristics of muffins

Samples	L*	a*	b*	ΔE
T A0	39.787±0.11^{a}	11.70±0.31^{a}	14.29±0.24^{a}	0.20±0.04^{e}
T A1	37.263±0.25^{b}	9.38±0.08^{b}	13.76±0.17^{b}	3.60±0.14^{d}
T A2	34.313±0.21^{c}	6.14±0.21^{c}	13.43±0.36^{c}	8.36±0.19^{c}
T A3	31.65±0.22^{d}	2.35±0.51^{d}	13.11±0.57^{c}	17.50±0.23^{a}
T A4	29.42±0.51^{e}	−2.72±0.11^{e}	11.88±0.31^{d}	13.44±0.15^{b}
T A5	25.35±0.18^{f}	−4.25±0.48^{f}	10.42±0.19^{e}	14.16±0.11^{b}

Muffins containing different concentration of guava leaves extract (GLE) underwent sensory assessments which displayed major ($p<0.05$) differences for appearance, taste, firmness, mouthfeel, aroma along with overall acceptability. The control sample (TA0) obtained high sensory scores across all features though firmness evaluations reached the highest point (7.65±0.24). Muffins prepared with 6% GLE extract (TA2) demonstrated the greatest sensory acceptance among the samples because its optimal phytochemical composition achieved high ratings for overall acceptability, taste and aroma without sensory degradation. The sensory ratings diminished with increasing GLE amounts greater than 9% (TA3-TA5) causing TA5 to receive the lowest scores for appearance at 5.55±0.57 and both taste and firmness scores at 5.20±0.84 and 5.40±0.75 respectively, as shown in Table 5.

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Table 5. Sensory properties

Treatments	Appearance	Taste	Firmness	Mouth feel	Aroma	Overall Acceptability
T_{A0}	6.66±0.36^{b}	6.75±0.98^{b}	7.65±0.24^{a}	6.75±0.91^{b}	6.60±0.87^{bc}	6.75±0.22^{b}
T_{A1}	6.30±0.14^{b}	6.00±0.47^{c}	7.20±0.67^{b}	6.30±0.77^{bc}	6.00±0.85^{cd}	6.40±0.85^{bc}
T_{A2}	7.65±0.81^{a}	7.65±0.59^{a}	6.90±0.24^{c}	7.60±0.23^{a}	7.55±0.17^{a}	7.70±0.25^{a}
T_{A3}	6.75±0.92^{b}	6.75±0.24^{b}	6.20±0.55^{d}	6.90±0.21^{ab}	6.75±0.28^{b}	6.85±0.11^{b}
T_{A4}	6.10±0.13^{bc}	5.50±0.65^{cd}	5.80±0.46d^{e}	5.70±0.19^{c}	5.8±0.69d^{e}	6.05±0.14^{c}
T_{A5}	5.55±0.57^{c}	5.20±084^{d}	5.40±75^{e}	5.60±0.85^{c}	5.30±0.72^{e}	5.50±0.68^{d}

Table 6 revealed peroxide value of muffins increased at each storage period as evidence of lipid oxidative reactions. The control group with TA0 developed the most prominent PV level of 8.76±0.21 mEq/kg on day 21 as a result of extensive oxidative damage. Muffins containing guava leaves extract at different concentrations showed decreasing peroxide values during storage because GLE acts as an antioxidant. The muffins containing 15% GLE in TA5 had the most reduced PV during storage which amounted to 1.94±0.10 mEq/kg by day 21 followed by a significant decrease ($p<0.05$) relative to the control. The antioxidant properties of GLE proved capable of postponing lipid peroxidation and both TA4 and TA3 demonstrated meaningful inhibitory effects. The research verifies that polyphenolic components found in guava leaves extract successfully reduce oxidative rancidity which extends muffin shelf life.

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Table 6. Effect of guava leaves extract on PV in muffins during storage

Peroxide value (mEq/kg)					
Treatments	Days				
	0	7	14	21	Means
T_{A0}	0.97±0.04^{p}	3.52±0.01^{g}	7.51±0.14^{b}	8.76±0.21^{a}	**5.19±0.08^{a}**
T_{A1}	0.97±0.10^{p}	2.53±0.12^{i}	4.18±0.08^{e}	5.95±0.14^{c}	**3.61±0.08^{b}**
T_{A2}	0.97±0.13^{p}	2.32±0.08^{l}	3.24±0.03^{h}	4.55±0.06^{d}	**2.84±0.05^{c}**
T_{A3}	0.96±0.02^{q}	2.20±0.02^{m}	3.02±0.12^{ij}	4.22±0.02^{e}	**2.65±0.06^{d}**
T_{A4}	0.96±0.11^{q}	1.85±0.06^{n}	2.97±0.01^{jk}	3.94±0.02^{f}	**2.43±0.04^{e}**
T_{A5}	0.96±0.01^{q}	1.02±0.13^{o}	2.81±0.13^{k}	2.97±0.01^{g}	**1.94±0.10^{f}**
Means	**0.965±0.06^{d}**	**2.52±0.12^{c}**	**3.80±0.09^{b}**	**4.98±0.09^{a}**	

During the 21-day storage period all muffin samples presented increasing TBA values because they served as indicators of secondary lipid oxidation. The TBA value of the TA0 control group reached a maximum measurement point (1.14±0.02 mg MDA/kg) while undergoing storage. The muffins with the addition of guava leaves extract (GLE) at 15% displayed the minimum TBA values which remained stable during storage (0.56±0.01 mg MDA/kg on day 21 and mean: 0.39±0.04 mg MDA/kg). Muffins containing lower amounts of GLE (TA3 and TA4) showed considerable inhibition of oxidative degradation relative to the control sample throughout the storage period, as shown in Table 7.

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Table 7. Effect of guava leaves extract on TBA in muffins during storage

Thiobarbituric value (mg MDA/Kg of sample)					
Treatments	Days				
	0	7	14	21	Means
T_{A0}	0.22±0.03^{k}	0.51±0.03^{gh}	0.89±0.07^{b}	1.14±0.02^{a}	**0.69±0.04^{a}**
T_{A1}	0.22±0.01^{k}	0.48±0.07^{h}	0.70±0.04^{d}	0.87±0.03^{b}	**0.56±0.04^{b}**
T_{A2}	0.21±0.04^{k}	0.39±0.02^{i}	0.62±0.05^{f}	0.75±0.16^{c}	**0.49±0.08^{c}**
T_{A3}	0.22±0.01^{k}	0.31±0.01^{j}	0.54±0.01^{g}	0.66±0.05^{e}	**0.43±0.02^{d}**
T_{A4}	0.21±0.01^{k}	0.31±0.03^{j}	0.51±0.03g^{h}	0.62±0.02^{f}	**0.41±0.02^{e}**
T_{A5}	0.21±0.03^{k}	0.30±0.10^{j}	0.49±0.02^{h}	0.56±0.01^{f}	**0.39±0.04^{f}**
Means	**0.21±02^{d}**	**0.38±0.03^{c}**	**0.62±0.05^{b}**	**0.77± 0.12^{a}**	

The moisture content in muffins demonstrated decreasing values during the 21-day storage duration because different treatments revealed significant differences. The initial moisture content of 16.90±0.15% across days was maintained by the TA0 control while all muffins containing GLE experienced a decline in moisture levels. Muffins containing 15% GLE (TA5) demonstrated the highest rate of water evaporation during storage since their moisture content decreased to 7.19±0.03% by day 21 (mean 11.58±0.18% during storage period). The decreasing moisture levels in every sample during storage are a result of both evaporation and starch retrogradation processes. Tests of GLE-enriched muffins indicate that the extract can decrease product moisture which may affect both texture and storage life of the product, as shown in Table 8.

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Table 8. Effect of guava leaves extract on moisture content of muffins during storage.

Moisture content (%)					
Treatments	Days				
	0	7	14	21	Means
T_{A0}	18.56±0.21^{a}	17.22±0.12^{b}	16.55±0.06^{c}	15.28±0.04^{d}	**16.90±0.15^{a}**
T_{A1}	18.34±0.02^{ab}	17.01±0.11^{bcd}	15.02±0.08^{d}	13.51±0.07^{f}	**15.96±0.12^{b}**
T_{A2}	18.25±0.08^{ab}	16.29±0.09^{cd}	14.50±0.22^{e}	13.19±0.14^{f}	**15.52±0.19^{c}**
T_{A3}	18.29±0.36^{ab}	15.52±0.05^{d}	13.01±0.31^{ef}	12.20±0.17^{g}	**14.77±0.22^{d}**
T_{A4}	18.21±0.19^{ab}	13.04±0.15^{e}	12.31±0.13^{g}	11.27±0.01^{i}	**13.70±0.12^{d}**
T_{A5}	18.12±0.08^{ab}	11.75±0.31^{h}	9.27±0.15^{j}	7.19±0.03^{k}	**11.58±0.18^{e}**
Means	**18.23±0.20^{a}**	**15.14±0.14^{b}**	**13.44±0.13^{c}**	**12.11±0.08^{d}**	

In a study by Offor (2015) the proximate analysis of guava leaves were evaluated in which the values of moisture, ash, fat and fiber were 10.74±0.08, 4.35±0.21, 1.37±0.36, 10.37±0.05% respectively(Offor, 2015). In another study by Goje et al. 2023 the aqueous leaves of guava were estimated and higher amounts of carbohydrates (82.58%), moderate amount of crude protein (2.55), fiber (2.43), ash (3.22), moisture content (8.52%), and lower fat (0.70%) were reported(GOJE et al., 2023). These findings are in line with the current study's results. Similarly, Jassal and Sonia (2019) reported the presence of carbohydrates (60.85%), fiber content (14.40%), moisture (8.67%), ash (3.80%) and low level of fat as (1.45%) in a significant amount. These findings supports that the developed product is a good option for substantial part of a diet that helps people avoid micronutrient deficiencies and for human nutrition(Adrian et al., 2015; Jassal & Kaushal, 2019).

The study by Biswas et al. 2013 confirmed tannins, phenols, flavonoids, terpenoids and glycosides in methanol and ethanol extracts without saponins while n-hexane extracts showed no component activity (Biswas et al., 2013). According to another study by Raj et al. 2020 the quantity of phytocompounds within each extract showed variation, and an evaluation of phytochemical quantity

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demonstrated that extracts prepared by methanol, ethanol, acetone, and aqueous extract contained greater numbers of phytocompounds (Raj et al., 2020).

Study by Zhu et al. 2009 support the findings of the present study which examined wheat starch properties after incorporating phytochemical extracts obtained from pomegranate peel as well as green tea and chinese hawthorn and chinese gall extracts(Zhu et al., 2009). The four extracts prolonged breakdown events by increasing the values but simultaneously reduced final viscosity amounts relative to the control condition. The peak viscosity reached maximum points before beginning its decline phase due to all extract solutions. Each extract decreased the gel stiffness and improved the stickiness of the gel. The addition of phytochemical extracts caused notable modifications to wheat starch characteristics according to their study findings(Zhu et al., 2009).

In addition, Alves and Perrone 2015 examined the process of adding guava leaves flour to bread. Bread dough fermentation actively modified the phenolic substance presence according to their study(Alves & Perrone, 2015). The melanoidin amounts increased across baking time from 24.1 mg/g to 71.9 mg/g while their molecular weight went down momentarily before rising. Bread production with wheat flour and 10 or 20 percent guava leaves flour enhanced phenolic compounds by 240 percent. The study showed the phenolic content in control bread was 3.75 mg/gm GAE and reached 71.5 mg/gm and 85.3 mg/gm in GB10 and GB20 by incorporation of guava leaves extract(Alves & Perrone, 2015).

Moczkowska et al. 2022 report similar results that indicate the greatest extract addition in formulations generated the highest TPC values between 24.5 and 67.19 mg/gm GAE to 70.29 mg/gm GAE for MEx and PEx (Moczkowska-Wyrwisz et al., 2022). Khalifa et al. 2016 conducted a study substituting wheat flour with guava leaves and seeds pomace at 5-20% levels in cupcake production which showed changes to cupcake physical properties. The wheat flour cupcake delivered the largest results through volume measurements which reached 108.00 and 2.62 cm3/g in specific volume. The addition of higher levels of guava seeds and leaves flour decreased specific volume because the flour absorbed extra water resulting in increased weight (Khalifa et al., 2016). This

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increase in individual levels caused both reduced volume and specific volume because these amounts interfered with the low-strength gluten net and gas retention properties. Furthermore, Arslan et al. 2017 revealed that bread texture became softer when the amount of added guava pulp powder decreased. The bread hardness steadily increased from 2.40N to 2.73N when the GPP addition levels increased to 5%. The dietary fiber present in guava pulp powder holds onto available water thus making bread harder according to their findings. Bread becomes harder as the use of guava pulp powder in higher amounts reduces the bread's volume. The CO2 retention may decrease because methylcellulose and starch matrix becomes weakened when tested for this impact (Arslan et al., 2017).

Moreover, Khan et al. 2022 developed sugar free muffins to assess their influence on overall muffin height along with textural characteristics. The muffins containing sugar demonstrated softer texture than the muffins made without sugar. The results indicated that switching to sugar-free muffins increased their hardness between 224 to 562g possibly because the replacement disturbed how water moves during preparation and modified both gluten hydration and gelatinization temperatures(Khan et al., 2022). The results of present study support the findings presented by Mitharwal et al. 2022 regarding how cinnamon and clove additions affected lemongrass muffins' color properties. Addition of cinnamon and clove resulted in darkening (58.4) of the muffins while turning them more red (3.5) with lower lightness (73.5 to 58.4) (Mitharwal & Chauhan, 2022).Zahidah et al. 2011 conducted research very similar to current findings where they evaluated pink guava leaves extract for its antioxidant potential when used in cookies. The PV and TBA values showed continuous increase during storage without reaching the control values(Zahidah et al., 2011). The present research shows that guava leaf extract functions well as a nutritious ingredient in baked goods by boosting antioxidant qualities and nutritional value and increasing shelf stability. The addition of aqueous ethanolic extract boosted muffin nutritive content through increased phenolic and flavonoid levels and enhanced their oxidative resistance. Properties of wheat-barley composite flour improved both protein and fiber quantities to make the end product more nutritious. The

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sensory results showed a 6% guava leaf extract concentration achieved the most optimal point which balanced health advantages with consumer approval. The future research should examine extraction techniques that preserve bioactive compounds, study plant extracts beyond guava leaves in bakery products and understand product stability when stored at various temperatures. The exploration of gut microbiota modifications by guava leaf extract together with its metabolic health contributions will enhance scientific understanding about functional food uses of this extract.

Conclusion

In conclusion, the impact of incorporating guava leaves extract into muffins were examined in this study. The results highlighted it as a functional ingredient due to its antioxidant properties, bioactive compounds, capacity to improve oxidative stability, and nutritional benefits. The phytochemical composition of muffins and lipid stability during storage was enriched by incorporation of guava leaves extract. However, higher concentrations of guava leaf extract negatively influenced textural properties and moisture content together with consumer satisfaction. The combination of wheat-barley flour in baking improved product nutrition values. Further, the study indicates the effective use of guava leaves extract in functional foods but future research is necessary to improve its application in bakery products.

Conflict of Interest

The author(s) declare no conflict of interest.

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