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Kinetic Analysis of Extraction Processes and Characterization of Nutrient Composition, Heavy Metals, and Caffeine Content in Malaysian Guarana Seeds (*Paullinia cupana*)

(Analisis Kinetik Proses Pengekstrakan dan Pencirian Komposisi Nutrien, Logam Berat dan Kandungan Kafein dalam Biji Guarana Malaysia (*Paullinia cupana*))

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ABSTRACT

The demand for bioactive compounds derived from plants has been steadily increasing. Alongside traditional solid-liquid extraction, ultrasonic extraction has emerged as a technique to enhance the recovery of bioactive compounds. Guarana seeds grown in Malaysia may exhibit distinct nutrient profiles compared to those from other regions due to environmental factors such as location, temperature, and climate. This study aims to determine the exhaustive extraction time for conventional and ultrasonic methods by evaluating the total phenolic content of guarana seeds using Peleg's mathematical model and Solver. Additionally, proximate analysis, heavy metal analysis (via ICP-MS), and caffeine content determination (via HPLC) were performed. The results indicate that ultrasonic extraction significantly reduced extraction time (1.30 ± 0.027 h) compared to conventional extraction (35.07 ± 8.036 h) (p<0.05). Although this shorter extraction time enhances processing efficiency, it was associated with slightly lower yields of certain bioactive compounds such as caffeine. However, shorter extraction times resulted in a lower reaction rate and reduced yield. Proximate analysis showed that guarana seed extract is a poor source of protein (0.6%) but contains high moisture content (97.9%). The extract also showed low levels of ash (<0.1%), fat (<0.1%), and carbohydrates (1.5%). ICP-MS analysis confirmed that the concentrations of heavy metals, including arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), and antimony (Sb), were within the safe limits set by the Ministry of Health Malaysia (MOH) (1 mg/kg for As, 2 mg/kg for Pb, 0.05 mg/kg for Hg, 1 mg/kg for Cd, and 1 mg/kg for Sb). Specifically, conventional extraction yielded 0.456 mg/kg As, 0.046 mg/kg Pb, 0.043 mg/kg Hg, 0.010 mg/kg Cd, and 0.005 mg/kg Sb, while ultrasonic extraction yielded 0.369 mg/kg As, 0.026 mg/kg Pb, 0.022 mg/kg Hg, 0.006 mg/kg Cd, and 0.003 mg/kg Sb. Caffeine content was 23% higher in extracts obtained using the conventional method (4.75 ± 0.001% w/w) compared to ultrasonic extraction (3.65 ± 0.002% w/w) (p<0.05) in powdery dried seed. Despite this, ultrasonic extraction was identified as the more efficient method due to its shorter extraction time and sufficient yield of bioactive compounds, supporting its use for future guarana-based applications. Overall, guarana seeds are a safe and nutrient-rich source with potential applications in the food industry for the commercial development of guarana-based products.

Keywords: Caffeine; guarana; maceration; Peleg model; proximate analysis; ultrasonic extraction

ABSTRAK

Permintaan terhadap sebatian bioaktif yang diperoleh daripada tumbuhan semakin meningkat. Selain kaedah pengekstrakan pepejal-cecair tradisional, pengekstrakan ultrasonik telah muncul sebagai teknik yang berkesan untuk meningkatkan pengeluaran sebatian bioaktif. Biji guarana yang ditanam di Malaysia mungkin mempunyai profil nutrien yang berbeza berbanding biji dari kawasan lain disebabkan oleh faktor persekitaran seperti lokasi, suhu, dan iklim. Kajian ini bertujuan menentukan masa pengekstrakan menyeluruh untuk kaedah konvensional dan ultrasonik dengan menilai kandungan fenol keseluruhan dalam biji guarana menggunakan model matematik Peleg dan Solver. Analisis lain yang turut dijalankan termasuk analisis proksimat, analisis logam berat (menggunakan kaedah ICP-MS) dan penentuan kandungan kafein (menggunakan kaedah HPLC). Keputusan menunjukkan bahawa pengekstrakan ultrasonik mengurangkan masa pengekstrakan dengan ketara (1.30 ± 0.027 jam) berbanding pengekstrakan konvensional (35.07 ± 8.036 jam) (p<0.05). Walau bagaimanapun, masa pengekstrakan yang lebih pendek menyebabkan kadar tindak balas lebih rendah dan hasil yang berkurangan. Analisis proksimat mendedahkan bahawa ekstrak biji guarana adalah sumber protein yang rendah (0.6%) tetapi mengandungi kandungan lembapan yang tinggi (97.9%). Ekstrak ini juga menunjukkan kandungan abu (<0.1%), lemak (<0.1%), dan karbohidrat (1.5%) yang rendah. Analisis ICP-MS mengesahkan bahawa kepekatan logam berat seperti arsenik (As), merkuri (Hg), plumbum (Pb), kadmium (Cd) dan antimoni (Sb) berada dalam had selamat yang

ditetapkan oleh Kementerian Kesihatan Malaysia (KKM) (1 mg/kg untuk As, 2 mg/kg untuk Pb, 0.05 mg/kg untuk Hg, 1 mg/kg untuk Cd dan 1 mg/kg untuk Sb). Secara khusus, pengekstrakan konvensional menghasilkan 0.456 mg/kg As, 0.046 mg/kg Pb, 0.043 mg/kg Hg, 0.010 mg/kg Cd dan 0.005 mg/kg Sb, manakala pengekstrakan ultrasonik menghasilkan 0.369 mg/kg As, 0.026 mg/kg Pb, 0.022 mg/kg Hg, 0.006 mg/kg Cd dan 0.003 mg/kg Sb. Kandungan kafein adalah 23% lebih tinggi dalam ekstrak yang diperoleh melalui kaedah konvensional (4.75 ± 0.001% w/w) berbanding kaedah ultrasonik (3.65 ± 0.002% w/w) (p<0.05) di dalam serbuk kering biji. Walau bagaimanapun, pengekstrakan ultrasonik tetap memberikan kelebihan kerana gelombang ultrasonik yang mengganggu dinding sel mempercepatkan proses pengekstrakan sebatian bioaktif daripada biji guarana. Secara keseluruhannya, biji guarana adalah sumber yang selamat dan kaya dengan nutrien serta berpotensi untuk diaplikasikan dalam industri makanan bagi pembangunan produk guarana secara komersial.

Kata kunci: Analisis proksimat; guarana; kafein; model Peleg; pengekstrakan ultrabunyi; proses perendaman

INTRODUCTION

Guarana (*Paullinia cupana*) is renowned for its stimulating properties, primarily due to its high caffeine content. The seeds of the guarana fruit are the most commercially valuable part of the plant, widely utilized for their bioactive properties. On average, guarana provides approximately 50 mg of caffeine per gram, although the actual concentration can vary depending on the preparation method. The physiological effects of guarana consumption closely mirror those of caffeine, including enhanced alertness and energy levels (Marques et al. 2019). Beyond its stimulant effects, guarana has been associated with numerous health benefits, such as improved cognitive performance and anti-cancer properties (Cadona et al. 2016; White et al. 2017).

To harness the bioactive compounds in guarana seeds, an effective extraction process is essential. Key stages in the isolation of bioactive compounds from plant materials include sample preparation, extraction, and purification. Choosing an appropriate extraction method is critical as the sample preparation stage can consume more than 60% of the total processing time. Moreover, the extraction method significantly impacts the quality of the extract and the retention of target compounds (Yahya, Attan & Wahab 2018).

Hot water extraction has traditionally been the most commonly employed technique for extracting bioactive compounds. However, it has notable limitations, such as prolonged extraction times and the need for high temperatures, which can adversely affect the physicochemical properties of bioactive compounds. Prolonged exposure to heat often leads to the degradation of these compounds, thereby reducing their biological activity (Shang et al. 2019). To address these challenges, innovative extraction techniques have been developed, including ultrasound-assisted extraction (UAE).

Ultrasound-assisted extraction has gained attention for its efficiency and eco-friendliness. This technique generates cavitation effects through ultrasonic waves, where the collapse of microscopic bubbles releases significant energy, facilitating the breakdown of plant cell walls and enhancing the extraction of bioactive compounds. Despite its advantages, UAE has limitations, including challenges in maintaining precise temperature control during the extraction process (Li & Wang 2016).

In this study, guarana seeds will be subjected to a systematic process of drying and grinding into powder for detailed analysis. Total phenolic content will be evaluated using the Folin-Ciocalteu method, while high-performance liquid chromatography (HPLC) will be employed to determine caffeine content. The content of heavy metals in guarana extracts will be analyzed using the inductively coupled plasma-mass spectrometry (ICP-MS) method. Additionally, the nutrient composition of guarana seeds will be assessed through proximate analysis based on the AOAC standards (2000).

This study holds significant importance as it not only elucidates the nutrient composition of guarana seeds but also expands the understanding of their bioactive properties. Furthermore, it aims to contribute valuable insights into the potential applications of guarana seeds in various industries, particularly the food industry. By improving extraction methods and understanding the composition of guarana, this research could support the development of guarana-based products with enhanced functional and nutritional benefits.

MATERIALS AND METHODS

MATERIALS AND REAGENTS

The guarana seeds used in this study were sourced from guarana farms located in Berapit, Penang (GPS: 5.39774, 100.19416) and Simpang Renggam, Kluang, Johor (GPS: 1.830432, 103.304278). The chemicals utilized in the study included ethanol, petroleum ether, boric acid, potassium sulfate, and copper sulfate, which were procured from ChemAR System. Sulfuric acid and hydrochloric acid were obtained from Sigma-Aldrich, USA. All chemicals used were of analytical grade, ensuring reliability and accuracy in the experimental procedures.

SAMPLE PREPARATION

The guarana seeds were crushed as a pre-preparation step prior to extraction. Initially, a pestle and mortar were used to break the seeds into fragments smaller than 10 mm. The crushed seeds were then placed on a tray and dried in an oven at 60 °C for 24 h, with slight modifications to the method

described by Zubairi et al. (2014). The guarana seeds were initially crushed manually using a pestle and mortar into fragments smaller than 10 mm, instead of mechanical crushing, to prevent excessive heat generation that could degrade thermolabile bioactive compounds. Following crushing, the seeds were dried at a lower temperature of 60 °C for 24 h (modified from Zubairi et al. 2014, who used 80 °C for 48 h) to minimize the loss of volatile and phenolic compounds while ensuring sufficient moisture removal. These adjustments were made to optimize the retention of bioactive compounds and improve the uniformity of seed particle size prior to extraction (Zubairi et al. 2014). After drying, the seeds were further ground using an electric grinder (Waring J-SPEC 7011BUJ) at low speed for 1 min to produce a fine powder with a particle size of less than 1 mm. The resulting powder was stored in an airtight container at 4 °C until the extraction process was performed.

CONVENTIONAL EXTRACTION (MACERATION)

A total of 30 g of guarana seed powder was added to a 1 L beaker containing 300 mL of distilled water, maintaining a solid-to-solvent ratio of 1:20 (w/v). Prior to the extraction process, the sample was wrapped in muslin cloth (10 cm × 15 cm) to prevent the powder from mixing with the extract, following the method outlined by Othman, Hasan and Zubairi (2017) and Zubairi et al. (2014). The beaker containing the wrapped guarana seed sample was placed in a water bath set at 60 °C, and the extraction process was conducted for 12 h. At 1-h intervals, 2 mL of extract was collected using a micropipette, and this step was repeated for up to 12 h. Each experiment was performed in triplicate (n = 3) to ensure reproducibility. The maximum total phenolic content of the extract and the time required for exhaustive extraction were determined using the Peleg and Excel Solver model.

ULTRASONIC EXTRACTION

A total of 30 g of the sample was weighed and placed into a 1 L beaker containing 600 mL of distilled water. Prior to the extraction process, the sample was wrapped in muslin cloth (10 cm × 15 cm) to prevent the powder from mixing with the extract, following the method described by Zubairi et al. (2014). The extraction was carried out in an ultrasonic bath with a frequency of 42 kHz and a power of 250 W for 60 min, as per the methods outlined by Ahmad et al. (2021) and Salamatullah et al. (2021). These parameters were selected based on prior studies that successfully optimized ultrasound-assisted extraction of phenolic compounds and caffeine from plant materials (Ahmad et al. 2021; Salamatullah et al. 2021). A frequency of 42 kHz was chosen to ensure effective cavitation without causing excessive thermal degradation of sensitive bioactive compounds. The extraction time of 60 min was established to achieve an optimal balance between extraction efficiency and compound stability. Maintaining the bath temperature at 22 °C was important to protect thermo-labile compounds, as higher temperatures could lead to degradation. This approach aimed to maximize the yield of phenolic compounds and caffeine while preserving the integrity of the extract. During the extraction, 2 mL of extract was collected at 5-min intervals using a micropipette, and this process was repeated until the 60-min mark. Each experiment was performed in triplicate (n = 3) to ensure the reliability of the results. The maximum total phenolic content of the extract and the time required for exhaustive extraction were determined using the Peleg model and Excel Solver.

EXTRACTION PELEG MODEL

The Peleg model is used to determine the maximum concentration and the time required for exhaustive extraction. This model was selected due to its high accuracy, as reported by Ocieczek and Zieba (2020). The Peleg model incorporates two constants, K1 and K2. K1 represents the y-intercept value, while K2 corresponds to the slope of the linear graph. The extraction rate (1/K1), maximum concentration (1/K2), and the time for exhaustive extraction (K1/K2) can be accurately determined through Peleg model calculations. The kinetic curve in the Peleg model mirrors the shape of the absorption curve (Rathod, Keerthiga & Gharat 2021). Therefore, the concept of the Peleg model in the solid-liquid extraction process is expressed through Equation (1).

$$C(t) = C_0 + t/[K_1 + K_2 . t] \quad (1)$$

where C is the concentration of total phenolic content (mg GAE/g powder) and C_0 is the total phenolic content at the time t = 0 (mg GAE/g powder). The unit for K_1 is hrs.g/mg while the unit for K_2 is g/mg. At the time t = 0, the concentration of the extract (C_0) is considered to be 0. Therefore, after mathematical linearization, Equation (2) appears as follows.

$$t/[C(t) - C_0] = K_1 + K_2 . t \quad (2)$$

K_1 is related to the extraction rate (B_0) at a given time as shown in Equation (3).

$$B_0 = 1/K_1 \quad (3)$$

K_2 is related to the maximum concentration of extract. Equation (4) shows the relationship between the equilibrium concentration of extract and the constant K_2 at the time $t \rightarrow \infty$.

$$C_{t \rightarrow \infty} = C_e = 1/K_2 \quad (4)$$

EXTRACTION EXCEL SOLVER MODEL

Microsoft Excel, with the aid of the Solver tool, is used to estimate the optimal values of parameters. Solver has been widely applied in various fields to find the maximum or

minimum value of one cell by adjusting another. The data fitting approach utilizing Solver follows the steps outlined by Elkholy and El-Ela (2019): (i) The sum of squared residuals (SSR) between the actual data and predicted data is computed using Excel, (ii) Excel Solver is employed to minimize SSR, thereby obtaining the optimal parameter values simultaneously.

Solver is a highly effective tool for solving complex optimization problems, with the main advantage of being user-friendly and time efficient. It provides a reliable and fast method to determine the optimal value of any variable. Solver delivers results that are comparable to those of other models, but with less time and effort. Unlike the Peleg model, Solver can determine the most effective solutions to achieve the study's objectives, such as minimizing SSR to meet the desired goals (Karmaker 2017).

TOTAL PHENOLIC CONTENT ANALYSIS

Phenolic content was determined using the Folin-Ciocalteu method, as described by Skerget et al. (2004). The oxidizing agent used in this analysis was the Folin-Ciocalteu reagent (AOCS 1990). A 0.5 mL aliquot of the diluted extract (25 mg in 10 mL of distilled water) was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and 2 mL of sodium carbonate (Na_2CO_3) solution (75 g/L). The mixture was incubated at 50 °C for 5 min, then cooled. For the control sample, 0.5 mL of distilled water was used. Absorbance was measured at 760 nm using a UV spectrophotometer (Varian-UV-VIS spectrophotometer). The phenolic content was calculated using Equation (5) and expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g extract).

$$\text{Total phenolic content (mg GAE/g)} = \frac{R \times DF \times TV}{SV \times wt \times 1{,}000{,}000} \quad (5)$$

where R is the reading from standard curve (mg/mL); DF is the dilution factor; TV is the total volume (volume of extract made); SV is the sample volume (volume of extract used for analysis); and wt is the sample weight (g).

PROXIMATE ANALYSIS

Proximate analysis was conducted on guarana seed extracts obtained from the conventional maceration extraction method. Therefore, proximate composition data are only available for the conventional extraction method. Ultrasonic extraction samples were reserved for the optimization and analysis of bioactive compound yield (phenolic content and caffeine), in line with the primary objectives of this study. The extracts prepared using ultrasonic extraction were primarily intended for bioactive compound analysis and were not subjected to proximate profiling. In this study, the proximate analysis of guarana seeds was conducted following the standard methods outlined by AOAC (2000). The components identified in this analysis include moisture, ash, protein, and fat content.

MOISTURE CONTENT ANALYSIS

The moisture content of the sample is determined by measuring the weight difference before and after dehydration. The weight after dehydration is considered as the dry matter. This is achieved using the oven drying method. A 2 g sample was weighed and dried overnight in an oven at 105 °C until a constant weight was reached. The sample was then placed in a desiccator to cool completely before being reweighed. The moisture content is calculated using Equation (6).

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1} \times 100\% \quad (6)$$

where W1 is the sample weight before drying (g); and W2 is the sample weight after drying (g).

ASH CONTENT ANALYSIS

A crucible containing 3 g of the sample was first burned until the sample turns black and ceases to produce white fumes. The sample was then placed in a muffle furnace and heated overnight at 550 °C. After cooling in a desiccator, the sample was weighed (AOAC 2000). The ash content is determined using Equation (7).

$$\text{Total ash (\%)} = \frac{\text{Weight of crucible and ash (g)} - \text{Weight of crucible (g)}}{\text{Weight of sample (g)}} \times 100\% \quad (7)$$

FAT CONTENT ANALYSIS

The fat content was determined using Soxhlet extraction with petroleum ether. Sample (2 g) was placed in a thimble, and 70 mL of petroleum ether was added to the extraction cup. The extraction process was performed using the Soxtec HT6 System. After extraction, the cup was dried in an oven for 15 min, then transferred to a desiccator to cool before weighing (AOAC 2000). The fat content is determined using Equation (8).

$$\text{Fat content (\%)} = \frac{(\text{Weight of cup + fat (g)}) - \text{Weight of cup (g)}}{\text{Weight of sample (g)}} \times 100\% \quad (8)$$

PROTEIN CONTENT ANALYSIS

The Kjeldahl method, widely used and approved by AOAC (2000), is the most commonly employed technique for determining nitrogen content due to its high accuracy and good repeatability. The protein content is calculated using a conversion factor of 6.25. To begin, 7 g of potassium sulfate and 2 g of copper sulfate were added to a digestive tube containing 2 g of the sample. Subsequently, 12 mL of concentrated sulfuric acid was added, and the mixture was heated in the Tecator Digestive System for 1 h until the solution turns greenish-blue. Afterward, 75 mL of distilled

water was added to the digestive tube before distillation, and 25 mL of boric acid was used as a receiver. The distilled solution was titrated with 0.2 M hydrochloric acid until it turns pink. The protein content is then determined using Equation (9).

$$\text{Nitrogen content (\%)} = \frac{\text{HCl used (sample} - \text{blank)} \times \text{N HCl} \times 14.007}{\text{Weight of sample (g)}} \times \frac{100}{1000} \quad (9)$$

$$\text{Protein content (\%)} = \text{Nitrogen content (\%)} \times 6.25$$

where 6.25 is the nitrogen conversion factor for all foods except cereals, legumes, milk and dairy products.

CARBOHYDRATE CONTENT ANALYSIS

The carbohydrate content is calculated by subtracting the moisture, ash, fat, and protein contents from 100%. The carbohydrate content is determined using Equation (10).

$$\text{Carbohydrate content (\%)} = 100\% - \text{\% (Moisture + Ash + Protein + Fat)} \quad (10)$$

HEAVY METALS ANALYSIS

The heavy metals content was analyzed using the ICP-MS method, as described by Lewen et al. (2004). A sample solution with a concentration of 1 mg/mL was prepared by weighing 25 mg of the sample into a 25 mL volumetric flask, adding 25 ng/mL each of cobalt (Co), gold (Au), and rhodium (Rh) as internal standards, and diluting with a 2-butoxyethanol solution (25:75). Spiked sample solutions were prepared at a concentration of 10 µg/g for each analyte element. After calibrating the ICP-MS system, the sample solution was analyzed for arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg), and antimony (Sb). Standard solutions were tested after every fifth sample, and their concentration should be within ± 20% of the theoretical value; otherwise, the instrument was recalibrated.

CAFFEINE CONTENT ANALYSIS

Caffeine content in the guarana seed extract was determined using high-performance liquid chromatography (HPLC, Agilent 1100, Germany), following the method outlined by Majhenič, Škerget and Knez (2007). A 20 mg the extracts was dissolved in 10 mL of distilled water and filtered through a Teflon membrane filter with a pore size of 0.45 µm. The aqueous extract (20 µL) was then injected into an Eclipse XDB-C18 column (5 µm pore size, 4.6 mm × 150 mm). Two mobile phase components were used: solvent A, prepared by adding 10 mL of acetonitrile to 1 L of 5% acetic acid, and solvent B, which was acetonitrile. The analysis was conducted under gradient elution conditions, with a flow rate of 1 mL/min. The elution profile was as follows: 0-20 min, 0-14% B in A; 20-28 min, 14-40% B in A; 28-30 min, 40-50% B in A; 30-35 min, 50-0% B in A. Caffeine was detected at 280 nm, and its concentration in the extract was determined using a calibration curve generated with analytical-grade caffeine (98% purity). The yield of caffeine in the extract was calculated using Equation (11).

$$\text{\% Caffeine} = \frac{\text{Caffeine content (g)}}{\text{Weight of extract (g)}} \times 100\% \quad (11)$$

STATISTICAL ANALYSIS

All analyses were performed in triplicate (n = 3) and the results are presented as mean ± standard deviation. An independent sample t-test was conducted to assess the difference in mean extraction times between conventional extraction and ultrasonic extraction. A 95% confidence interval (p<0.05) was used to determine statistical significance. Data were analyzed using Minitab software version 19.1 (Minitab Pty Ltd, Sydney).

RESULTS AND DISCUSSION

KINETIC ANALYSIS OF GUARANA SEEDS

The results of the independent t-test analysis showed a significant difference (p<0.05) in the maximum total phenolic content of guarana seed extracts between conventional and ultrasonic extraction methods. Figures 1 and 2 demonstrate that the total phenolic content (mg GAE/g) of the extract increases with prolonged extraction time. The determination of total phenolic content and the maximum extraction time were obtained through the Peleg mathematical model (Figures 3 & 4).

Table 1 presents the kinetic parameters of the experimental data and the predicted Peleg model for guarana seed extraction. The K1 value calculated from the experimental data (60.56 ± 7.128 hr.g/mg) was not significantly different (p>0.05) from the K1 value predicted by the Peleg model (58.59 ± 11.109 hr.g/mg). Similarly, the K2 values from the experimental data (16.21 ± 0.375 g/mg) and the Peleg model (16.28 ± 0.958 g/mg) did not differ significantly (p>0.05). Furthermore, the time for exhaustive extraction, derived from the experimental data (40.00 ± 5.495 h), did not show a significant difference (p>0.05) compared to the predicted Peleg model value (35.07 ± 8.036 h).

In contrast, all K1, K2 values, and exhaustive extraction times for ultrasonic extraction showed significant differences (p<0.05) between the experimental data and the predicted Peleg model. The K1 value from the Peleg model (2.85 ± 11.968 hr.g/mg) was significantly lower (p<0.05) than the experimental data (4.75 ± 18.053 hr.g/mg). The K2 value from the experimental data was 30.09 ± 1.059 g/mg, while the Peleg model predicted a value of 33.70 ± 1.205 g/mg. The exhaustive extraction time calculated from the experimental data was 2.68 ± 0.110 h, while the Peleg model predicted 1.30 ± 0.027 h.

The Solver method produced faster exhaustive extraction times than the experimental data for both extraction methods. The Solver predictions were chosen due to their high accuracy in predicting optimal data. One advantage of using Solver is its ability to quickly set up and run without the need for tuning control or behavioral metrics (Ghorbani et al. 2018). Moreover, the kinetic graphs of the Peleg model predictions showed a high R^2 value of 0.999 for conventional extraction and 0.967 for ultrasonic extraction, whereas the experimental data yielded lower R^2 values of 0.724 and 0.715 for conventional and ultrasonic extractions, respectively. R^2 values indicate the degree of accuracy in fitting the results to the expected regression line, with higher R^2 values reflecting more accurate results (Roy et al. 2017).

Lower K1 and K2 values indicate a faster extraction rate and a higher maximum concentration at equilibrium, respectively (Patil et al. 2021). When comparing the Peleg model predictions for guarana seed extraction, conventional extraction showed a significantly higher K1 value (58.59 ± 11.109 hr.g/mg) compared to ultrasonic extraction (2.85 ± 11.968 hr.g/mg), although the K2 value for conventional extraction (16.28 ± 0.958 g/mg) was significantly lower (p<0.05) than that for ultrasonic extraction (33.70 ± 1.205 g/mg). This discrepancy is due to the saturation of the liquid phase as the concentration of the extract increases, which reduces the extraction rate (Yang et al. 2019). Higher extract concentrations lead to slower extraction rates of guarana seeds into the solvent (Zubairi et al. 2014). On the other hand, ultrasonic extraction significantly increases the extraction rate by reducing the activation energy, enthalpy, Gibbs free energy, and activation entropy (Huang et al. 2017).

This phenomenon is clearly reflected in the longer extraction time required for conventional extraction (35.07 ± 8.036 h) compared to ultrasonic extraction (1.30 ± 0.027 h) (p<0.05). Ultrasonic waves induce changes in the internal structure of the seed matrix (Rahaman et al. 2019), facilitating the release of bioactive compounds after disrupting the cell walls (Hadidi, Ibarz & Pagan 2020). Consequently, ultrasonic extraction generally requires shorter extraction times, resulting in higher yields and minimizing the degradation of thermo-sensitive compounds (Fu et al. 2021). However, it is important to note that while extraction time was reduced, ultrasonic extraction yielded slightly lower concentrations of caffeine, suggesting that extraction goals (e.g., maximizing time efficiency versus maximizing compound yield) should guide the choice of method).

The K1 and K2 values observed in this study were notably higher than those reported in other studies, indicating a slower extraction rate and lower maximum concentration. For example, in Patil et al. (2021), K1 values ranged from 0.0006 to 0.0156 min.g/mg GAE, and K2 values ranged from 0.0108 to 0.0162 g/mg GAE for curry leaf extraction. These differences are likely due to variations in solvent selection, as alcohol-water mixtures are found to be more effective for extracting phenolic compounds than single-component solvents (Belwal et al. 2016). Similarly, in the study by Xavier, Freire and González-Álvarez (2019), the use of ethanol at 75 °C for phenolic extraction from lignocellulose subproducts resulted in high K1 and K2 values (0.154 min.g/mg GAE and 0.048 g/mg GAE), which can be attributed to the high solubility of ethanol and the enhanced diffusivity at higher temperatures.

Plant extraction is the process of isolating soluble compounds from plant materials using solvents. While conventional extraction uses solvents to soften plant materials, thus, releasing bioactive compounds (Wang et al. 2019), it is often time-consuming. Ultrasonic extraction, on the other hand, employs ultrasound energy to generate cavitation bubbles, which create mechanical and thermal effects on plant cells, leading to cell wall disruption and the release of bioactive compounds into the solvent medium through diffusion (Bi et al. 2019). Ultrasound frequencies above 20 kHz are commonly used to extract bioactive compounds from plants (Qian et al. 2020). Studies, such as Ampofo and Ngadi (2020), have shown that ultrasonic energy (360 W, 40 kHz) significantly enhances the biosynthesis of phenolic compounds over a 60-min extraction period.

Many researchers prefer ultrasonic energy for the extraction of phenolic compounds, which are secondary metabolites produced through the shikimate or propanoic acid pathways (Faujan, Baker & Zubairi 2023; Sim, Ong & Nyam 2019). Ultrasonic extraction enhances chemical reactions and absorption, provided the ultrasound intensity is correctly matched with other processing variables to optimize the results (Yusoff et al. 2022).

The non-exponential Peleg model is widely used to model extraction yields and optimal extraction times, owing to its simplicity and effectiveness in describing the kinetics of bioactive compound extraction (Aguiló-Aguayo et al. 2017). According to Milićević et al. (2021), the Peleg model has been successfully adapted to describe solid-liquid extraction of various plant metabolites due to its similarity to the absorption curve in extraction processes. By incorporating the Solver add-in, the data becomes more accurate, as Solver minimizes the sum of squared residuals (SSR) (Karmaker 2017).

In conclusion, the Peleg model, combined with Solver, offers a reliable method for understanding extraction kinetics and operational parameters. The optimal time for exhaustive extraction was determined to be 1.30 h, with ultrasonic extraction being more efficient than conventional extraction due to its reduced extraction time and higher yield (Nie et al. 2021). It is an effective method for accelerating chemical reactions (Ojha et al. 2019). Considering both extraction efficiency and processing time, ultrasonic extraction presents a superior method for future applications. Despite yielding a slightly lower caffeine

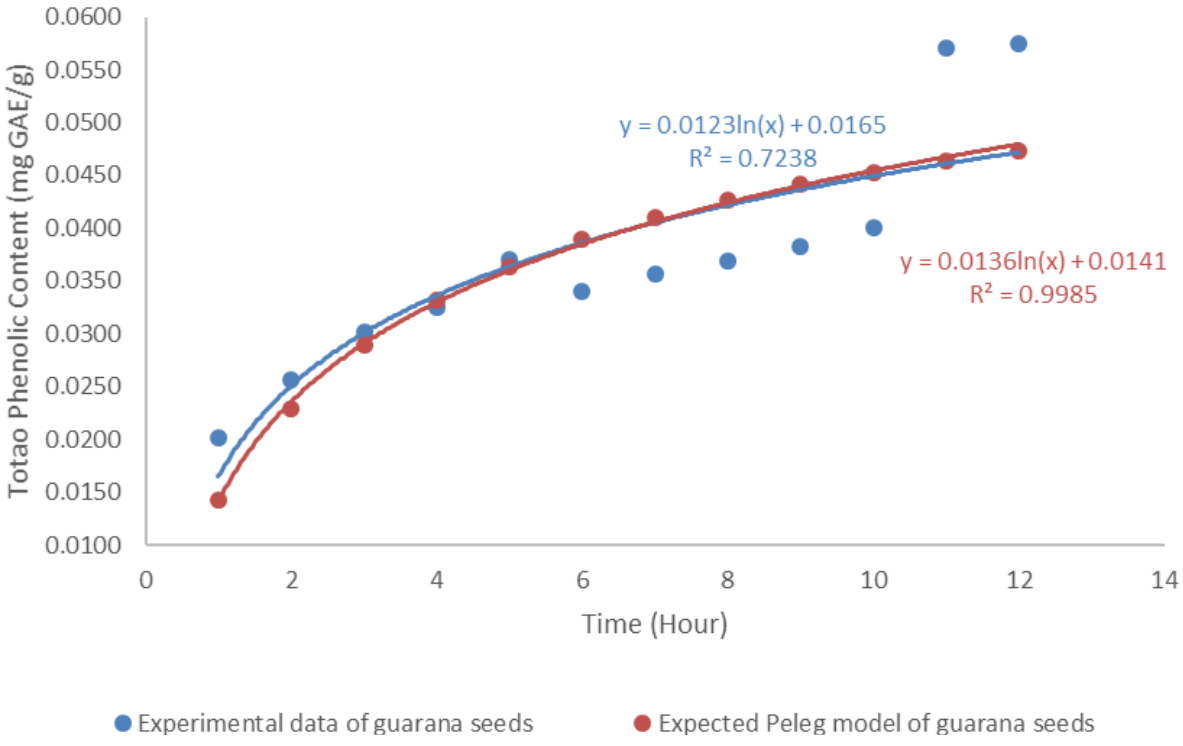


FIGURE 1. Experimental data and expected Peleg model of conventional extraction of guarana seeds

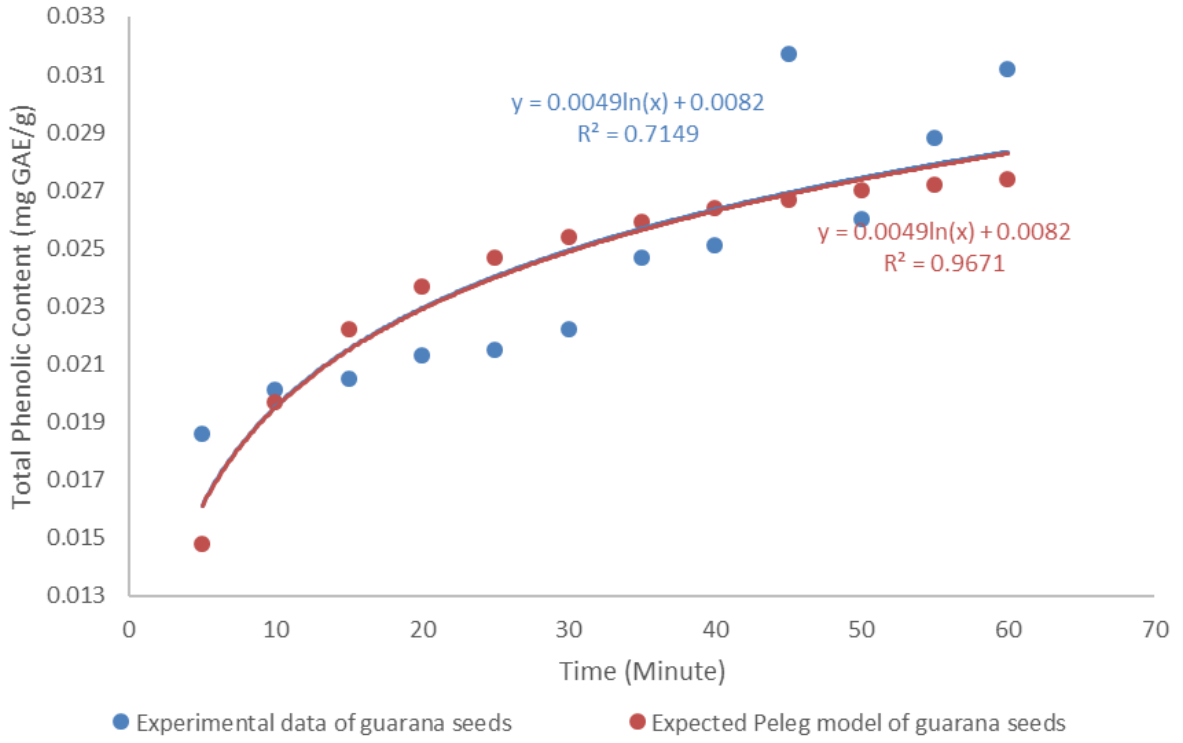


FIGURE 2. Experimental data and expected Peleg model of ultrasonic extraction of guarana seeds

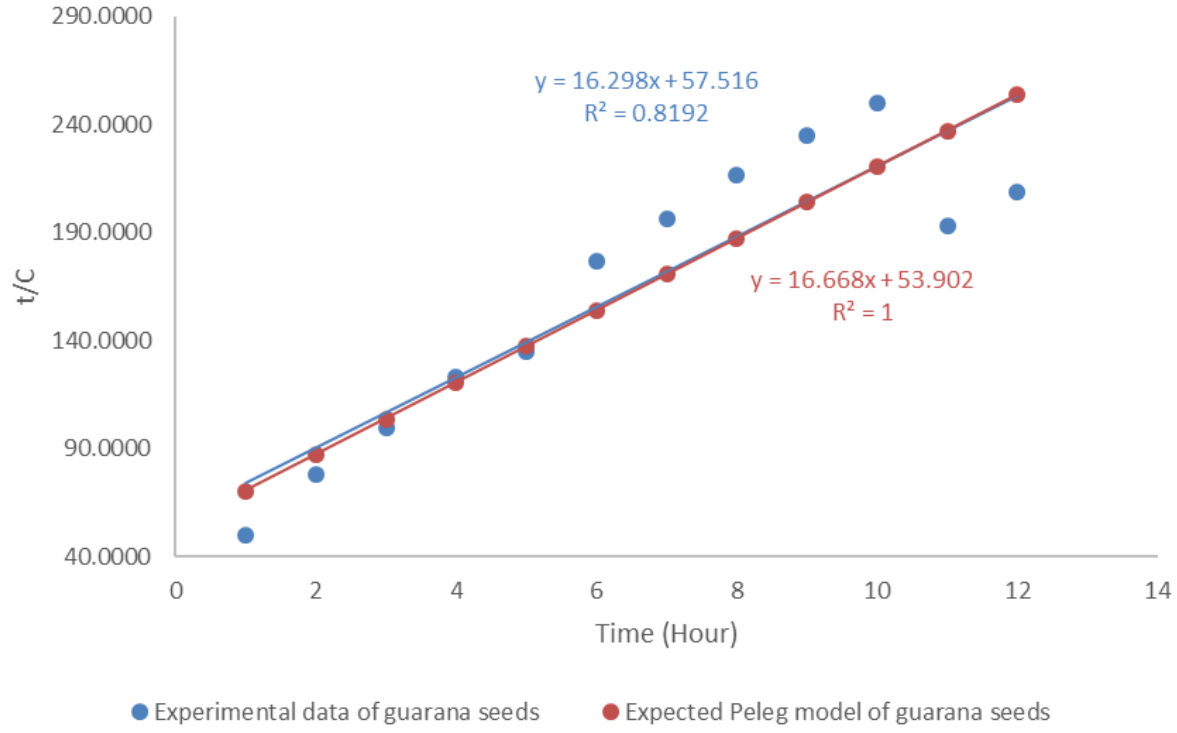


FIGURE 3. Kinetic extraction model of guarana seeds for conventional extraction

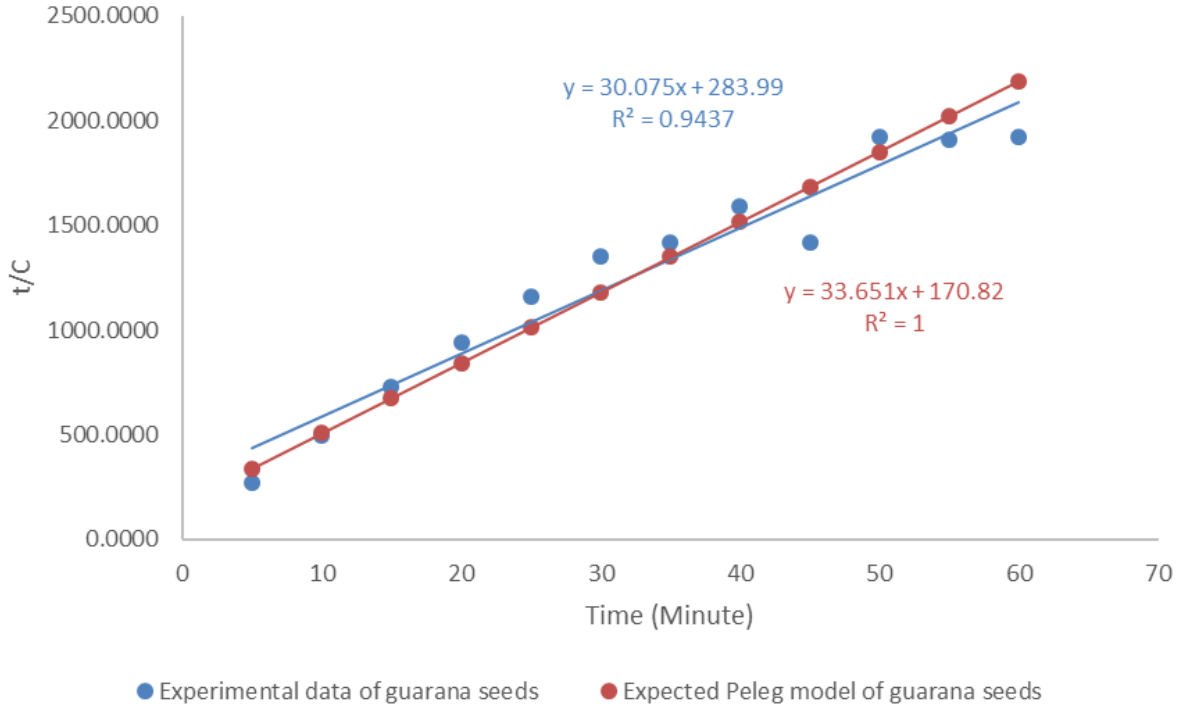


FIGURE 4. Kinetic extraction model of guarana seeds for ultrasonic extraction

concentration compared to conventional extraction, its significantly reduced extraction duration (1.30 ± 0.027 h vs. 35.07 ± 8.036 h) and ability to retain essential bioactive compounds make it an optimal technique for industrial-scale operations. Its eco-friendly profile, minimal thermal degradation, and enhanced diffusion efficiency support its recommendation as the preferred method for future guarana extract studies and product development.

PROXIMATE PROFILING OF GUARANA EXTRACT

Guarana seeds have long been recognized for their nutrient-rich composition. The proximate composition results are summarized in Table 2. In this study, proximate analysis was conducted exclusively on extracts obtained through conventional maceration, as ultrasonic extraction was primarily optimized for bioactive compound recovery (phenolic content and caffeine). Therefore, proximate composition comparison between conventional and ultrasonic extracts was not performed. According to Santana and Macedo (2019), the moisture content in raw guarana seed extract is 10.82 ± 0.1 g/100 g, while the extract from waste guarana seeds has a higher moisture content of 34.96 ± 0.06 g/100 g. Marques, Klein and de Mello (2019) reported that the moisture content in guarana ranges from 4.3% to 10.5%, with guarana powder containing 8.80%. In contrast, the guarana seed extract in this study had a much higher moisture content of 97.9%, as indicated in Table 2. This significant difference suggests that the guarana seed extract in this study contains far more water than those reported in other studies. Moisture content is a key indicator of water activity and serves as a measure of both stability and vulnerability to microbial contamination. This high moisture content suggests that guarana seed extract may have a relatively short shelf life (Okokon & Okokon 2019).

Carbohydrate content was calculated through subtraction. In raw guarana seeds, the carbohydrate content is 65.03 ± 0.14 g/100 g, while the waste guarana seed extract contains 47.64 ± 0.51 g/100 g (Santana & Macedo 2019). In this study, the carbohydrate content of the guarana seed extract was much lower, at just 1.5%, as shown in Table 2. This was significantly lower than values found in other studies. Typically, the stimulating effect of energy drinks is attributed to caffeine, often in combination with carbohydrates and sugars present in the drinks but not heavily dependent on its polysaccharides content (Marques, Klein & de Mello 2019).

Protein is an essential component of guarana, as it plays a key role in the development of flavors, such as those found in coffee, where proteins are involved in the Maillard reaction, producing volatile compounds like furans, pyridines, and pyrazines (Arya et al. 2021). Santana and Macedo (2019) reported that raw guarana seeds contain 16.29 ± 0.07 g/100 g of protein, while the protein content in waste guarana seed extract is 11.80 ± 0.22 g/100 g. Marques, Klein and de Mello (2019) found protein content in guarana ranging from 7.0% to 8.0%. However, in this study, the protein content in the guarana seed extract was much lower at 0.6%, which is significantly lower than reported in other studies. This protein content is also lower than that found in fresh coffee beans, which contain around 9% protein, though dried coffee beans contain between 11% and 15% protein (Arya et al. 2021).

The ash content in raw guarana seed extract was reported by Santana and Macedo (2019) to be 1.6 ± 0.1 g/100 g, while waste guarana seeds contain 1.18 ± 0.11 g/100 g. Marques, Klein and de Mello (2019) noted that ash content in guarana ranges from 1.06% to 2.88%, with guarana powder having an ash content of 1.51%. In contrast, the ash content in the guarana seed extract from this study was less than 0.1%, which is significantly lower than values found in other studies. The ash content represents the amount of minerals present in the sample (Okokon & Okokon 2019).

Santana and Macedo (2019) reported a fat content of 6.25 ± 0.10 g/100 g in raw guarana seeds, and 4.42 ± 0.42 g/100 g in the waste guarana seed extract. Marques, Klein and de Mello (2019) found that guarana contains 0.16% fat. In this study, the fat content in the guarana seed extract was less than 0.1%, which is significantly lower than in other studies. The fat content in the guarana seed extract is also much lower than that found in green coffee beans, which have a fat content ranging from 14.7% to 16.4% (Caporaso et al. 2018).

TABLE 1. Kinetic parameters of experimental data and expected Peleg model of conventional extraction and ultrasonic extraction of guarana seeds

Guarana seeds extraction	K_1 (hr.g/mg GAE)	K_2 (g/mg GAE)	R^2	SE	Exhaustive extraction time (h)
Experimental data					
Conventional	60.56 ± 7.128	16.21 ± 0.375	0.724	0.000	40.00 ± 5.495
Ultrasonic	4.75 ± 18.053*	30.09 ± 1.059*	0.715	0.000	2.68 ± 0.110*
Expected Peleg model					
Conventional	58.59 ± 11.109	16.28 ± 0.958	0.999	0.000	35.07 ± 8.036
Ultrasonic	2.85 ± 11.968*	33.70 ± 1.205*	0.967	0.000	1.30 ± 0.027*

*Referring to significant differences between conventional and ultrasonic extraction process (p<0.05)

In summary, the proximate composition of guarana seed extract in this study demonstrates significant differences in moisture, carbohydrate, protein, ash, and fat content compared to previous studies. The notably higher moisture content and lower carbohydrate, protein, ash, and fat levels suggest that the guarana seed extract studied and obtained locally in Malaysia differs from traditional guarana products, with implications for its stability and nutritional profile as compared to its native species mostly taken from the south America region (e.g., Brazil) with substantial different climate and soil composition which may lead to these huge anomalies. However, the caffein content (secondary metabolite) is considered the main indicator of the seed quality that need to be used for food and beverages (F&B) product development.

HEAVY METALS PROFILING OF GUARANA EXTRACT

Heavy metals analysis was conducted on guarana seed extracts from Simpang Renggam, Johor (Malaysia) to determine the concentration of heavy metals and assess their potential risk to human health. Table 3 presents the heavy metal concentrations in guarana seed extracts obtained through conventional and ultrasonic extraction methods. The order of heavy metal concentration is as follows: As > Pb > Hg > Cd > Sb. According to Yu et al. (2021), the maximum allowable concentrations for arsenic (As) and cadmium (Cd) are 1.0 mg/kg and 1.5 mg/kg, respectively. The Food and Agriculture Organization (FAO) (1995) sets the maximum allowable mercury (Hg) concentration at 0.1 mg/kg, while lead (Pb) in vegetable legumes is also limited to 0.1 mg/kg. The maximum permissible level for antimony (Sb) is 35 mg/kg (Nishad & Bhaskarapillai 2021). According to the Ministry of Health Malaysia's Food Regulations (1985), the maximum allowable levels for heavy metals in coffee-related products are 1 mg/kg for As, 2 mg/kg for Pb, 0.05 mg/kg for Hg, 1 mg/kg for Cd, and 1 mg/kg for Sb. In this study, the concentrations of As (0.456 mg/kg), Pb (0.046 mg/kg), Hg (0.043 mg/kg), Cd (0.010 mg/kg), and Sb (0.005 mg/kg) in the conventional extraction method were all below the regulatory limits set by the Ministry of Health. Similarly, in the ultrasonic extraction method, the concentrations of As (0.369 mg/kg), Pb (0.026 mg/kg), Hg (0.022 mg/kg), Cd (0.006 mg/kg), and Sb (0.003 mg/kg) were also within the safe limits. Thus, the heavy metal content in guarana seed extracts is within a safe range and does not pose a significant health risk to humans (Yu et al. 2021).

The cadmium (Cd) content in this study is comparable to the findings of da Silva et al. (2017), who reported Cd levels of 0.01 mg/kg in Arabica coffee samples. However, the Pb content in their coffee samples (0.75 mg/kg) was higher than the levels found in this study. Similarly, the study by Semen et al. (2017) found that Arabica coffee samples contained 0.010 mg/kg As, 0.061 mg/kg Hg, 0.120 mg/kg Pb, 0.005 mg/kg Cd, and 0.003 mg/kg Sb, with only the Hg content exceeding the limits set by the Ministry of Health. The variation in heavy metal content is influenced by factors such as the growth location, soil type, climate, and processing methods. Additionally, soil and water contamination, as well as the use of fertilizers and pesticides, can contribute to the accumulation of heavy metals in crops (Semen et al. 2017).

Heavy metals are a significant concern as food contaminants, originating from natural, industrial, and environmental sources. Certain heavy metals pose serious health risks through the food chain, especially as their concentrations increase in food products (Guo et al. 2021). Chronic consumption of plant materials with high heavy metal content is a major health consideration. Among these metals, the accumulation of Pb and As is particularly relevant due to their potential health impacts (Wakshlag et al. 2020). Arsenic (As) and other metals such as cadmium (Cd), mercury (Hg), and lead (Pb) have no known biological function and can be toxic even at very low levels (Sánchez-Quiles, Marbà & Tovar-Sánchez 2017). Arsenic pollution has three main sources: Natural groundwater, industrial effluents, and food or drink contamination (Paul & Saha 2019). Populations living in areas contaminated with arsenic are at risk for severe health issues. Arsenic is a potent carcinogen, and both acute and chronic exposure can lead to severe health effects. Long-term ingestion of even small amounts of arsenic can result in skin damage, peripheral nerve damage, anemia, liver disease, circulatory issues, and cancer (Guo et al. 2021).

Mercury (Hg) is one of the most toxic and common food contaminants. Even small exposures to $Hg2^{+}$ can cause irreversible damage to the central nervous system, leading

TABLE 2. Proximate analysis for guarana seeds extract

Test description	Unit	Results (%)
Moisture	g/100 g	97.9
Ash	g/100 g	< 0.1
Protein	g/100 g	0.6
Fat	g/100 g	< 0.1
Carbohydrate	g/100 g	1.5

to symptoms such as tremors, hearing loss, coordination problems, and memory loss. Increased mercury levels also negatively affect the endocrine system in both men and women (Guo et al. 2021).

The World Health Organization (WHO) asserts that there is no safe level of lead (Pb) exposure. Even minimal lead concentrations in human blood can increase the risk of hypertension, a cardiovascular disease. Pb exposure is also linked to neurodevelopmental problems in children, with long-term effects that can lead to reduced academic performance, higher risks of substance abuse, incarceration, and diminished economic productivity (Guo et al. 2021). Cadmium (Cd) is another highly toxic pollutant, primarily absorbed by the public through food. The accumulation of Cd in human organs can last for 10 to 30 years, with the potential for gradual buildup through the food chain, even at low daily doses (Zuo et al. 2018). Chronic exposure to cadmium can result in severe damage to the lungs, kidneys, liver, and other organs (Tinkov et al. 2018). Antimony (Sb) is a metalloid with both toxicity and carcinogenic potential, widely present in the environment. Antimony pollution has become a significant global issue (Wen et al. 2018). Exposure to Sb, whether directly or indirectly, can have detrimental effects on human health. Sb and its compounds readily bind with sulfhydryl groups in the body, disrupting enzyme activity, disturbing ion balance in cells, and causing hypoxia (Li et al. 2018).

In conclusion, while the guarana seed extracts in this study contained detectable levels of heavy metals, these concentrations were well below the maximum allowable limits (MAL) established by regulatory bodies, suggesting that the risk to human health is minimal. However, continued monitoring of heavy metal contamination in food products remains crucial to ensuring public safety.

CAFFEINE CONTENT OF GUARANA EXTRACT

Guarana is a significant source of caffeine, which has wide applications in the food and pharmaceutical industries. Table 4 presents the caffeine content in guarana seed extracts obtained through conventional and ultrasonic extraction methods, while Figure 5(a), 5(b) and 5(c) shows the HPLC chromatograms of the caffeine standard and the guarana seed extract sample, respectively. In this study, the caffeine content of guarana seed extract from conventional extraction was found to be 4.75 ± 0.001% (w/w) (23% higher than the ultrasonic extraction: $p<0.05$), while that from ultrasonic extraction was 3.65 ± 0.002% (w/w) in powdery dried seed. These values are consistent with the findings of Marques, Klein and de Mello (2019), where caffeine content in guarana ranged from 2.41% to 5.07%. Various studies have analyzed the caffeine content in guarana, with Machado et al. (2018) reporting an average caffeine content of 2.94%, and Schimpl et al. (2013) stating that the caffeine content in guarana seeds ranges from 2.5% to 6%. This caffeine concentration in guarana remains higher than that in other common caffeine sources, such as coffee and tea (Schimpl et al. 2013).

The higher caffeine content observed in the conventional extraction method compared to ultrasonic extraction may be attributed to the longer extraction time of 12 h for the conventional method, while ultrasonic extraction lasted only 60 min. This reduction in caffeine content with ultrasonic extraction may also be attributed to factors such as the higher frequency (42 kHz), shorter extraction time (1 h), and lower temperature (22 °C) used. Extraction efficiency is highly time-dependent, with longer extraction time's generally yielding higher caffeine concentrations (Fazil, Azzimi & Zubairi 2018; Goula et al. 2017). Additionally, the lower caffeine yield from ultrasonic extraction could be due to the high frequency (42 kHz) used in this study. High-frequency ultrasound leads to a greater number of bubbles forming during extraction. This increased cavitation effect, however, can diminish the energy transfer into the extraction medium, reducing the overall extraction efficiency. Furthermore, the high ultrasound intensity may degrade bioactive compounds; further lowering yields (Kumar, Srivastav & Sharanagat 2021). According to Jordens et al. (2016), lower ultrasound frequencies produce stronger shockwaves, which can enhance extraction yields. Low-frequency ultrasound (28 kHz) has been shown to extract 1.8 times more caffeine compared to high-frequency ultrasound (40 kHz) under identical conditions (Wang et al. 2011).

Temperature is another key factor influencing caffeine content. In this study, the ultrasonic bath was maintained

TABLE 3. Heavy metals concentration in guarana seeds extract for conventional and ultrasonic extraction

Heavy metals	Concentration (mg/kg)		Maximum MOH limit (mg/kg)
	Conventional extraction	Ultrasonic extraction	
Arsenic (As)	0.456	0.369	1.00
Mercury (Hg)	0.043	0.022	0.05
Plumbum (Pb)	0.046	0.026	2.00
Cadmium (Cd)	0.010	0.006	1.00
Antimony (Sb)	0.005	0.003	1.00

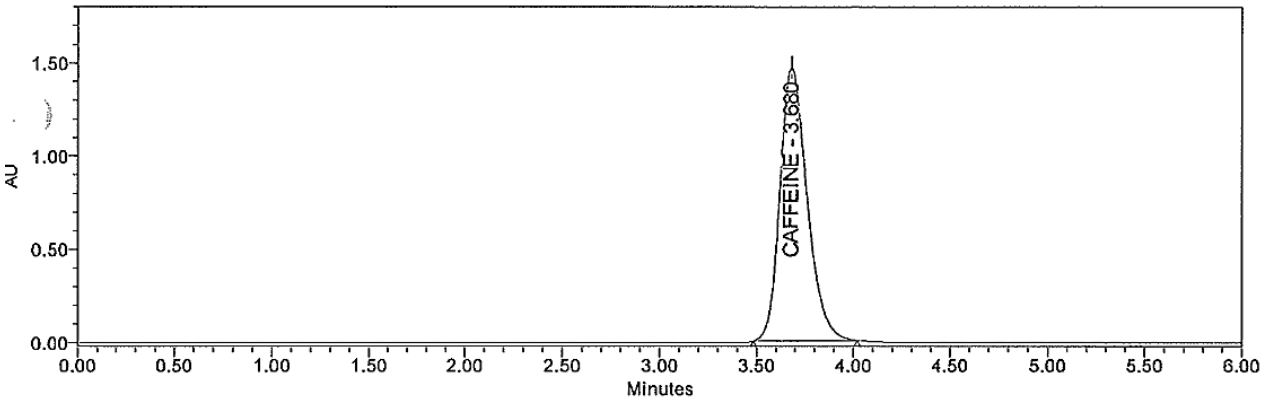
at 22 °C, while conventional extraction was carried out at 60 °C. The lower temperature in ultrasonic extraction likely contributed to its lower caffeine content. To increase caffeine yield, higher temperatures should be considered, as increased temperature enhances desorption and solubility of the solute in the solvent. Higher temperatures also reduce solvent viscosity, thereby increasing the diffusion of the solvent into the tissue matrix (Kumar, Srivastav & Sharanagat 2021).

The caffeine content of guarana seed extract obtained in this study ranged from 3.65% (ultrasonic extraction) to 4.75% (conventional extraction), which corresponds to approximately 36.5-47.5 mg of caffeine per gram of dried extract. These values are consistent with prior literature on guarana, which reports caffeine levels ranging from 2.5% to 6% (Marques et al. 2019; Schimpl et al. 2013). From a safety perspective, the European Food Safety Authority (EFSA) considers single caffeine doses of up to 200 mg and daily intakes of up to 400 mg as safe for healthy adults (EFSA 2015). Likewise, the U.S. Food and Drug Administration (FDA) identifies 400 mg/day as the upper limit for safe consumption in healthy individuals (FDA 2022). Based on our findings, a daily intake of 8-11 grams of guarana seed extract would approach this threshold, thus emphasizing the need for careful formulation in commercial food or nutraceutical products. While the Malaysian Food Regulations (1985) do not stipulate specific caffeine limits for herbal extracts, any product containing caffeine must adhere to labeling and consumer safety requirements. Therefore, guarana-derived products should clearly disclose caffeine content per serving and include appropriate health advisories, particularly for sensitive populations such as children, pregnant women, or individuals with cardiovascular conditions. This positions guarana extract as a potent natural stimulant, suitable for functional food applications when used within recommended intake levels.

TABLE 4. Caffeine content in guarana seeds extract for conventional and ultrasonic extraction

Caffeine	Conventional extraction	Ultrasonic extraction
Caffeine content (%, w/v)	0.18 ± 0.001*	0.13 ± 0.002
Caffeine content (g)	0.9508 ± 0.002*	0.7318 ± 0.001
Caffeine yield/dried seed (%, w/w)	4.75 ± 0.001*	3.65 ± 0.002

*Referring to significant differences between conventional and ultrasonic extraction process (p<0.05)



The retention time for caffeine was observed at approximately 3.68 and 3.75 min, consistent with the standard, confirming the accuracy of caffeine identification and quantification

FIGURE 5(a). HPLC chromatogram of caffeine standard at 280 nm showing a retention time of 3.68 min) The caffeine peak was identified by comparing the retention time of the sample with that of the standard

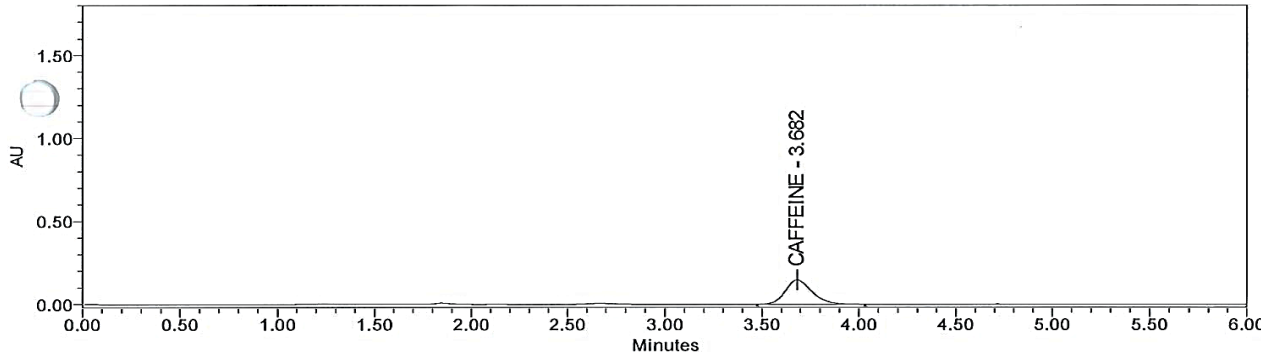


FIGURE 5(b). HPLC chromatogram of caffeine in guarana seed extract (conventional extraction) at 280 nm, showing a retention time of 3.68 min

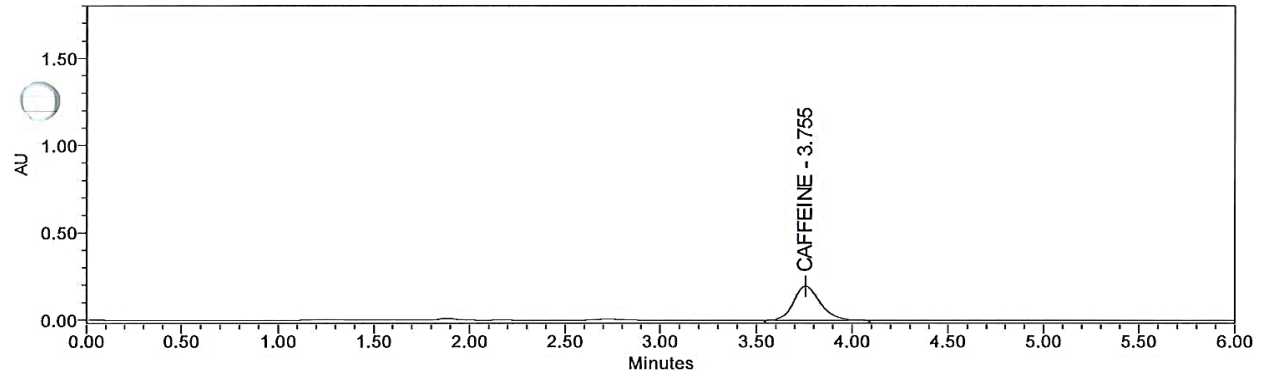


FIGURE 5(c). HPLC chromatogram of caffeine in guarana seed extract (ultrasonic extraction) at 280 nm, showing a retention time of 3.75 min

CONCLUSIONS

Peleg's mathematical model provides an efficient and accurate way to determine key processing parameters. The analysis using this model revealed that ultrasonic extraction significantly reduced extraction time (1.30 ± 0.027 h) compared to conventional extraction (35.07 ± 8.036 h) (p<0.05), indicating greater time efficiency. However, while ultrasonic extraction accelerated the process, it also resulted in a slightly lower caffeine content compared to conventional extraction. Therefore, the selection of extraction method should consider both the required extraction time and the desired yield of bioactive compounds. Overall, guarana seeds extracted using both methods were shown to be safe, with heavy metal contents within regulatory limits. The guarana seed extract in this study exhibited a high moisture content of 97.9%, while the concentrations of ash (<0.1%), fat (<0.1%), protein (0.6%), and carbohydrates (1.5%) were relatively low. The study also measured the concentrations of arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), and antimony (Sb) in guarana seed extract using the ICP-MS method. The concentration of heavy metals followed the order: As > Pb > Hg > Cd > Sb. For conventional extraction, the concentrations were 0.456 mg/kg for As, 0.046 mg/kg for Pb, 0.043 mg/kg for Hg, 0.010 mg/kg for Cd, and 0.005 mg/kg for Sb. In the case of ultrasonic extraction, the concentrations were 0.369 mg/kg for As, 0.026 mg/kg for Pb, 0.022 mg/kg for Hg, 0.006 mg/kg for Cd, and 0.003 mg/kg for Sb. All measured heavy metals were below the maximum allowable concentrations set by the Ministry of Health Malaysia (MOH), which are 1 mg/kg for As, 2 mg/kg for Pb, 0.05 mg/kg for Hg, 1 mg/kg for Cd, and 1 mg/kg for Sb, indicating that the levels of these metals in the extract are safe for consumption. Additionally, this study successfully extracted a significant amount of caffeine from guarana seeds. The conventional extraction yielded 4.75 ± 0.001% (w/w) caffeine with 23% higher than the ultrasonic extraction in which resulted in 3.65 ± 0.002% (w/w) caffeine content in powdery dried seed (p<0.05). Although conventional extraction yielded slightly higher caffeine content, ultrasonic extraction is considered the better method overall due to its rapid extraction rate and preservation of bioactive, making it highly suitable for future studies and commercial production.

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