Comparative Study of the Characterisation and Extraction Techniques of Polyphenolic Compounds from *Acacia seyal* gum

Ahmed A. M. Elnour\textsuperscript{ab}\textsuperscript{c}\textsuperscript{*}; Mohamed E. S. Mirghani\textsuperscript{a}; Nassereldeen. A. Kabbashi\textsuperscript{b}; Khalid Hamid Musa\textsuperscript{d}; Fahimeh Shahabipour\textsuperscript{e}; Nureddin Ashammakhi\textsuperscript{fg}; and Nour Hamid Abdurahman\textsuperscript{h}

\textsuperscript{a}International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia (IIUM), P. O. Box 10, Gombak, 50728 Kuala Lumpur, Malaysia;

\textsuperscript{b}Bioenvironmental Engineering Research Centre (BERC), Biotechnology Engineering Department, Kulliyyah of Engineering, International Islamic University, Malaysia (IIUM), P. O. Box 10, Gombak, 50728 Kuala Lumpur, Malaysia;

\textsuperscript{c}Institute of Gum Arabic & Desertification Studies (IGADS), University of Kordofan, Sudan, Box 160, Elobied, Sudan;

\textsuperscript{d}Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Kingdom of Saudi Arabia;

\textsuperscript{e}Skin Research Centre, Shahid Beheshti University of Medical Sciences, Tehran 19857-17443, Iran;

\textsuperscript{f}Department of Bioengineering, Henry Samueli School of Engineering, University of California, Los Angeles, CA 90095, USA;

\textsuperscript{g}Department of Biomedical Engineering, College of Engineering, Michigan State University, East Lansing, MI 48824, USA;

\textsuperscript{h}Centre of Excellence for Advanced Research in Fluid Flow (CARIFF), University Malaysia Pahang Gambang, Malaysia.

\textcopyright{} The Author(s) 2022. Published by Oxford University Press on behalf of Zhejiang University Press.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Abstract

Acacia seyal gum is an abundant source of natural polyphenolic compounds (NPPCs) and antioxidant activity with numerous benefits and is often used in cancer treatment. The type of extraction technique can significantly impact the yield and isolation of NPPCs from Acacia seyal gum (ASG). The traditional use of maceration extraction reportedly yields fewer NPPCs. Objectives: This study investigates five extraction techniques for NPPCs and ASG antioxidant activity, namely: homogenisation, shaking, ultrasonication, magnetic stirring, and maceration. Materials and Methods: The evaluation of the antioxidant activity (AoA) of the extracted NPPCs from ASG used five assays, namely: Total Flavonoids Content (TFC), Folin-Ciocalteu index (FCI), 2,2-Diphenyl-1-Picrylhydrazyl radical scavenging activity (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Cupric Reducing Antioxidant Capacity (CUPRAC). Results: To minimise the dataset dimensionality requires Principal Component Analysis. The ultrasonic and maceration techniques were the best techniques to extract NPPCs and examine the AoA of ASG, with a high correlation between the NPPCs and AoA. However, the maceration process was slow (12 h) compared to ultrasonication (1 h). Slow extraction can result in a decline of the NPPCs due to polyphenol oxidase-enzyme and impact productivity. Conclusions: These findings provide an essential guide for the choice of extraction techniques for the effective extraction of NPPCs from ASG and other plant materials.

Keywords: Polyphenolic extraction; Gum Arabic; Comparison of techniques; Principal Component Analysis (PCA); Ultrasonication.
Graphical Abstract

Acacia seyal gum (ASG) is sieved to 1.40 mm and then macerated for 24 h, at 23.0°C. The resulting powder is extracted with methanol 100% and ultrasonicated for 1 h, at 12 kHz, at 23.0°C. The optimum method for ultrasonic assisted extraction is shown.

The extracts are analyzed using DPPH, FRAP, TPC, and TFC. The supernatants are measured using a Microplate Reader and stored at 4°C.
Abstract: Comparative Study of Extraction Techniques of Natural Polyphenolic Compounds (NPPCs) from *Acacia seyal* gum (ASG). (Graphical Abstract location)

Note: Steps of the extraction techniques of natural polyphenolic compounds (NPPCs) from *Acacia seyal* gum.

Highlights

- Compares five methods for the extraction of polyphenol compounds from Gum Arabic.
- Discusses how extraction techniques influence antioxidant extraction, factors affecting NPPCs efficacy, and methods to determine AoA.
- Provides information about the role of relative standard deviation (RSD) and Principal Component Analysis (PCA) as powerful and effective repeatability tools in alleviating NPPCs.
1. Introduction

The role of mediators of intracellular signalling cascades is reactive oxygen species (ROS), which come through cellular metabolism and have both favourable and detrimental effects on living systems [1]. However, total production of ROS may lead to oxidative stress (OS), failure of cell function (CFs), and eventually apoptosis or necrosis. Therefore, a balance between oxidative and intracellular polyphenolic compounds (NPPCs) is vital for CFs, regulation, and adaptation to diverse growth conditions [2]. However, ROS benefits include suitable defence mechanisms in the interactions between cells and their surrounding environments and responding to infections [3]. Besides, non-enzymatic oxidants and an imbalance effect between enzymatic activities and ROS would result from high-level traces of ROS in biological systems. Also referred to as OS, this imbalance is an outcome of damage to such parts of biological systems, as proteins, DNA, or lipids. In turn, this hinders the biological system's functionality negatively in the human body. In this sense, some health problems, including cancer, cystic renal failure, and hypertension of synthetic phenolic compounds (SPCs), including; tertbutyl hydroquinone (TBHQ), butylated hydroxyanisole, and butylated hydroxyl toluene (BHT), may occur due to the chemical composition as a result of the rapid consumption of SPCs [4]. A huge concern is the lack of natural antioxidants in preventing epidemics and degenerative diseases [5]. Hence, sustainable natural polyphenolic compounds (NPPCs) are becoming extraordinarily crucial based on desirable purification and extraction techniques. The detrimental effects of free radicals can be reduced by applying antioxidants. However, due to the possible toxic effects of SPCs on human health, much attention has been focused on applying novel NPPCs, especially plant-based ones [5]. Thus, the extraction of NPPCs from natural plants is urgently needed.

*Acacia seyal* gum, Del. variety seyal, derived from the (*Acacia seyal*) tree, is commonly used in folk medicine. It has several applications in the therapeutics, food, and pharmaceutical industries. ASG provides a potential source of bioactive components such as natural polyphenol compounds (NPPCs), flavonoids, terpenoids, coumarins, quinones, tannins, and lignans that can provide antiproliferative and antioxidant properties beneficial for medical applications, including cancer treatment [6]. Therefore, extracting polyphenols from ASG under optimised techniques is an essential step toward enhancing its medical applications.

Chemically, ASG is a composite blend of macromolecules, including proteins and carbohydrates. It has plenty of non-viscous soluble fibres and minerals such as calcium, potassium, and magnesium with high dietary benefits [7]. ASG promotes the metabolism of lipids [8], and it is helpful for the treatment of multiple pathological disorders, including kidney failure [9], heart diseases [10] and gastrointestinal ailments [11]. Thus, the NPPCs of *Acacia seyal* gum (ASG) is promising in the medicinal and pharmaceutical industries.
Preliminary studies reveal that a tremendous number of NPPCs still exist in the ASG [12]. There are several studies regarding the NPPCs of ASG. However, there are no studies regarding the impact of extraction methods on the extraction of NPPCs; optimising the extraction process to reach the optimum amount of these compounds is the main objective of the current study. The choice of an appropriate technique to extract the NPPCs with the highest efficiency and maximum purity is based on the NPPCs’ nature, stability thermal, and the processed raw substrate nature [13]. Thus, NPPCs exist in plant cells as free and bound forms. The solvent system can quickly obtain the free ones, whereas the bound forms are covalently associated with other plant materials. Therefore, they cannot efficiently be extracted by conventional and aqueous extraction techniques/organic solvents [14]. Hence, seeking an optimum extraction technique is highly needed.

As mentioned earlier, the extraction process has influenced the biological properties of NPPCs. The mechanism of the solvent used for separating the NPPCs efficiently, which depends on the extraction technique and solvent system due to the variations in NPPCs polarity [15]. Sequentially, NPPCs extraction is harrowing because of the covalent linkage among NPPCs, polysaccharides, pectin, and cellulosic constituents [16]. Therefore, an appropriate extraction technique can significantly preserve bioactive components and NPPCs from ASG. However, only a few studies have reported the extraction of NPPCs, including antioxidants from ASG.

Many extraction technique have reported NPPCs extraction from various plant substrates using different solvent polarities [17]. Recently, novel ETs such as ultrasound-assisted extraction (UAE), which is defined as a non-thermal and novel technique, maceration, magnetic stirring, homogenisation, and shaking, which are defined as traditional techniques, have been demonstrated [18]. Hence, maceration has been commonly used as an extraction technique. However, maceration has some disadvantages, including the low yield of NPPCs and time consumption [19]. Consequently, seeking advanced ETs is necessary.

Among these techniques, UAE is one of the crucial techniques for NPPCs extraction from plants, due to its operation at the lowest time and temperature, simplest, cheapest, and most effective ones, which increased the efficiency of NPPC’s extraction, provided more processing efficiency, and reduced extraction time [20]. The UAE mechanism is qualified by the cavitation phenomenon resulting from the UAE’s growth, generating sound waves and collapsing the gas pressure. The bubbles burst with physicochemical and mechanical influences, leading to the destruction of biological cells, the maximum releasing ratio of extractable NPPCs, and increasing mass transfer within solvents and plant matrices [21]. Hence, the UAE is recommended for natural polyphenolic compound extraction.
Recently, the UAE technique has been reported by Elnour et al. [22] it took only a short time to extract antioxidants from ASG, which resulted in a significantly higher yield of NPPCs than other techniques. Standard techniques for extracting NPPCs from other plant materials and various foodstuffs include vibrating, homogenisation (at high-speed), maceration, and agitating. Novel ETs, including microwave-assisted methods, ultrasonically agitated processes, the application of enzymes and supercritical fluid, have recently been designed to extract antioxidants from plant materials [23, 24]. Besides maceration and ultrasonic-assisted extraction, these techniques have not been investigated for ASG or compared for extraction yield. Some of the drawbacks of each technique, such as low product quality, essential safety hazards, and prolonged removal time, were investigated in various isolated studies [25]. Although the enzyme-assisted supercritical technique provides an environmentally friendly condition, it requires a low-temperature range, which raises the cost [23, 24, 26]. To the best of the author’s knowledge, there are no studies regarding the impact of the UAE on the natural polyphenolic compounds from *Acacia seyal* gum. This study aimed to investigate the impact of five ETs, namely: ultrasonication, maceration, homogenisation, magnetic stirring, and shaking techniques, on the NPPCs contents in ASG. The total flavonoids content (TFC), Folin-Ciocalteu Index (FCI), 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) were used to assess the efficacy of various techniques. Each of these methods surveys different features of the antioxidant capacity of the ASG extract. To minimise the dataset, Principal Component Analysis (PCA) was applied to lower the dimensionality of the variables in the ETs and adequately account for the solvent’s different factors of extraction process, such as planning or evaluation.

2. Materials and Methods

2.1 Materials

The analytical grade compounds and reagents used in the paper include Folin-Ciocalteu phenol reagent, ferric chloride (FeCl₃·6H₂O), and HCl acquired from Merck (Darmstadt, Germany), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), Neocuproine (2,9-dimethyl-1,10-phenanthroline), gallic acid, Trolox, and sodium acetate trihydrate from Sigma. Sodium carbonate from RDH (Germany) and glacial acetic acid from Mallinckrodt Baker. To obtain spectrophotometric measurements requires a SPECTROstar Nano spectrophotometer (BMG LABTECH, Offenburg, Germany).
2.2 Sample Collection and Preparation
The ASG nodule samples were obtained from the Blue Nile state in Sudan. All samples were cleaned from impurities such as bark and sand. Then samples were randomly selected and divided into two parts. Each part was uniformly grained and ground mechanically into a powder using a USA standard, a testing sieve of 1.40 mm (Fisher, Lenexa, Kansas, United States).

2.3 Extraction of Antioxidants
One gram of ASG powder was introduced into several vials with a capacity of 20 mL, and 10 mL of methanol was added to each vial as an extracting solvent. Five extraction techniques were applied, namely: homogenisation, shaking, ultrasonication, magnetic stirring, and maceration. According to the procedure highlighted by Musa et al. [27], the extraction was performed with minor modifications. After the extraction, the samples were filtered using 0.45 µm Sartorius polytetra-fluorethylene (PTFE) membrane filters, and the supernatants were collected for further evaluation (Figure 1). (Figure 1 location)

Figure 1. Techniques for extracting natural polyphenolic compounds (NPPCs) from Acacia seyal gum (ASG) and the extract's evaluation using different antioxidant activity (AoA) assays.

2.4 Determination of Total Flavonoids Content (TFC)
To determine the TFC, a colourimetric technique was used, as outlined by Zhang et al. [28]. The evaluation of 0.5 mL of each sample extract diluted with 2.25 mL of distilled water in a test tube. After 6 minutes elapsed, 0.3 mL of 10% AlCl₃·6H₂O solution was added to ensure that a reaction was altered by adding 1.0 mL of 1M NaOH after 5 minutes. Then, the mixture was blended by vortexing, and the absorbance was measured at a 510nm wavelength. Milligram units of quercetin equivalents (QE) per 100g sample was used to express the results (mg QE/100 g of dray weight (DW)).

2.5 Determination of Total Phenolic Content using Folin-Ciocalteu Index (FCI)
The method espoused matches the steps highlighted by Musa et al. [27] for the FCI assay. Approximately 0.5 mL of thinned Folin-Ciocalteu reagent was added to 100 µL of each sample extract and allowed to sit for 5 minutes before adding 1 mL (7.5%) of sodium carbonate
(Na₂CO₃) (w/v). The results of the absorbance measurements were analysed at the 765 nm wavelength after two hours and were recorded per mg of gallic acid equivalent (GAE).

2.6 Determination of DPPH Radical-Scavenging Activity (DPPH%)

Based on the procedure highlighted by Musa *et al.* [29], the AoA of each sample extract is evaluated using DPPH. The DPPH was prepared by dissolving 40 mg of DPPH in 1000 mL of methanol (CH₃OH) to absorb the 1.00±0.01 unit at 517 nm wavelength. Approximately 100 μL of each extract was combined with 1 mL of freshly prepared DPPH reagent and kept in the dark for 30 minutes. The following equation then defines the DPPH radical scavenging activity.

\[
\text{DPPH\%} = \left( \frac{A_{\text{conc}} - A_{\text{sample}}}{A_{\text{conc}} \times 100} \right)
\]

Where \( A_{\text{conc}} \) is defined as absorbance of the control, and \( A_{\text{sample}} \) is defined as the absorbance of the sample.

2.7 Determination of Cupric Reducing Antioxidant Capacity (CUPRAC)

The CUPRAC technique was carried out according to the method described by Apak [30]. The method was performed by mixing 1mL of copper [31] chloride, neocuproine (2,9-dimethyl-1,10-phenanthroline), ammonium acetate buffer, and water, adding 0.1mL of each sample extract. After 30 minutes, the 450 nm absorbance was recorded against a reagent blank, and the result was recorded in mg of Trolox equivalent per 100 g of fresh sample (mg TE/100g of fresh weight (FW)).

2.8 Determination of Ferric Reducing Antioxidant Power (FRAP)

According to Musa *et al.* [27]. The FRAP reagent was prepared fresh by mixing 300mM acetate buffer of pH 3.6 [composed of 3.1g sodium acetate trihydrate, and 16mL glacial acetic acid, which was prepared up to 1L with an equal parts ratio of distilled H₂O]; 10mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCl, and 20 mM FeCl₃H₂O, in the ratio of 10:1:1, to give the working reagent. About 1mL of the FRAP reagent was added to 100μL of each sample extract, and the absorbance was measured after 30 minutes at 595 nm wavelength. The result is reported in milligram of Trolox equivalent per 100g of fresh sample (mg TE/100 g of FW).
2.9 Statistical Analysis

Each assay was carried out in triplicates and the variations were analysed using Minitab® Software version 18. Fisher's novel varied range of experiments were used to ascertain meaningful variations, whereas the correspondences among recorded data were computed using Pearson’s correlation coefficient.

3. Results and Discussion

3.1 Effect of Extraction Techniques on the Antioxidants Activity of Acacia seyal gum

The antioxidant activity (AoA) of ASG obtained using five different ETs is presented in Table 2 and Figure 2. The results showed each extract’s total flavonoids content (TFC), total phenolic content measured by FCI, DPPH, FRAP, and CUPRAC. The mean efficacy of ETs based on TFC, DPPH, and CUPRAC values in ASG extracts proved that both maceration and ultrasonic-assisted extraction were significantly better (P≤0.05) than other techniques. Having considered TFC and DPPH values, we figured out that the ultrasonically assisted extract produced a significantly greater yield (P≤0.05) than other methods, including maceration. The FCI and FRAP values were significantly (P≤0.05) decreased after using homogenisation and shaking, respectively, confirming the effect of the ETs on AoA.

Regarding the time required to achieve the extraction, maceration seems to take the longest time of (24 h), whereas the UAE requires about 1 hour to obtain an equivalent AoA of the extract. Improved extraction by using the homogenisation method (24,000 rpm for 1 h) or by vibration (300 rpm for 1 h) resulted in significantly (P≤0.05) higher AoA in comparison with magnetic stirring for 1 h (1000 rpm). Even though shaking and magnetic stirring are fast sample and solvent mixing processes, their AoA is still lower than the UAE and maceration; this is likely due to the extreme vibration and power of the UAE (12 kHz at 1 h) compared to the speeds for homogenisation and shaking (300 rpm) and magnetic stirring (1,000 rpm). The AoA was notably (P≤0.05) higher when the UAE method was used compared to homogenisation and maceration extraction with equal solvent concentrations at room temperature for both cases.

Musa et al. [27] reported that UAE and homogenisation were the most convenient methods to obtain antioxidants from guava fruits. However, the authors did not mention the contributory effect of UAE power on the extraction outcome. Thaipong et al. [32] derived guava fruit’s antioxidant using the homogenisation technique, yet they did not report the speed of the process. Chuah et al. [33] applied the magnetic stirring technique at a rate of 1100 rpm for 3 h in the extraction process of antioxidants from Clinacanthus nutans leaves. Using maceration...
significantly lowers the AoA compared to methods using homogenisation. However, increasing the extraction time led to significant improvement in polyphenol oxidase-enzyme values.

Previously, Gordillo et al. [34] adopted extended maceration at an ambient temperature for hazelnut bye-product treatment. For instance, Shortle et al. [35] and Sharif et al. [36] used maceration for 8 and 48 h, respectively, to extract antioxidants from various plants, including guava. The authors showed that extended maceration at room temperature seemed as effective as hot extraction in obtaining a rich extract with a high phenolic concentration value. Unlike the outcomes obtained by Musa et al. [27], our findings explain that the maceration technique produced the lowest value (P≤0.05) of AoA.

Although the cause of the discrepancies has not been analysed, it has been shown in a separate study that the extraction techniques [37-40]. The UAE extraction technique includes two physical aspects, particularly diffusion through the cell walls and rinsing (washing-out) of the cell contents once the cell walls have split [22, 41]. The ultrasonic treatment enhances extraction by intensifying mass transference and allowing more access to the cell’s solvent [42, 43]. The critical advantage of the homogenisation technique is that increasing the speed improves extraction efficiency.

High-speed mixing yields more active bioactive compounds; the maceration technique depends on the compound’s solubility in the product’s solvent and mass transfer kinetics. One might predict that the maceration technique should obtain the same results as homogenisation. In the current study, the maceration technique significantly (P≤0.05) increased FCI values, DPPH, and CUPRAC after 24 h, coinciding with the Musa et al. [27] report. Thus, the maceration technique takes more time as compared to the UAE technique.

In contrast, the maceration technique yielded significantly (P≤0.05) lower AoA values within three days than the homogenisation technique [44]. They lost their AoA after being exposed to unfavourable lighting and oxygenation conditions for an extended period of time. Those conditions are known as potential causes of chemical degradation or long-term oxidation of polyphenols due to the accumulation of polyphenol oxidase enzymes. Therefore, in this study, the sample concentration was calculated based on the inhibition concentration, translating to the sample scavenging rate against the DPPH reagent. Finally, the other antioxidant assays were determined based on the standard curve, and the findings are presented in Table 1. (Table 1 location)
Table 1. Effect of extraction techniques on the antioxidant activities (AoA) of Acacia seyal gum (ASG) determined using total flavonoids content (TFC), folin-ciocalteu index (FCI), DPPH radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC).

Results are depicted as a mean ± standard deviation (SD). Values in each row marked by the same small letter are not significantly different at P≤0.05.

A Milligrams of quercetin equivalent (QE) per 100 g of dry weight.

B Milligrams of gallic acid equivalent (GAE) per 100 g of dry weight.

C % Compared to DPPH without added samples.

D & E Milligrams of Trolox equivalent (TE) per 100 g of dry weight.

F RSD % relative standard deviation.

Figure 2. The influence of extraction techniques on antioxidant activity (AoA) of methanolic extract from Acacia seyal gum (ASG). M (maceration for 24h), UAE (ultrasonication for 1h at 12kHz power), SH (shaking for 1h at 300rpm), HO (homogenisation for one minute at 24000rpm), MS (magnetic stirring for 1h at 1000 rpm).

3.2 Correlations and Repeatability for Antioxidant Assays of Extraction Techniques

The calculation of the repeatability of each extraction techniques used the relative standard deviations (RSD%) values presented in Table 2. All antioxidant assays presented standard deviation (SD)% values lower than 10%, implying good repeatability for the ETs. RSDs for TFC were about 0.49% for shaking (1 h) and 4.38% for magnetic stirring (1 h).
Furthermore, the FCI was approximately 0.37% for homogenisation (1 h) and maceration (24 h), while DPPH RSDs were approximately 0.24% for UAE (1 h) and 6.31% for magnetic stirring technique (1 h). For FRAP, RSDs were about 0.70% for homogenisation (1 min), 5.64% for both maceration (24 h) and ultra-sonication (1 h) techniques. For CUPRAC, RSDs were about 4.42% for maceration (24 h) and 13.4% for homogenisation (1 h) techniques.

It can be concluded that RSD is a powerful and the most effective repeatability tool in the separation and effectiveness of extraction of NPPCs from ASG. Table 3 shows strong correlations between TFC and values for AoA (FRAP, FCI, and DPPH), respectively. Overall, the correlation between FCI and TFC was remarkably higher than the correlation between TFC and each assay: i.e., FRAP, DPPH, and CUPRAC, respectively. This value was also higher than the correlation between FCI and FRAP and between DPPH and CUPRAC. For the homogenisation technique (1 h), the correlation between CUPRAC and FRAP and between CUPRAC and DPPH were the highest (1.000). The maceration technique (24 h) showed a negative correlation (-0.197) between DPPH and TFC, while the homogenisation technique showed the least correlation, just under (0.577) between FCI and each assay, including FRAP, DPPH, and CUPRAC, respectively. In addition, the UAE technique displayed high correlations between TFC and each assay: FCI (0.991), DPPH (0.974), and CUPRAC (0.995), respectively. However, magnetic stirring and homogenisation exhibited the lowest correlation (0.567) between FCI and CUPRAC.

There is a discrepancy between the relationship between phenolic compound concentration and AoA in the literature. Several authors have observed high correlations between FCI, TPF, and DPPH [45], while others have reported none or weak direct correlation [46, 47]. Thus, screening should be carried out individually on every single piece of work during antioxidant extraction. Finally, the correlation between antioxidant activities is presented in Table 2 and Figure 2. (Table 2 location)

**Table 2.** Pearson’s correlation coefficients of antioxidant activities of various extraction techniques

|                        | FCI   | FRAP  | DPPH  | CUPRAC |
|------------------------|-------|-------|-------|--------|
| Total Flavonoids Content | a     | b     | c     | d      |
| Ferric reducing antioxidant power |       |       |       |        |
| DPPH radical scavenging activity |       |       |       |        |
| Cupric reducing antioxidant capacity |       |       |       |        |
| Folin-Ciocalteu index (FCI) |       |       |       |        |

*Significant, and **highly significant different respectively at P≤0.05* and P≤0.01**.
3.3 Principal of Component Analysis

Principal Component Analysis (PCA) is a mathematical tool used to reduce data dimensionality and visually represent the underlying structure of experimental data and the relationship between data samples. This tool is used based on a standard procedure for defining values of the analysed parameters of ASG, using five extraction techniques (homogenisation, maceration, magnetic stirring, shaking, and ultrasonication) of five AoA detection assays (TFC, FCI, DPPH, FRAP, and CUPRAC). Setting the standard was necessary due to the different magnitudes of the analysed values shown in Table 1. Thus, every parameter equally contributed to dataset variance and equal weight in the PC following calculation by setting the standard.

As shown in Figure 3, the cumulative total variance (CTV) is 70.22%, 94.12%, 97.89% and 100%, for PC1, PC2, PC3, and PC4 respectively. The first PC (PC1) had a value of 4.7426, which was among the highest eigenvalues, accounting for 94.85% of the dataset variability. The 2nd PC (PC2) had an eigenvalue of about 0.2328 and accounted for 4.6569% of the data variance. Moreover, the 3rd PC eigenvalue is 0.0236 and reveals a variance of about 0.4729 %. Finally, the 4th PC eigenvalue accounts for only 0.0009% of the dataset variability. Generated NPPCs progressively yielded smaller eigenvalues with relatively remarkable data variability (less than 3% of the total). (Figure 3 location).

**Figure 3.** Eigenvalues of principal component numbers for the extraction of polyphenolic compounds (NPPCs) from Acacia senegal gum (ASG).

The PC1 vs PC2 sample score plot is presented in Figure 4, where the biplot of the PC1 x PC2 for ASG reveals approximately 95.50%. Moreover, as shown in Figure 4, different groups (G1, G2, G3, and G4) were detectable for different techniques of ASG extraction. By considering PC1, ultrasonication, maceration, and magnetic stirring techniques using methanol were categorised as G1, shaking techniques as G2, and homogenisation as G3. Values presented by G1 and G3 for all analyses were better than those of other groups. PCA techniques are beneficial for data separation and orthogonality. Orthogonal regression concerning the ASG extracts analysed the relationship between G1, G2, and different ETs such as G3. Thus, PCA can be used for a differential relationship between groups. A visual representation of the ASG biplot model can be seen in Figure 4, when different techniques, such as shaking and homogenisation, were distributed through PC2. Moreover, FRAP values,
CUPRAC, TFC, FCI, and DPPH in G1 (maceration, magnetic stirring, and ultrasonic techniques) were split due to their significantly (p≤0.5) being accepted as an optimal technique for the extraction of NPPCs from ASG.

Furthermore, as shown in Figure 4, a vast majority of ETs are located on the right half of the plot, except for shaking and homogenisation techniques, which are located near the zero points of PC1. Interestingly, UAE and maceration techniques (G1) are located above all other techniques, suggesting that their techniques enable measuring and comparing some of the measured variations remarkably. (Figure 4 location).

Figure 4. Biplot of eigenvalues of PC1 (94.85%) and PC2 (4.66%) for the techniques used to extract phenolic compounds from Acacia seyal gum (ASG) using TFC, FCI, DPPH, FRAP, and CUPRAC.

4. Conclusions

This study investigates the antioxidant activities (AoA) of *Acacia seyal* gum (ASG) using five different natural polyphenolic compounds (NPPCs) extraction techniques [48] and five different stable antioxidant evaluation assays. The results show that the UAE technique can be used as the best technique for extracting the NPPCs from ASG. Compared to other techniques, the UAE technique's use resulted in a significant increase in extraction yield in a short time (less than 1 h). However, maceration can produce a high yield of extraction, but it takes a relatively long time. The use of the UAE extraction technique has an economic benefit, as it is a time-consuming and low-cost procedure, especially for the food and pharmaceutical industries.
Acknowledgement

This research is supported by the postdoctoral scholarship merit programme from the Islamic Development Bank (IsDB) (grant number ID: 2020-276278). The authors also acknowledge the International Institute for Halal Research and Training (INHART) and the Biotechnology Engineering Department for Laboratory, IIUM.

Funding

This research was funded by the Islamic Development Bank (IsDB) as a merit postdoctoral fellowship to the main author (Dr. Ahmed Adam Hassan Mohammedelnour, grant number ID: 2020-276278).

Conflicts of Interest

The authors declare no conflicts of interest.
References

1. Sachdev, S., et al., (2021). Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. Antioxidants. 10(2): p. 277.
2. Arfin, S., et al., (2021). Oxidative Stress in Cancer Cell Metabolism. Antioxidants. 10(5): p. 642.
3. Silwal, P., et al., (2020). Mitochondrial reactive oxygen species: double-edged weapon in host defense and pathologic inflammation during infection. Frontiers in Immunology. 11: p. 1649.
4. Ousji, O. and L. Sleno, (2020). Identification of In Vitro Metabolites of Synthetic Phenolic Antioxidants BHT, BHA, and TBHQ by LC-HRMS/MS. International journal of molecular sciences. 21(24): p. 9525.
5. Ullah, R., et al., (2019). Natural antioxidant anthocyanins—A hidden therapeutic candidate in metabolic disorders with major focus in neurodegeneration. Nutrients. 11(6): p. 1195.
6. Elnour A. M. Mohamed E. S. Mirghani, N.A.K., Djabir Daddiouaissa, Khalid Hamid Musa, Md Z. Alam, and A. Nour Hamid Abdurahman., (2020). Active Fractions of Methanol Crude Obtained from Acacia Seyal Gum and their Antiproliferative Effects against Human Breast Cancer Cells. Global Journal of Science Frontier Research.
7. Ali, Use of Acacia Gum in the Treatment of Skin Lesions of Two Children With Kwashiorkor, in Gum Arabic: Structure, Properties, Application and Economics. 2018, Elsevier. p. 221-228.
8. Mohamed, R.E, M.O. Gadour, and I. Adam, (2015). The lowering effect of Gum Arabic on hyperlipidemia in Sudanese patients. Frontiers in physiology. 6: p. 160.
9. Farman, M.S., M.I. Salman, and H.S.H. Hamad, (2020). Effect of Gum Arabic Administration on Some Physiological and Biochemical Parameters in Chronic Renal Failure Patients. Systematic Reviews in Pharmacy. 11(6): p. 697-701.
10. Mohamed, R.E, M.O. Gadour, and I. Adam, (2015). The lowering effect of Gum Arabic on hyperlipidemia in Sudanese patients. Frontiers in Physiology. 6(MAY).
11. Wapnir, R.A., et al., (2008). Modulation of rat intestinal nuclear factor NF-κB by gum Arabic. Digestive diseases and sciences. 53(1): p. 80-87.
12. Elnour, A.A., et al., (2019). Active Fractions of Methanol Crude Obtained from Acacia seyal gum: Antioxidant Capacity using FTIR Analysis. Borneo Journal of Pharmacy. 2(2): p. 94-107.
13. Xu, D.-P., et al., (2017). Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International journal of molecular sciences. 18(1): p. 96.
14. Alara, O.R., N.H. Abdurahman, and C.I. Ukaegbu, (2021). Extraction of phenolic compounds: A review. Current Research in Food Science.
15. Nawaz, H., et al., (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Brazilian Journal of Pharmaceutical Sciences. 56.
16. Bermúdez-Oria, A., et al., (2019). Strawberry dietary fiber functionalized with phenolic antioxidants from olives. Interactions between polysaccharides and phenolic compounds. Food Chemistry. 280: p. 310-320.
17. Alcântara, M.A., et al., (2019). Effect of the solvent composition on the profile of phenolic compounds extracted from chia seeds. Food Chemistry. 275: p. 489-496.
18. Wang, L. and C.L. Weller, (2006). Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology. 17(6): p. 300-312.
19. Yusuf, M., et al., (2020). Optimization ultrasonic assisted extraction (UAE) of bioactive compound and antibacterial potential from sea urchin (diadema setosum). Current Research in Nutrition and Food Science. 8(2): p. 556-569.
20. Vinatoru, M., T. Mason, and I. Calinescu, (2017). Ultraslonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. TrAC Trends in Analytical Chemistry. 97: p. 159-178.
21. Wen, C., et al., (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops–A review. Ultrasonics sonochemistry. 48: p. 538-549.

22. Elnour, A.A., et al., (2018). Gum Arabic: An Optimization of Ultrasonic-Assisted Extraction of Antioxidant Activity. Studia Universitatis Babes-Bolyai, Chemia. 63(3).

23. Ashraf, W., et al., (2020). Technological Advancement in the Processing of Lycopene: A Review. Food Reviews International.

24. Magangana, T.P., et al., (2020). Processing factors affecting the phytochemical and nutritional properties of pomegranate (punica granatum L.) peel waste: A review. Molecules. 25(20).

25. Yusof, N., M.S. Abdul Munaim, and R. Veloo Kutty. (Year) Ultrasound-assisted extraction propolis and its kinetic study. Institute of Physics Publishing.

26. Khalid, N., et al., (2017). A comprehensive characterisation of safflower oil for its potential applications as a bioactive food ingredient—a review. Trends in Food Science and Technology. 66: p. 176-186.

27. Musa, K.H., et al., (2011). Antioxidant activity of pink-flesh guava (Psidium guajava L.): effect of extraction techniques and solvents. Food Analytical Methods. 4(1): p. 100-107.

28. Zhang, H., et al., (2010). Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (Ziziphus jujuba Mill.) from China. Food and chemical toxicology. 48(6): p. 1461-1465.

29. Musa, K.H., et al., (2013). A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity. Food chemistry. 141(4): p. 4102-4106.

30. Apak, R., et al., (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. Microchimica Acta. 160(4): p. 413-419.

31. Salleh, A.S., et al., Embedding Automobile Safety Rating in Malaysia’s E-Hailing Policy – An Analysis of NCAP-Rated Fleet. p. 8.

32. Thaipong, K., et al., (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of food composition and analysis. 19(6-7): p. 669-675.

33. Chuah, P.N., et al., (2020). Comparative conventional extraction methods of ethanolic extracts of Clinacanthus nutans leaves on antioxidant activity and toxicity. British Food Journal. 122(10): p. 3139-3149.

34. Gordillo, B., et al., (2016). Optimisation of an oak chips-grape mix maceration process. Influence of chip dose and maceration time. Food Chemistry. 206: p. 249-259.

35. Shortle, E., et al., (2014). Influence of extraction technique on the anti-oxidative potential of hawthorn (Crataegus monogyna) extracts in bovine muscle homogenates. Meat science. 98(4): p. 828-834.

36. Sharif, M.F. and M.T. Bennett, (2016). The effect of different methods and solvents on the extraction of polyphenols in ginger (Zingiber officinale). Jurnal Teknologi. 78(11-2): p. 49-54.

37. Dowlath, M.J.H., et al., (2020). Effect of solvents on phytochemical composition and antioxidant activity of cardiospermum halicacabum (L.) extracts. Pharmacognosy Journal. 12(6): p. 1241-1251.

38. Muhammad, D.R.A., et al., (2021). Phytochemical composition and antioxidant activity of Cinnamomum burmannii Blume extracts and their potential application in white chocolate. Food Chemistry. 340.

39. Nagoor Gunny, A.A., T.W. Xiang, and M.H. Che Mat. (Year) Deep Eutectic Solvent for extraction of natural antioxidant from a medicinal plant, Coleus aromaticus. 1st International Conference on Science, Engineering and Technology, ICSET 2020. IOP Publishing Ltd.

40. Yu, L., et al., (2021). Enhanced extraction performance of iridoids, phenolic acids from Eucommia ulmoides leaves by tailor-made ternary deep eutectic solvent. Microchemical Journal. 161.
41. Karabegović, I.T., et al., (2014). The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel (Prunus laurocerasus) leaf and fruit extracts. Industrial Crops and Products. 54: p. 142-148.

42. Bhagya Raj, G.V.S. and K.K. Dash, (2020). Ultrasound-assisted extraction of phytocompounds from dragon fruit peel: Optimization, kinetics and thermodynamic studies. Ultrasonics Sonochemistry. 68.

43. Rakshit, M., P.P. Srivastav, and K. Bhunia, (2020). Kinetic modeling of ultrasonic-assisted extraction of punicalagin from pomegranate peel. Journal of Food Process Engineering. 43(11).

44. Gali, L., et al., (2020). High-pressure homogenization-assisted extraction of bioactive compounds from Ruta chalepensis. Journal of Food Measurement and Characterization. 14(5): p. 2800-2809.

45. Trujillo-Mayol, I., et al., (2020). The relationship between fruit size and phenolic and enzymatic composition of avocado byproducts (Persea americana mill.): The importance for biorefinery applications. Horticulturae. 6(4): p. 1-13.

46. Kurt-Celebi, A., et al., (2020). Accumulation of Phenolic Compounds and Antioxidant Capacity during Berry Development in Black 'Isabel' Grape (Vitis vinifera L. x Vitis labrusca L.). Molecules. 25(17).

47. Zitouni, H., et al., (2020). Phytochemical components and bioactivity assessment among twelve strawberry (arbutus unedo l.) genotypes growing in morocco using chemometrics. Foods. 9(10).

48. Ejikeme, A.R., et al., (2021). Characteristics of Lassa Fever Outbreak in Ondo State, Nigeria, Year 2019. East African Journal of Health and Science. 3(1): p. 115-125.
Table 1. Effect of extraction techniques on the antioxidant activities (AoA) of *Acacia seyal* gum (ASG) determined using total flavonoids content (TFC), folin-ciocalteu index (FCI), DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC).

|                  | Homogenisation | Maceration | Magnetic Stirring | Shaking | Ultrasonication |
|------------------|----------------|------------|-------------------|---------|-----------------|
| TFC              | 12.72±2.26d    | 51.84±1.22c| 58.71±3.07b       | 10.11±0.57d | 66.65±1.65a     |
| RSD%             | 2.22           | 2.36       | 4.38              | 0.49    | 2.47            |
| FCI              | 2.68±0.26b     | 10.86±0.92a| 9.59±1.74a        | 2.75±0.34b | 10.96±0.73a     |
| RSD%             | 0.37           | 0.83       | 0.73              | 0.73    | 0.64            |
| DPPH             | 28.31±0.34c    | 90.55±0.0a | 66.31±0.92b       | 26.63±0.15d | 90.57±0.04a     |
| RSD%             | 1.29           | 0.24       | 6.31              | 0.53    | 0.24            |
| FRAP             | 5.69±0.62c     | 23.23±1.31b| 34.76±1.64a       | 5.74±0.93c | 25.19±2.42b     |
| RSD%             | 0.7            | 5.64       | 4.72              | 1.56    | 5.64            |
| CUPRAC           | 12.79±1.71b    | 57.49±2.54a| 63.10±3.33a       | 10.38±1.04b | 59.36±5.43a     |
| RSD%             | 13.4           | 4.42       | 5.28              | 10.01   | 9.15            |

Results are depicted as a mean ± standard deviation (SD).

Values in each row marked by the same small letter are not significantly different at P≤0.05.

A Milligrams of quercetin equivalent (QE) per 100 g of dry weight.

B Milligrams of gallic acid equivalent (GAE) per 100g of dry weight.

C % Compared to DPPH without added samples.

D & E Milligrams of Trolox equivalent (TE) per 100g of dry weight.

F RSD % relative standard deviation.
Table 2. Pearson’s correlation coefficients of antioxidant activities of various extraction techniques

| Correlation coefficient | TFC  | FRAP  | DPPH  | CUPRAC |
|-------------------------|------|-------|-------|--------|
| **Homogenisation 1 min** |      |       |       |        |
| FRAP                    | 0.845** |       |       |        |
| DPPH                    | 0.826** | 0.999** |       |        |
| CUPRAC                  | 0.839** | 1.000** | 1.000** |        |
| FCI                     | 0.924** | 0.577* | 0.548* | 0.567* |
| **Maceration 24 h**     |      |       |       |        |
| FRAP                    | 0.835** |       |       |        |
| DPPH                    | -0.197 | 0.371ns |       |        |
| CUPRAC                  | 0.805** | 0.999** | 0.422ns |        |
| FCI                     | 0.886** | 0.995** | 0.273ns | 0.988** |
| **Magnetic Stirring 1 h** |  |       |       |        |
| FRAP                    | 0.999** |       |       |        |
| DPPH                    | 0.957** | 0.947** |       |        |
| CUPRAC                  | 0.968** | 0.976** | 0.854** |        |
| FCI                     | 0.756*  | 0.732*  | 0.913** | 0.567* |
| **Shaking 1 h**         |      |       |       |        |
| FRAP                    | 0.979** |       |       |        |
| DPPH                    | 0.999** | 0.988** |       |        |
| CUPRAC                  | 0.965** | 0.893** | 0.951** |        |
| FCI                     | 0.866** | 0.747* | 0.840** | 0.967** |
| **Ultrasonication 1 h** |  |       |       |        |
| FRAP                    | 0.699*  |       |       |        |
| DPPH                    | 0.995** | 0.765* |       |        |
| CUPRAC                  | 0.974** | 0.517* | 0.947** |        |
| FCI                     | 0.991** | 0.598* | 0.974** | 0.995** |
a Total Flavonoids Content, b Ferric reducing antioxidant power, c DPPH radical scavenging activity, d Cupric reducing antioxidant capacity, f Folin-Ciocalteu index (FCI).

*Significant, and **highly significant different respectively at P≤0.05* and P≤0.01**.
Figure 1

Acacia seyal gum (ASG)

1. Sieving 1.40 mm
2. Homogenization for 1 min, & 24,000 rpm, at 23.0°C
3. Acacia seyal gum (ASG) Powder
4. Maceration for 24 h, at 23.0°C
5. Magnetic Stirrer for 1 h, & 1,000 rpm, at 23.0°C
6. Shaking for 1 h, & 300 rpm, at 23.0°C
7. Ultrasonication for 1 h, & 12 kHz, at 23.0°C

SCREENING METHODS OF EXTRACTION USING MACHINE INTEGRATION

DPPH
FRAP
TPC
TFC

PTFE 0.45 μm

Supernatants

ASG extract after adding reagents

Microplate Reader

Microplate 96

Store at 4°C

Downloaded from https://academic.oup.com/fqs/advance-article/doi/10.1093/fqsafe/fyab034/6506486 by guest on 19 January 2022
Figure 3

Scree Plot

Cumulative Variability

Eigenvalue Variability (%) Cumulative Cumulative

Principal Component Number

PC1 PC2 PC3 PC4
Figure 4

Biplot (axes F1 and F2: 99.51 %)

- Shaking 1h
  - Homogenizer 1min
- Magnetic Stirrer 1h
- Ultrasound 1h
- Maceration 24h
- FRAP
- CUPRAC
- DPPH
- TFC
- FCI

G1
G2
G3