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Extraction and Determination of Bioactive Compounds with Mineral Evaluation from Grape and Date Seeds: Concerning their Applications in Antioxidant and Antibacterial Activity

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ABSTRACT

An optimized extraction method incorporating maceration has been employed to isolate bioactive compounds from four seed types: black and white grape seeds, and ajwa and barhi date seeds. These extracted materials were evaluated for antioxidant and antibacterial activities, along with quantification analysis performing of total phenolics, flavonoids, betalains, chlorophyll (a and b), carotenoids, anthocyanins, and mineral content. UV-VIS spectrophotometry was revealed a high phenolic concentration across all samples, showing (729.49±14.50 mg/g dry Weight (DW)), (660.80±8.82 mg/g of DW), (658.24±0.37 mg/g of DW), (643.78±2.39 mg/g of DW) black grape seed, white grape seed, barhi date seed and ajwa date seed respectively. Additionally, flavonoids showed significant levels, particularly in black grape (41.99±0.06 mg/g DW) and ajwa date seeds (41.5±0.05 mg/g DW). While other phytochemicals were revealed in lower concentrations. Phytochemicals such as anthocyanin, betalain, carotenoid, chlorophyll a and b were found in smaller amounts. Also, GC–MS analysis was employed to identify and characterize bioactive compounds in the extracts. The results revealed a variety of phytochemicals, including several previously unreported compounds. The evaluated antioxidant activity of the four seed extracts varied between 72.43% and 91.28%, signifying substantial radical scavenging capability. Antibacterial effect was evaluated using five bacterial strains confirmed inhibitory effects of the extracts. Mineral profiling via ICP-MS identified potassium, calcium, phosphorus, sulfur, and magnesium as dominant elements across all seed types. These findings underscore the nutritional and therapeutic potential of grape and date seeds as sources of natural antioxidants and antimicrobial agents.

1.Introduction

Research on seeds is crucial for various fields, including population survival, adaptation to environmental changes, food production, native plant conservation, and restoration efforts. Additionally, consumer demand for healthy food options has been risen in recent years, prompting the food industry to proactively address this challenge (Everingham et al., 2023). In general, the plant seeds regarded as by-products are secondary products, derived from primary agro-food production processes. They serve as a valuable and cost-effective source of potentially beneficial ingredients, such as peptides, carotenoids, and phenolic compounds, thereby promoting the concept of a circular economy (Elkatry et al., 2022) .

Grape seeds (*Vitis vinifera L.*) are the main industrial by-products generated from grape processing industries, including grape juice and wine production (Dávila et al., 2017). It is a prospective source of bioactive chemicals capable of preventing primary and secondary thrombotic diseases (El-Baky, Amara and Redwan, 2023). Grape seed is a heterogeneous matrix of fiber, oil, protein, complex phenols, tannins, sugars, and minerals (Nowshehri, Bhat and Shah, 2015). Significant promise has been shown for this by-product as a source of nutraceutical products (El-Baky, Amara and Redwan, 2023). Epicatechin and catechin are two of the polyphenols they contain (Pozzo et al., 2023), gallocatechin, epicatechin 3-*O*-gallate, epigallocatechin, and gallic acid (El-Baky, Amara and Redwan, 2023). Furthermore, flavonoids like procyanidin trimers, dimers, and more widely polymerized procyanidins (Aldubayan, 2018; El-Baky, Amara and Redwan, 2023), and anthocyanin (Ahmed et al., 2022; Pozzo et al., 2023). Polyphenols from grape seeds have shown promise for health, including cardio-protection (Nunes et al., 2016), antioxidative (Libera, Latoch and Wójciak, 2020), immunomodulatory anti-inflammatory (Lai et al., 2023), and antitumor effects (El-Baky, Amara and Redwan, 2023). An important reservoir of minerals, such as calcium, phosphorus, potassium, and iron, has been the subject of numerous investigations (Elkatry et al., 2022). The seeds of grape have the most antioxidant activity among its multiple components, followed by the peel and pulp, according to numerous studies (Yilmaz et al., 2014). According to (Elkatry et al., 2022), grape seeds have a great deal of biological potential that can be used to extract bioactive substances and produce semifinished products and extracts that are useful for agronomy, dermatological applications, and cosmetics (Tao et al., 2022). This seed is rich in certain minerals, including calcium, phosphorus, potassium, and iron (Elkatry et al., 2022). Some phytochemicals and their applications derived from grape seed are illustrated in Figure 1.

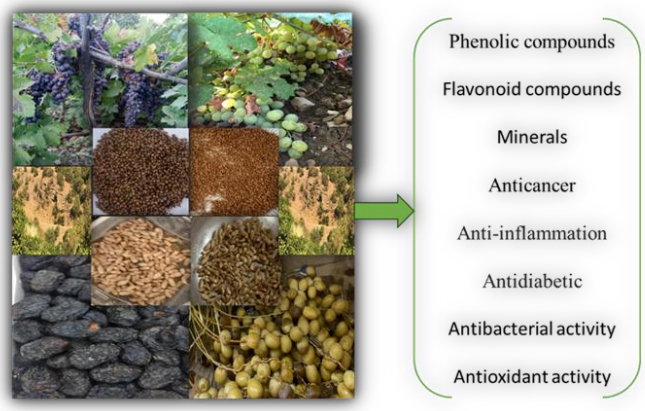

Figure 1: Phytochemical properties and applications of grape and date seed.

Phoenix dactylifera, showed in Figure 1, commonly known as the date palm, is a species of plant in the arecaceae family of palms. It is a significant plant in dry and semiarid regions of many Middle Eastern and North African nations (Mrabet et al., 2022). Date by-products, including date seeds, are abundant in nutraceuticals (Ghafoor et al., 2022). Date seeds comprise moisture, protein, fat, and ash (Hribesh, 2020). Consequently, these seeds may be regarded as a significant source of phenolics, predominantly in the form of phenolic acids, particularly gallic acid (Adheem et al., 2021), syringic acid (Adheem *et al.*, 2021; Alfaleh and Sindi, 2024), and catechins (Hilary et al., 2021), and flavonoids with a higher anthocyanin (Zarie et al., 2023), and contents (Himanshu et al., 2024), and also a rich source of carotenoids (Zarie et al., 2023). Date seeds

contain various essential minerals, including potassium, magnesium, calcium, phosphorus, sodium, and iron, making them a valuable mineral source (Achour et al., 2022). Extensive research has been conducted on the pharmacological benefits of date seeds, including their antioxidant properties (Shams Ardekani et al., 2010), anti-inflammation, antidiabetic, antibacterial, and antiviral properties (Saryono et al., 2020). They are essential components of the cosmetics industry and offer a significant supply of bioactive compounds (Alharbi, Raman. Ph.D and Shin, 2021). Bioactive components are extracted, isolated, identified, and quantified as part of the development of nutraceuticals. When recovering bioactive compounds from plant matrices, extraction is a crucial step. (Ghafoor et al., 2022). Various extraction methods, from conventional to modern, each with unique benefits and drawbacks. Because of their simplicity and ease of use, conventional methods are favored; however, they have drawbacks, such as lower extraction efficiency and longer extraction times, which negatively impact biomolecules. By breaking down cell walls and increasing mass transfer rates by creating microcavities, the ultrasonication-assisted extraction method (UAE) solves these problems and shortens extraction times while using less solvent (Niroula et al., 2024). Extraction is often conducted by a solvent-based method (Ghafoor et al., 2022).

In order to improve the extraction of bioactive ingredients from various grape and date seeds that are sourced from different regions, this project aims to develop and optimize a new, straightforward, and cost-effective combination extraction method that uses dynamic maceration and ultrasonication. This is because different extraction techniques, such as Soxhlet extraction, cold maceration, microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), exhibit variations in efficiency, solvent utilization, energy demands, selectivity, and cost. The amount of different metabolites, including betalain, carotenoid, chlorophyll, polymeric anthocyanin, and total phenolic and flavonoid were measured using spectrophotometric techniques. Bioactive chemicals in the extracts were identified and characterized using GC-MS analysis. The mineral compositions were thoroughly analyzed using inductively coupled plasma mass spectrometry (ICP-MS), and assess antioxidant activity along with biological activities.

2. Materials and Methods

2.1. Instruments

Using an ultraviolet-visible (UV-VIS) spectrophotometer (UV-6100PC; EMCLAB; Germany) equipped with a tungsten halogen light source with a wavelength range of (200–1010) nm and 1 cm matched quartz cells, as well as gas chromatography-mass spectrometry (GC-MS) (GC 7890A, AGILENT), (MS 5975C, MODE EI), infrared spectroscopy (IR Spectroscopy SHIMADZU), and inductively coupled plasma mass spectrometry ICP-MS (Agilent 7900 USA RF generator Power). A DENVER instrument model, rotary evaporator, ultrasonic cleaner (VCLEAN 1- L2, Iran), centrifuge (BIOFUGE PRIMO), shaker (Heidolph Unimax 1010), electronic balance (KERN, ABS), hotplate, incubator (HERA THERM,Thermo scientific), thermometer, and micropipettes measuring 1-10 µl (United), 20-200µl, and 100-1000µl (LAMTEK).

2.2. Samples and Chemicals

2.2.1. Chemicals

All of the compounds were of the analytical reagent quality: deionized water (DIW) was used to create the solutions, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetine, iodine, potassium iodide, potassium hydroxide, ferric chloride, sodium hydroxide, alpha-Naphthol, ninhydrin reagent, sodium carobonate, aluminum chloride, potassium acetate, potassium chloride, sodium acetate, Ethanol (99%), chloroform, ammonia, acetic anhydride, acetic acid, benzene, acetone, hydrochloric acid, and sulfuric acid.

2.2.2. Plant Materials

White grape seed (WGS) and black grape seed (BGS) were collected (36.54313° N, 44.53497° E) from Rwandz city, Kurdistan Region, Iraq (August 2024), see Figure 2. Two Varieties of the date were used, one of them is the barhi date seed (BDS) (33°41'22.6"N 44°39'32.6"E) from Baqubah city, Iraq (September 2024), and other ajwa date seed (ADS) (24.4969587, 39.6030082) from

Madinah city in Saudi Arabia (August 2024), see Figure 3.

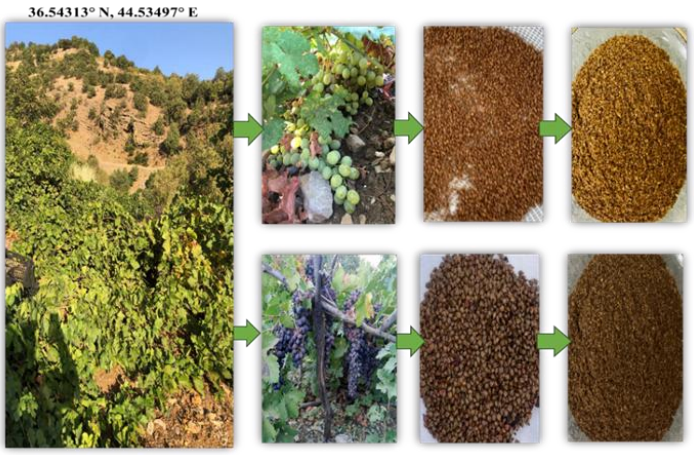

Figure 2: Preparation of grape seed powder.

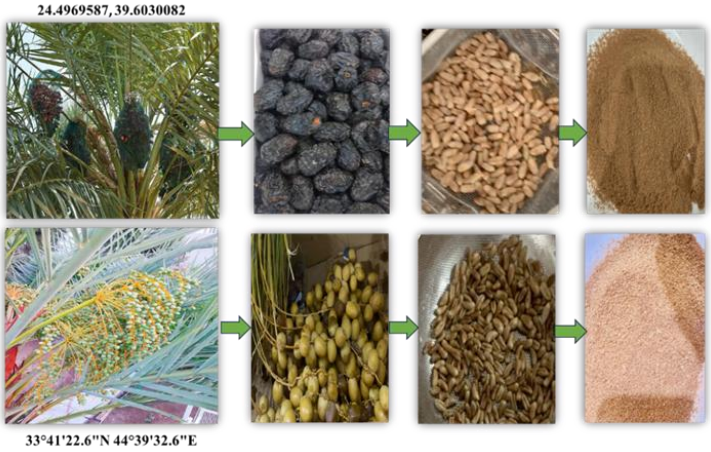

Figure 3: Preparation of date seed powder.

2.3. Production of Grape and Date Seed Powder

Grape and date seeds were separated from the skin. Then the seeds were washed by deionized water (DIW), dried at room temperature for seven days in a dark place, then stored at 4℃ for further analysis. For each experiment, a certain amount of dried seeds was grinded to powder by using (Multi-purpose High-speed Crusher).

2.4. Preparation of Date and Grape Seed Extracts

2.4.1. Preparation of Date and Grape Seed Extracts by Conventional Method

An amount of 1 g of each seed powder BDS, ADS, BGS, and WGS was dissolved in various solvents including ethanol and deionized water (Et: DIW, 1:1), methanol (Me), and a methanol and DIW mixture (1:1) in four separate beakers. In all extraction methods, the mixtures underwent an initial extraction process followed by centrifugation at 4000 rpm for 10 minutes. The resulting supernatants were decanted, filtered using filter paper, concentrated using a rotary evaporator, and the concentrated extracts were transferred into pre-weighed, clean glass containers. These were then incubated at laboratory temperature until dry powders were obtained. The extraction yield was calculated using Equation (Eq.) (1). While the general procedure remained consistent, variations existed among the methods: in method (a), the solutions were heated at 60–67 °C for 1 hour and then left at room temperature on a shaker 120 rpm for 30 minutes; in method (b), the mixtures were shaken overnight at room temperature 120 rpm followed by heating at 30–40 °C for 30 minutes; and in method (c), the solutions were shaken overnight at room temperature 120 rpm, followed by incubation in the dark at room temperature for five days to enhance the extraction of bioactive compounds. These procedural differences were designed to evaluate the impact of extraction conditions on yield and bioactivity of the seed extracts.

$$\text{Yield } \% = \frac{\text{weight of extract}}{\text{weight of seed sample}} \times 100 \qquad \text{Eq. (1)}$$

2.4.2. Preparation of Date and Grape Seed Extracts by Ultrasonication-Assisted Extraction (UAE) Method

An amount of 1 g of BDS, ADS, BGS, and WGS seed powder was dissolved in various solvents as described in section 2.4.1 with each sample prepared in a separate beaker. The solutions were placed in a water bath at 30 °C and subjected to sonication using a VCLEAN 1-L2 ultrasonic cleaner (Iran) for 90 minutes to enhance the extraction of bioactive compounds. Following sonication, the samples were immediately centrifuged at 4000 rpm for 10 minutes, and the supernatant was collected by decantation. The resulting liquid was filtered using filter paper, and the filtrate was concentrated using a rotary evaporator. The concentrated extracts were then transferred into clean, pre-weighed glass containers and left to dry at laboratory

temperature in an incubator. The extraction yield of each sample was calculated using Eq. (1).

2.5. Phytochemical Tests

Phytochemical screening of seed extracts (SEs) was carried out using various qualitative tests to identify bioactive compounds based on color changes or precipitate formation. Alkaloids, tannins, flavonoids, carbohydrates, saponins, phenolics, cardiac glycosides, and amino acids were successfully detected using standard reagents. Tests such as Wagner's, ferric chloride, Shinoda, Molisch, frothing, and ninhydrin indicated their respective compounds. coumarins and steroids were identified through the Coumarin and Salkowski tests, while terpenoids were assessed via the Liberman test (Govindappa, 2014; Sumanta et al., 2014; Priya and Ravindhran, 2015; Jaganathan, Shanmugavadivu and Ganesh, 2018; Dahanayake et al., 2019a; Dahanayake et al., 2019b; Shaikh and Patil, 2020; Ourradi et al., 2022; Krasteva et al., 2023; Nalado and Tijjani, 2023; Ahmed and Saddam, 2024). The detailed procedures for all phytochemical analyses are provided in the electronic supplementary information (ESI), section materials and method.

2.6. The Total Phenolic Content (TPC)

The total phenolic content (TPC) in the SEs were determined by the Folin-Ciocalteu reagent by using UV–VIS spectrophotometer (UV-6100PC; EMCLAB; Germany), which method as described by (Ourradi et al., 2022) with some modifications. A 0.5 mL aliquot of suitably diluted extracts was introduced into a 10 mL volumetric flask. The seed extract was combined with 2.5 ml of Folin-Ciocalteu reagent, and after 2 minutes, 2 ml of 7% Na_2CO_3 was added and diluted to the volumetric (10 mL) with DIW. Subsequently, incubated for one hour, and the absorbance was measured at 755 nm against the prepared blank by UV–VIS spectrophotometer. Different concentrations of gallic acid (5, 10, 25, 50, 100, 150, 200, 250, 300 µg/mL) were generated to establish the standard calibration curve.

2.7. Total Flavonoid Content (TFC)

The quantification of total flavonoids was conducted via a UV–VIS spectrophotometer (UV-6100PC; EMCLAB; Germany),which method as described by (Hogan et al., 2009) with some modifications, A 0.5 ml aliquot of suitably diluted SEs were introduced into a 5 mL volumetric flask, 0.2 mL of 10% aluminium chloride ($AlCl_3$) and 0.2 mL of 1M potassium acetate were added to the solution, followed by the addition of 3 mL of ethanol. The mixture was then diluted to a 5 mL volumetric flask and incubated for 1 hour. The absorbance was measured at 420 nm against the prepared blank. The outcome of the determination was quantified in terms of quercetin present in the extract. Different concentrations of quercetin (1, 3, 5, 10, 15, 25, 30, 40, 50 µg/mL) were generated to establish the standard calibration curve.

2.8. Chlorophyll a and b and Carotenoid Content

Precisely weighed 0.01g of SEs and homogenized in a tissue homogenizer with 5 mL of various extractant solvents such as 80% acetone, and 95% ethanol. The homogenized sample mixture was centrifuged at 10,000 rpm for 15 minutes. The solution combination was tested for chlorophyll a (Ch a), chlorophyll b (Ch b), and carotenoid (C) concentration using UV-VIS spectrophotometer (UV-6100PC; EMCLAB; Germany). The equations for quantifying Ch a, Ch b, and C using various extractant solvents are described in Table 1.

Table 1: Equations for calculating concentrations of chlorophyll a (Ch a), chlorophyll b (Ch b), and total carotenoids (C) using two different extractant solvents in a spectrophotometer (Sumanta et al., 2014).

Solvents	Equations
80% Acetone	$Ch\ a=12.25\ A_{663.2} - 279\ A_{646.8}$ Eq. (2) $Ch\ b=21.5\ A_{646.8} - 5.1\ A_{663.2}$ Eq. (3) $C = (1000\ A_{470} - 1.82\ C_a - 85.02\ C_b)/198$ Eq. (4)
95% Ethanol	$Ch\ a=13.36\ A_{664} - 5.19\ A_{649}$ Eq. (5) $Ch\ b=27.43\ A_{649} - 8.12\ A_{664}$ Eq. (6) $C = (1000\ A_{470} - 2.13\ C_a - 97.63\ C_b)/209$ Eq. (7)

2.9. The Betalain (BL) Content

The quantity of betacyanins and betaxanthins in the extracts was quantified spectrophotometrically, measured at 538 nm for betacyanins and 480 nm for betaxanthins.The betalain (BL) content was determined using the following Eq:

BLC [mg L^{-1}] = (A × DF × MW × 1000) / (ϵ × 1) Eq. (8)

where A represents the absorption value, DF denotes the dilution factor, and 1 indicates the path length (1 cm) of the cuvette. The molecular weights (MW) and molar extinction coefficients (ϵ) for betacyanins (Bc) and betaxanthins (Bx) are as follows: Bc has a MW of 550 g mol^{-1} and a ϵ of 60,000 L $mol^{-1}cm^{-1}$ at λ = 538 nm in H_2O, whereas Bx has a MW of 339 g mol^{-1} and a ϵ of 48,000 L $mol^{-1}cm^{-1}$ at λ = 480 nm in H_2O.

2.10. Total Anthocyanin (AC) Content

Determination of total anthocyanin (AC) content was determined using the pH-differential method as by (Krasteva et al., 2023). An amount of 250 µL SEs solutions were mixed with 150 µL of 0.2% (w/v) potassium chloride buffer solution (pH 1.0), and 250 µL of SEs solution was mixed with 3% (w/v) sodium acetate buffer solution (pH 4.5). Both solutions were left for 15 min. The absorbance of each solution was measured at 520 nm and 700 nm. The absorbance (A) of the diluted sample was calculated as follows:

$A = (A_{520} – A_{700})$ pH 1.0 – $(A_{520} – A_{700})$ pH 4.5 Eq. (9)

The AC pigments concentration was calculated using this equation:

Total Anthocyanin = A x MW x DF x 1000 / ε x 1 Eq. (10)

The total AC content was calculated as cyanidin-3-glucoside equivalents using the extinction coefficient of 26,900 L cm^{-1} mg^{-1}, molecular weight (MW) of 449.2 g mol^{-1}, and the appropriate dilution factor (DF).

2.11 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The process was carried out by using GC-MS in accordance with the methodology described by (Hagr, Adam and Mohammed, 2021), with some modifications. The quantitative analysis of the grape and date seed extracts was conducted using the GM-MS technology and a capillary column (HP-5 ms-30 m×0.25 mm×0.25 µm). 1 µL of the sample was injected using split mode (80.1), with helium as the carrier gas at a flow rate of 1 mL/min. The temperature program commenced at 60 °C for 4 minutes, increasing at a rate of 3 °C/min to 100 °C for 2 minutes, followed by a rate of 4 °C/min for 5 minutes until reaching a final temperature of 260 °C. The injection port temperature was set at 260 °C, and the auxiliary (transfer line) temperature was maintained at 280 °C. The material was analysed in scan mode within the range of 50-550 m/z, with a total run time of 60 minutes. The components of the sample were identified by comparing their retention durations. Mass fragmentation patents were compared with those accessible in the library of the National Institute of Standards and Technology (NIST), and the findings were documented.

2.12. Evaluation of Antioxidant Activity

The hydrogen atom and electron donation properties of the seed extracts (SEs) were assessed using the decolorization of a purple ethanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl). This spectrophotometric assay was conducted with the stable radical DPPH as a reagent, as described by (Fazeli-Nasab et al., 2023) with some modifications. In summary, 1 mL of each sample (75-50-25-12.5 µg/mL) was combined with 1 mL of a freshly prepared 0.1 mmol/L DPPH ethanolic solution in the absence of light. Following a 30-minute incubation at ambient temperature, the absorbance was measured at 516 nm. Ascorbic acid was employed as a positive control. The radical-scavenging activities (RSA %) of the samples were assessed as an inhibition ratio utilizing Eq. (11):

Antioxidant Activity % = (A blank – A sample / A blank) × 100 Eq. (11)

A blank represents the absorbance of the control, which includes all reagents except the test compound, whereas A sample indicates the absorbance of the test compounds. The inhibition percentages of all samples at different

concentrations were plotted and evaluated to ascertain the extract concentration resulting in 50% inhibition (IC50).

2.13. Determination of Minerals by ICP-MS

The determination of minerals was conducted by ICP-MS following the approach outlined by (Sadee, 2022), with some modifications. Transfer 0.1 grams of the SEs into four uncontaminated falcon tubes. Introduce 5 mL of nitric acid (HNO_3) to the samples and maintain them at room temperature for 24 hours to facilitate complete reaction with the acid. Heating: After 24 hours, immerse the falcon tubes in a beaker containing water and apply heat with a laboratory heater until the sample is completely dissolved in the acid. After the sample is completely dissolved, filter the solution and dilute the filtrate with distilled water to get a final volume of 15 mL. The preliminary data acquired from the ICP-MS apparatus are then analyzed, and to derive the final results, these values are multiplied by the dilution factor. The dilution factor is determined by dividing the final volume 15 mL by the initial mass of the sample (0.1g).

2.14. Antibacterial Activity

A well diffusion assay was performed to assess the antibacterial activity of grape seed and date seed extracts against five bacterial strains: *Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli.* Each bacterial strain was inoculated into nutrient broth and incubated at 37 °C for 18–24 hours. Using a sterile cotton swab, the bacterial cultures were evenly spread over Mueller-Hinton agar plates. Wells of approximately 8 mm diameter were created on the agar surface using a cork borer or sterile micropipette tip. Various concentrations of the seed extracts (1, 10, 50, and 100 mg/mL) were added to the respective wells. Standard antibiotic discs served as positive controls to validate and compare the results: Imipenem (IPM) and Gentamicin (GN) were applied against *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Tetracycline (TE) was used for *Staphylococcus aureus* and *Enterococcus faecalis*; Amoxicillin–Clavulanic Acid (AMC) was also tested on the Gram-positive strains but showed no inhibitory effect. Ethanol and distilled water, without extracts, served as negative controls. All plates were incubated aerobically at 37 °C for 24 hours, after which antibacterial activity was determined by measuring the zones of inhibition surrounding each well.

2.15. Fourier-Transform infrared Spectroscopy (FTIR)

Fourier-transform infrared (FTIR) spectroscopy was used to identify the functional group present in the sample using Shimadzu, ATR-FTIR spectrophotometer (Single bounce diamond ATR). Attenuated Total Reflectance (ATR) is a measurement technique used in IR spectroscopy. The powdered sample of each extract was kept on the sample holder and scanned at a resolution of 4 cm^{-1} with a scan range of 4000–400 cm^{-1}.

2.16. Statistical Analysis

Statistical analyses were performed using the (Excel Software version (2021), and OriginPro 2025). The experiments were conducted in triplicate. The results are shown as mean values ± standard deviation (SD). One way analysis of variance (ANOVA) was considered significant difference among unequal variance between BGSE and WGSE, and also between ADSE and BDSE for phenolic, flavonoid content, total AC content, BL content and Ch a and b and carotenoid content. One way analysis of variance (ANOVA) at a 5% significance level to determine statistically significant difference to antioxidant activity between all SEs were applied. Correlations between parameters were determined by using Pearson's correlation coefficient (r) between antioxidant activity for extracts with vitamin c.

3. Result and Discussion

3.1. Different Extraction Conditions

The results of the UV-VIS spectrophotometric analysis clearly indicate that the maceration method was more effective than ultrasound-assisted extraction (UAE) in isolating bioactive compounds from both grape seed (WGSE) and date seed (ADSE) samples. WGSE samples extracted with aqueous ethanol (Et + DIW, 1:1) over five days showed the highest absorbance

intensity, particularly within the 250–300 nm and 300–400 nm ranges, corresponding to phenolic compounds and flavonoids or non-flavonoid polyphenols, respectively. Similarly, optimal extraction of ADSE was achieved by maceration at 60 °C for one hour using the same solvent system, as shown in Figures 4 and 5. The peak numbers, absorbance strength, and spectral shifts (red or blue) across different solvent conditions further highlight how extraction parameters influence the chemical profile of the extracts. Compared to extracts prepared with only methanol (Me), ethanol (Et), or methanol-water mixtures (Me + DIW), aqueous ethanol consistently yielded superior spectral results. These findings are consistent with *(Philippidis et al.*, 2020), who linked spectral bands to specific bioactive compound groups. Quantitative data support these observations: maceration yielded between 12.3–18.6%, whereas UAE produced lower yields (5.9–11%), as shown in Table 2. This trend was observed across all four seed samples, including WGSE, ADSE, BGSE, and BDSE. These results align with (Krasteva et al., 2023), who reported similar yields (12–18%) from various grape seed varieties using conventional extraction methods. Additionally, (Jaganathan, Shanmugavadivu and Ganesh, 2018), emphasized that the choice of solvent significantly affects yield in date seed extractions, reporting 32.8% with ethanol and 23.6% with methanol, which supports the high efficiency of ethanolic solvents observed in this study.

Furthermore, the variation in extract composition and yield emphasizes the need to optimize extraction parameters depending on the plant source and desired compounds. (Silva et al., 2021) noted, the solvent type, extraction method, plant variety, and plant fraction all play vital roles in influencing the recovery and quality of phytochemicals.

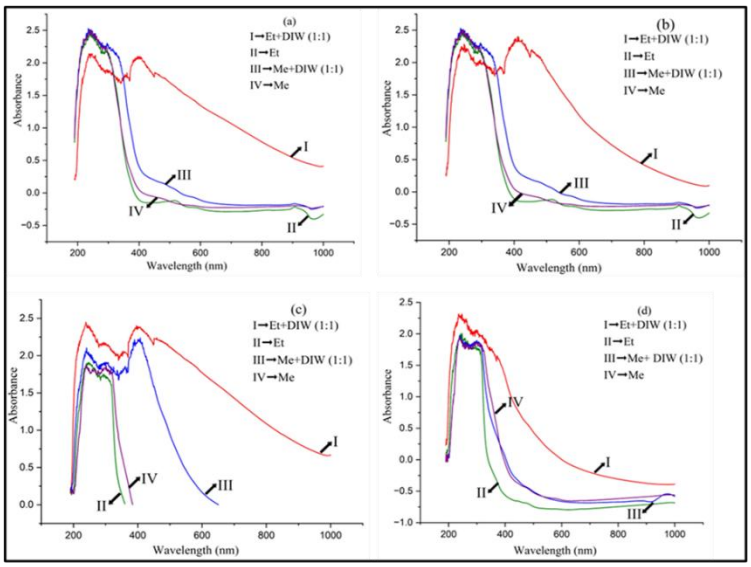

Figure 4: WGSEs' UV-VIS spectra using two techniques and various solvents. The techniques are: (a-c) maceration under various circumstances. The methods are: (a) heating the sample (1 hr at 60 °C), (b) incubating the sample for 24 hrs (heating for 30 min at 60 °C), (c) incubating the sample for 5 days (heating for 30 min at 60 °C), and (d) UAE.

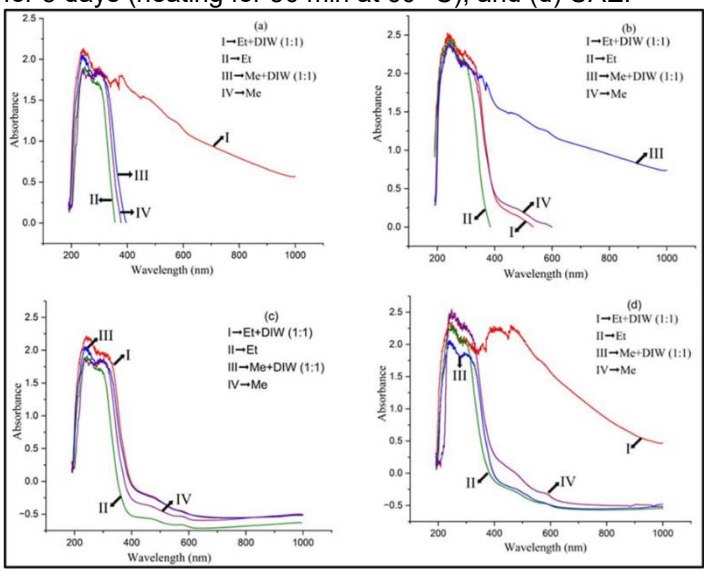

Figure 5: The UV-VIS spectra of ADSEs using two methods and different solvents. The methods include: (a-c) maceration with different conditions (a) heating the sample (1 hr at 60 °C), (b) incubating the sample for 24 hrs (heating for 30 min at 60 °C), (c) incubating the sample for 5 days (heating for 30 min at 60 °C), and (d) UAE.

Table 2: The extraction yield of four different seed samples (BGSE, WGSE, ADSE, and BDSE) using maceration and UAE and 1:1 ratio of Et+ DIW.

Extracts	Yield %	
	Maceration extraction	UAE
BGSE	12.3%	6.20%
WGSE	11.1%	5.93%
ADSE	18.56%	11.03%
BDSE	17%	10.54%

3.2. Phytochemical Screening

Phytochemicals are non-nutritive compounds derived from plants that possess protective or disease-preventive attributes. Plants synthesize these chemical compounds for self-defense, and they are also thought to prevent from certain illnesses (Hassan, Mahmoud and Mahmoud, 2015). *Table S 3.1 in ESI* presents the findings of the qualitative phytochemical analysis of four chosen samples, including BGSE, WGSE, ADSE, and BDSE, utilizing Et+DIW (1:1) as an extracted solvent.

The examination of all extracted seeds indicates the presence of all analyzed phytochemicals: alkaloids, tannins, phlobatannins, free anthraquinones, combined anthraquinones, flavonoids, carbohydrates, saponins, phenols, cardiac glycosides, steroids, proteins, coumarins, and anthocyanins. The outcomes of this investigation align with prior research findings (Hassan, Mahmoud and Mahmoud, 2015; Ahmed and Saddam, 2024) who detected flavonoids, alkaloids, steroids, anthocyanins, saponins, and cardiac glycosides in the examination of grape seeds. The date seed examination by (Eze-Steven, Onyishi and Mamah, 2021), and by (Jaganathan, Shanmugavadivu and Ganesh, 2018) also showed the presence of most phytochemical compounds that is identified in our research.

3.3. Total Phenolic Content (TPC)

The estimation of TPCs in the four extracted samples named (BGSE, WGSE, ADSE, BDSE) were examined using (Folin –Ciocalteu). The calibration curve was plotted using a standard gallic acid (GA equivalent), see *(Figure S 3.2)* in *ESI.* Table 3 displays the TPC of several extracts (BGSE, WGSE, ADSE, BDSE). All extracts demonstrate a significant content of phenolic chemicals, ranging from 643.78 to 729.49 mg GAE/g of dry Weight (DW). The highest phenolic content was found in the seeds of BGSE was (729.49mg GAE/g of DW), while TPC in WGSE was (660.80 mg GAE/g of DW). In this study the ANOVA test performed on TPC of BGSE and WGSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). In the study by (Krasteva *et al.*, 2023), the TPC of various grape seed varieties was found to range from 79.06 to 111.22 mg GAE/g of DW; this result is lower than our study. The findings of this study are in line with other grape seed variety analyses conducted by (Guler and Turgut, 2021), which found phenolic levels between 646.50 and 1120.78 mg GAE/g of DW. But in this study, date seeds had the lowest TPC when compared to grape seeds, which had a BDSE of 658.24 mg GAE/g of DW and an ADSE of 643.78 mg GAE/g of DW. Additionally, in our study the ANOVA performed on the TPC of ADSE and BDSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). The TPC of date seed in the recent study by (Himanshu et al., 2024), which was 448 mg of GAE/100 g of DW. Additionally, (Shi et al., 2023) in their other study on date seed, they discovered that the TPC of samples extracted with their use of ultrasonic technology was 18.53 mg GAE/g.

Table 3: Total phenolic, flavonoid, and anthocyanin content in extracts (BGSE, WGSE, ADSE, BDSE).

Extracts	TPC mg/g of DW ±SD	TFC mg/g of DW ±SD	TAC mg/g of DW ±SD
BGSE	729.49±14.50	41.99±0.06	0.20±0.03
WGSE	660.80±8.82	19.48±0.06	0.115±0.02
ADSE	643.78±2.39	41.50±0.05	0.10±0.02
BDSE	658.24±0.37	21.55±0.05	0.01±0.00

3.4. Total Flavonoid Content (TFC)

Flavonoids are a category of polyphenolic chemicals commonly located in the flowers, leaves, and seeds of various plants as secondary metabolites. Glycosides are predominantly found in plant cell vacuoles and are classified into seven distinct subclasses based on their structure: flavanols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones. These polyphenols are receiving heightened interest owing to their biological characteristics (Shen et al., 2022). The estimation of TFCs in the four extracted samples named (BGSE, WGSE, ADSE, BDSE) were examined. The calibration curve was plotted using a standard quercetin (Q) see *(Figure S 3.3) in ESI*. Table 3 displays the TFC of several extracts (BGSE, WGSE, ADSE, BDSE). All extracts demonstrate a high concentration of flavonoid content, ranging from 19.48 to 41.99 mg QE/g of DW. In this study TFC determined in BGSE was 41.99 mg QE /g of DW while in WGSE was 19.48 mg QE /g of DW. In this study the ANOVA test was performed on TFC of BGSE and WGSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). The TFC in the previous work by (Kamah et al., 2025) was found to vary from 24.50 to 40.70 mg QE/g of grape seed, which is in line with our findings. Over the course of the study, TFC was also determined to be 41.50 mg QE/g of DW in ADSE and 21.55 mg QE/g of DW in BDSE. Additionally, in our study the ANOVA performed on TFC of ADSE and BDSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). The TFC in several date seeds, however, varied slightly from the findings of our analysis, ranging from 78.35 to 141.78 mg QE/100 g in the study published by (Ghafoor et al., 2022) and from 83.98 to 94.97 mg QE/100 g in a study by (Warnasih et al., 2020) due to the variations in date seed varieties and the role that environmental conditions play in producing a variety of results.

3.5. The Anthocyanins (AC) Content

Anthocyanins are pigmented chemicals and constitute a subclass of dietary flavonoids. Their coloration mostly relies on the pH of the fruit, chemical composition, and glycoside substituents on the anthocyanidin core. Research involving in vitro experiments demonstrate their significant biological qualities, including antioxidant, anti-inflammatory, antibacterial, and anti-carcinogenic actions(Padayachee et al., 2012). This work employed a spectrophotometric approach to quantify AC concentration in all extracts (BGSE, WGSE, ADSE, BDSE), as presented in Table 3. The study's results showed that the amount of AC was 0.11 mg/g of DW in WGSE and 0.20 mg/g of DW in BGSE. In this study the ANOVA performed on AC of BGSE and WGSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). In a recent study, (Krasteva et al., 2023) discovered that while one variety of grape seed extract had no AC at all, the amounts of AC in four different types of seed extracts varied from 0.04 to 0.06 mg/g DW for three varieties. Additionally, the AC in this study was found to be 0.10 mg/g DW in ADSE and 0.01 mg/g DW in BDSE. In our study the ANOVA performed on AC of ADSE and BDSE varieties revealed a statistically significant difference ($F > F$ critical, $p < 0.05$). The AC concentration in various date fruit seed kinds ranged from 0.24 to 1.00 mg/100g, according to a study by (Ghafoor et al., 2022), the results were derived using a variety of extraction techniques.

3.6. The Betalain (BL) Content

Plant pigments known as betalains, which belong to the Caryophyllales order, have both aesthetic and health-promoting properties (Gengatharan, Dykes and Choo, 2015). This study assessed the BL content of extracts spectrophotometrically. The results, presented as mean value and standard deviation, are reported in mg/g of DW in a Table 4. The BL content in the SEs was assessed as betacyanin and betaxanthin levels using two solvents: DIW and a 1:1 mixture of DIW and Et. Higher levels of BL were found in aqueous solvents compared to an equivalent ratio of DIW: Et. Since BLs are water-soluble pigments, as per this study (Gengatharan, Dykes and Choo, 2015) their concentration in water is substantially higher than that of a 1:1 mixture of water and ethanol in our study. The ANOVA test performed in this study on BL content in BGSE, WGSE, ADSE and BDSE varieties in both solvents revealed a statistically significant difference ($F > F$ critical, $p < 0.05$).

Table 4. The betalain content as betacyanin, and betaxanthins in extracts (BGSE, WGSE, ADSE, BDSE).

xtracts	DIW				Et+ DIW			
	BC (mg/g DW) ±SD (480 nm)	BX (mg/g DW) ±SD (480 nm)	BC (mg/g DW) ±SD (530nm)	BX (mg/g DW) ±SD (530nm)	BC (mg/g DW) ±SD (480 nm)	BX (mg/g DW) ±SD (480nm)	BC (mg/g DW) ±SD (530nm)	BX (mg/g DW) ±SD (530 nm)
BGSE	7.98±0.00	6.15±0.00	7.13±0.00	5.49±0.00	6.54±0.00	5.04±0.00	5.50±0.00	4.24±0.00
WGSE	4.32±0.00	3.33±0.00	3.65±0.00	2.81±0.00	2.62±0.00	2.02±0.00	2.14±0.00	1.65±0.00
ADSE	6.66±0.02	5.13±0.02	6.07±0.00	4.68±0.00	3.88±0.00	2.99±0.00	3.37±0.00	2.60±0.00
BDSE	4.14±0.01	3.19±0.01	3.47±0.00	2.67±0.00	2.95±0.01	2.27±0.00	2.32±0.00	1.79±0.00

3.7. Chlorophyll (a and b) and Carotenoid Content

Carotenoids are present in various plants, fruits, and vegetables as a distinct category of plant pigments, along with numerous documented health advantages (Gebregziabher et al., 2023). Chlorophyll is the pigment that facilitates light absorption during photosynthesis (Guo et al., 2022). Chlorophyll a is identified as the primary pigment that transforms light energy into chemical energy. Chlorophyll b functions as an auxiliary pigment in photosynthesis by indirectly passing the light it absorbs to chlorophyll a (Ramesh and Muthuraman, 2018). This study analyzed extracts from seeds of four distinct samples (BGSE, WGSE, ADSE, BDSE) for their carotenoid concentrations (C) and chlorophyll a and b using spectrophotometry with two solvents (acetone and ethanol). The results are reported in Table 5, which acquired by the mean value and standard deviation. The ANOVA test was also performed on the amount of carotenoid, chlorophyll a and b in BGSE, WGSE, ADSE and BDSE varieties in both solvents and revealed a statistically significant difference ($F > F$ critical, $p < 0.05$).

Carotenoid were detected at greater concentrations in acetone compared to ethanol, except for BGSE, which was significantly higher in ethanol than in acetone. The study by (Ignat et al., 2011) evaluated the concentration of assimilatory pigments (chlorophyll a and chlorophyll b) in grape seeds aqueous extract across various plant species, revealing a range of chlorophyll a from 189.20 to 661.10 µg/g, chlorophyll b from 164.38 to 339.51 µg/g, and total carotenoid content from 46.63 to 169.80 µg/g. In study described by (Zarie et al., 2023) establishes the carotenoid concentration (C) of several date seeds within the range of 0.89 to 1.98 mg/g.

Table 5: Chlorophyll (a and b) and Carotenoid (C) Content in extracts (BGSE, WGSE, ADSE, BDSE.

Extracts	Acetone			Ethanol		
	Ch a (662) mg/g DW±SD	Ch b (645) mg/g DW±SD	C (470) mg/g DW±SD	Ch a (662) mg/g DW±SD	Ch b (645) mg/g DW±SD	C (470) mg/g DW±SD
BGSE	0.96±0.00	1.70±0.00	0.068±0.00	0.24±0.00	0.80±0.01	0.13±0.00

WGSE	0.44±0.01	0.77±0.00	0.28±0.00	0.24±0.00	0.65±0.00	0.16±0.00
ADSE	0.62±0.00	1.12±0.00	0.25±0.00	0.34±0.00	0.90±0.00	0.07±0.00
BDSE	0.85±0.00	1.55±0.0	0.24±0.00	0.27±0.00	0.85±0.00	0.20±0.00

3.8. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Gas Chromatography–Mass Spectrometry (GC–MS) was employed to analyses the phytochemical composition of grape and date seed extracts, revealing a diverse array of bioactive constituents. The chromatograms obtained from the ethanol–water extracts (Figures 6 and 7) clearly illustrate the presence of numerous chemical components across all samples. Detailed spectral data and compound identification for the grape seed extracts BGSE and WGSE are presented in Tables 6 and 7. In total, 15 unique bioactive compounds were identified for BGSE, while 8 distinct compounds were characterized in WGSE. The relative abundance of each compound was calculated based on the average peak area compared to the total chromatographic area.

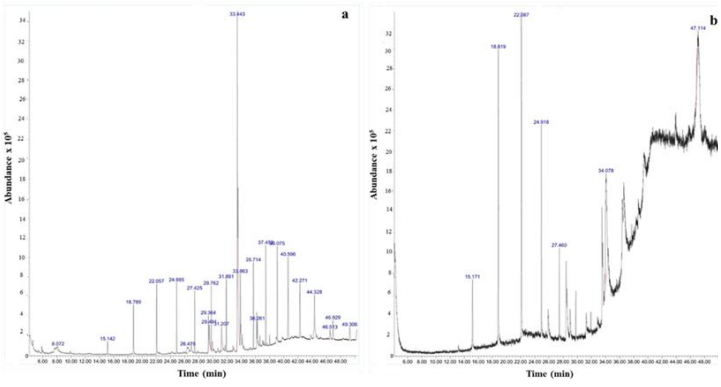

Figure 6: GC-MS total ion chromatogram of (a) BGSE and (b)WGSE.

In BGSE, the dominant compound was 13-tetradece-11-yn-1-ol (52.30%), followed by 13-tetradecenal (13.38%) and n-nonadecanoic acid pentamethyldisilyl ester (4.16%). In contrast, WGSE exhibited a different chemical fingerprint, with major constituents including Methyl ((24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl)amino)acetate (31.06%), Methyl glycocholate (22.13%), 1-monopalmitin (16.03%), dodecamethylpentasiloxane (9.83%), and 6-aza-5,7,12,14-tetrathiapentacene (9.60%). The chemical variability observed in the current study is consistent with previous research, though significant differences exist in compound profiles. For instance, (P.A. Raajeswari, 2016), analyzed methanolic grape seed extracts by using GC–MS and reported compounds such as 7-methoxy-2,2-dimethylchromone, hexadecanoic acid, 3-(4-hydroxy-3,5-dialkylphenyl)propanol,4-n-propylresorcinol, and 2-hydroxycyclopentadecanone. These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial properties, contributing to the recognized therapeutic potential of grape seed extracts. The absence of these specific compounds in our study is likely due to differences in solvent polarity, as methanol tends to extract more polar bio-actives than the ethanol–water mixture used here. Additionally, geographical and environmental factors, such as soil composition, climate, and cultivation practices, are well-known to influence phytochemical profiles in plants, possibly accounting for the observed variation. Supporting this (Gorodyska et al., 2018), investigated the chemical composition of industrially processed grape seed powders using hydroalcoholic solvents (ethanol and isopropanol). Their GC–MS analysis revealed a substantial presence of phenolic compounds and fatty acid esters, consistent with some of the lipophilic compounds observed in our extracts, such as 1-monopalmitin. Their findings also reinforce the idea that solvent system and processing scale can profoundly impact the range and yield of detectable compounds.

GC–MS analysis of grape seed extracts revealed distinct and novel phytochemical profiles between BGSE and WGSE. In BGSE, 13-Tetradece-11-yn-1-ol was the predominant compound (52.3%), representing a rare alkyne-containing alcohol scarcely reported in plant systems, suggesting a unique biosynthetic trait of this cultivar and highlighting its potential for bioactive compound discovery. In contrast, WGSE was dominated by two bile acid derivatives methyl ((24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl)amino)acetate (31.06%) and methyl glycocholate (22.13%) compounds typically associated with mammalian metabolism. Their unexpected presence in plant material raises the possibility of either unusual plant metabolic pathways or artifacts from sample derivatization during GC–MS analysis, warranting further investigation. Additionally, organosilicon compounds were consistently detected across all grape seed extracts. While these may result from external contamination, their recurrent occurrence suggests the potential existence of a little-known class of naturally occurring silicon-based secondary metabolites in grape seeds, meriting deeper exploration into their origin and biological relevance.

Table 6: List of identified chemical compounds in BGSE in the water-ethanol extracts of sample by GC-MS analysis.

n	RT (min)	Area %	Name of compounds	Molecular weight	Molecular formula	Class of compound
1	8.072	0.85	2-Ethylhexanol	130.23	$C_8H_{18}O$	Branched alcohol
2	9.3	0.10	Phenol	94.11	C_6H_6O	Aromatic compound
3	15.144	0.90	Dodecamethylcyclohexasiloxane	444.92	$C_{12}H_{36}O_6Si_6$	Organosilicon compound
4	22.055	3.48	Tetracosamethylcyclododecasiloxane	889.9	$C_{24}H_{72}O_{12}Si_{12}$	Organosilicon compound
5	24.883	3.30	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$	Organosilicon compound
6	26.476	1.53	1,E-8,Z-10-Pentadecatriene	206.2	$C_{15}H_{26}$	Polyunsaturated hydrocarbon
7	29.366	1.80	Linoleic acid ethyl ester	308.50	$C_{20}H_{36}O_2$	Fatty acid ethyl ester
8	29.491	1.45	Ethyl oleate	310.52	$C_{20}H_{38}O_2$	Fatty acid ethyl ester
9	29.76	2.86	Tetracosamethylcyclododecasiloxane	889. 9	$C_{24}H_{72}O_{12}Si_{12}$	Organosilicon compound
10	31.208	3.41	Lauric acid	200.32	$C_{12}H_{24}O_2$	Fatty acid
11	31.229	0.10	Phytol	298.55	$C_{20}H_{42}O$	Acyclic unsaturated alcohol
12	33.444	52.30	13-Tetradece-11-yn-1-ol	208.34	$C_{14}H_{24}O$	Unsaturated alcohol
13	33.527	13.38	13-Tetradecenal	210.36	$C_{14}H_{26}O$	Aldehyde

14	35.717	4.16	n-Nonadecanoic acid, pentamethyldisilyl ester	428.8	$C_{24}H_{52}O_2Si_2$	Silyl ester of fatty acid
15	36.262	0.88	Heptadecene-(8)-carbonic acid-(1)	268.4348	$C_{17}H_{32}O_2$	Unsaturated fatty acid

Table 7: List of identified chemical compounds in WGSE in the water-ethanol extracts of sample by GC-MS analysis.

n	RT (min)	Area %	Name of compounds	Molecular weight	Molecular formula	Class of Compound
1	15.17	2.98	Dodecamethylcyclohexasiloxane	444.92	$C_{12}H_{36}O_6Si_6$	Organosilicon compound
2	18.817	9.83	Dodecamethylpentasiloxane	384.84	$C_{12}H_{36}O_4Si_5$	Organosilicon compound
3	22.086	9.60	6-Aza-5,7,12,14-tetrathiapentacene	355.5	$C_{17}H_9NS_4$	Heterocyclic aromatic compound
4	24.919	5.88	Cyclononasiloxane, octadecamethyl-	667.4	$C_{18}H_{54}O_9Si_9$	Organosilicon compound
5	27.462	2.50	Bistrimethylsilyl N-acetyl eicosasphinga-4,11-dienine	511.38	$C_{28}H_{57}NO_3Si_2$	Sphingolipid derivative
6	34.077	16.03	1-Monopalmitin	330.5	$C_{19}H_{38}O_4$	Monoacylglycerol
7	36.568	22.13	Methyl glycocholate	479.7	$C_{27}H_{45}NO_6$	Bile acid ester
8	47.111	31.06	Methyl ((24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl)amino)acetate	519.83	$C_{30}H_{53}NO_4Si$	Amino acid conjugate

Tables 8 and 9 present the GC–MS profiling results of ADSE and BDSE, both obtained through ethanol–water extraction. The analysis revealed a total of 16 compounds in ADSE and 33 compounds in BDSE, reflecting significant chemical diversity between the two varieties. In ADSE, Glycerol trilaurate (75.16%) was identified as the dominant compound, followed by 1,4-diphenyl-2-butene (4.95%) and bis(2-ethylhexyl) phthalate (2.70%). The high content of trilaurin, a medium-chain triglyceride with known emollient, antimicrobial, and potential therapeutic properties, underscores the potential application of ADSEs in nutraceutical and cosmetic formulations. In contrast, BDSE exhibited a more chemically diverse profile, with key bioactive constituents including 9-octadecenal (Z-) (17.98%), N-ethyl-1,3-dithioisoindoline (12.95%), gibberellin A3 (10.49%), and 1-mono-olein (10.29%). The presence of gibberellin A3, a well-characterized plant hormone, indicates a potential role in seed development and physiological regulation, while the detection of sulfur-containing heterocycles such as N-ethyl-1,3-dithioisoindoline suggests possible involvement in defense mechanisms or signaling pathways. The high variability and broader range of compounds in BDSE compared to ADSE may reflect genotypic differences or ecological adaptations between date palm cultivars. These findings are consistent with further supported by previous research. For instance,(Eldeen et al., 2022) utilized GC–MS to identify 11 phytochemicals in date seed extracts, while the study by (Alsuhaymi et al., 2023) reported that date seeds are notably rich in hydroxycinnamic acid derivatives, such as caffeic,

ferulic, and sinapic acids metabolites known for their potent antioxidant and anti-inflammatory effects. While these specific phenolic compounds were not prominently detected in the current ethanol water extracts, differences in extraction solvent, seed origin, and cultivar type may account for this variation.

Ethanol water combinations tend to favor lipophilic or moderately polar molecules, as those found in this study, while methanol or aqueous extraction techniques are frequently more effective in extracting polar phenolic acids.

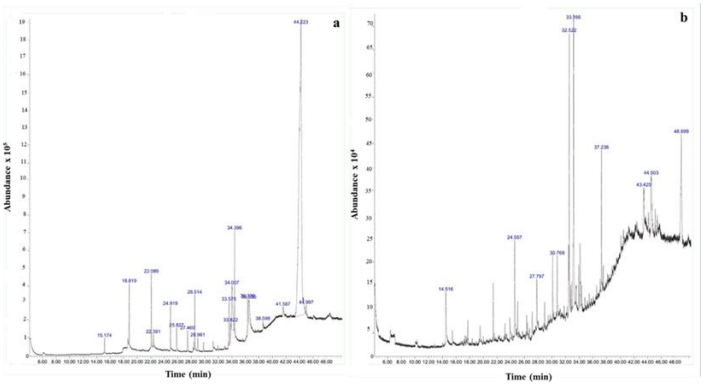

Figure 7: GC-MS total ion chromatogram of (a) ADSE and (b)BDSE.

In contrast, BDSE was characterized by the presence of gibberellin A3 (10.49%), a key plant hormone involved in growth and developmental regulation, and N-ethyl-1,3-dithioisoindoline (12.95%), a sulfur-containing heterocycle that may play a role in plant defense or signaling. These findings suggest that Barhi seeds may harbor metabolites with regulatory or protective functions.

Interestingly, the chemical composition of the extracts reveals the existence of many bioactive substances with potential antioxidant and biological activities, according to (Sławińska and Olas, 2023). These chemicals are recognized for their antibacterial, anti-inflammatory, and cytoprotective properties. Consequently, the discovered components substantiate the antioxidant and biological efficacy of BGSP, WGSE, ADSE, and BDSE extracts, indicating their potential application in pharmaceutical and nutraceutical fields.

Table 8: List of identified chemical compounds in ADSE in the water-ethanol extracts of sample by GC-MS analysis.

n	RT (min)	Area %	Name of compounds	Molecular weight	Molecular formula	Class of Compound
1	15.175	0.55	Dodecamethylcyclohexasiloxane	444.92	$C_{12}H_{36}O_6Si_6$	Organosilicon compound
2	22.086	1.48	6-Aza-5,7,12,14-tetrathiapentacene	355.5	$C_{17}H_9NS_4$	Heterocyclic aromatic compound
3	22.392	0.40	Methyl myristate	242.4	$C_{15}H_{30}O_2$	Fatty acid methyl ester
4	24.919	0.96	Cyclononasiloxane, octadecamethyl	667.4	$C_{18}H_{54}O_9Si_9$	Organosilicon compound
5	25.827	0.85	Pentadecanoic acid, 14-methyl-, methyl ester	270.5	$C_{17}H_{34}O_2$	Branched fatty acid methyl ester
6	27.462		Pentasiloxane, dodecamethyl-	384.84	$C_{12}H_{36}O_4Si_5$	Organosilicon compound
7	28.515	2.02	9-Octadecenoic acid, methyl ester	296.5	$C_{19}H_{36}O_2$	Unsaturated fatty acid methyl ester
8	28.961	0.48	Methyl stearate	298.5	$C_{19}H_{38}O_2$	Saturated fatty acid methyl ester
9	33.574	1.55	beta-Monoolein	356.54	$C_{21}H_{40}O_4$	Monoacylglycerol

10	33.823	0.51	4-Methyl-1H-indole	131.17	C_9H_9N	Heterocyclic aromatic compound
11	34.005	4.95	1,4-Diphenyl-2-butene	208.30	$C_{16}H_{16}$	Organic aromatic hydrocarbon
12	34.399	2.70	Bis(2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$	Phthalate ester
13	36.334	1.69	5-Methylindole	131.17	C_9H_9N	Heterocyclic aromatic compound
14	36.532	2.53	Methyl glycocholate	479.65	$C_{27}H_{45}NO_6$	Organic compound
15	41.585	0.99	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_3Si_3$	Organosilicon compound
16	44.221	75.16	Glycerol trilaurate	639	$C_{39}H_{74}O_6$	Bile acid ester

Table 9: List of identified chemical compounds in BDSE in the water-ethanol extracts of sample by GC-MS analysis.

n	RT (min)	Area %	Name of compounds	Molecular weight	Molecular formula	Class of Compound
1	14.516	4.11	2-Ethylhexyl cyanoacetate	197.27	$C_{11}H_{19}NO_2$	Ester
2	15.476	0.70	Tetradecane	198.39	$C_{14}H_{30}$	Hydrocarbon
3	17.302	0.18	Cycloheptane, methyl-	112.21	C_8H_{16}	Cycloalkane
4	17.479	0.17	Octadecane	254.49	$C_{18}H_{38}$	Hydrocarbon
5	17.728	0.78	butylated hydroxy toluene	220.35	$C_{15}H_{24}O$	Phenol
6	18.371	0.19	1H-Indole, 2-methyl-3-phenyl-	207.27	$C_{15}H_{13}N$	Heterocyclic aromatic compound
7	19.507	0.71	Hexadecane	226.44	$C_{16}H_{34}$	Hydrocarbon
8	19.985	0.20	Methyl 3,5-di-t-butylsalicylate	264.36	$C_{16}H_{24}O_3$	Ester
9	21.432	1.46	Triallylmethylsilane	166.34	$C_{10}H_{18}Si$	Organosilicon compound
10	21.572	0.14	n-Eicosane	282.55	$C_{20}H_{42}$	saturated hydrocarbon
11	23.861	1.98	1-Acetoxy-3-phenyl-3-phenoxyacetone	284.31	$C_{17}H_{16}O_4$	aromatic
12	24.556	5.88	Ethene, chlorotrifluoro-	116.47	C_2ClF_3	Haloalkene
13	25.002	1.03	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	276.36	$C_{17}H_{24}O_3$	Spirocyclic ketone
14	26.315	0.91	Ethyl margarate	298.49	$C_{19}H_{38}O_2$	fatty acid ester
15	26.445	0.35	n-Eicosane	282.55	$C_{20}H_{42}$	saturated hydrocarbon

16	27.124	1.19	1-(2-Methyl-2-propenyl)-5-carbethoxy-2-pyrrolidinone	211.26	$C_{11}H_{17}NO_3$	Lactam derivative
17	27.799	2.86	Pentadecanoic acid, 15-hydroxy-	258.40	$C_{15}H_{30}O_3$	Hydroxy fatty acid
18	28.935	0.92	9-Octadecenoic acid, ethyl ester	310.51	$C_{20}H_{38}O_2$	Unsaturated fatty acid ester
19	30.108	2.32	Myristoyl chloride	246.82	$C_{14}H_{27}ClO$	fatty acid derivative
20	30.767	3.38	Palmitoyl chloride	274.87	$C_{16}H_{31}ClO$	fatty acid derivative
21	32.443	2.66	beta-Monolinolein	354.52	$C_{21}H_{38}O_4$	fatty acid ester
22	32.52	10.29	Olein, 1-mono-	356.54	$C_{21}H_{40}O_4$	fatty acid ester
23	32.728	0.95	3-Benzyloxy-1,2-diacetyl-1,2-propanediol	266.29	$C_{14}H_{18}O_5$	Diacetate ester derivative
24	32.873	0.82	2-Oxecanone, 10-methyl-	170.25	$C_{10}H_{18}O_2$	Ketone
25	33.164	17.98	9-Octadecenal, (Z)-	266.46	$C_{18}H_{34}O$	Unsaturated aldehyde
26	33.916	2.10	methyl (Z)-3,3-diphenyl-4-hexenoate	280.36	$C_{19}H_{20}O_2$	unsaturated fatty acid derivative
27	34.108	3.61	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	332.46	$C_{22}H_{20}OS$	organosulfur compound
28	34.337	1.05	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-	~294	$C_{22}H_{22}$	Hydrocarbon
29	37.237	5.78	Monoplex dos	426.27	$C_{26}H_{50}O_4$	diester plasticizer
30	37.502	0.68	Trivinyl-s-striazine-2,4,6-(1H,3H,5H)-trione	207.11	$C_9H_9N_3O_3$	Triazine derivative
31	43.427	10.49	Gibberellin A3	346.37	$C_{19}H_{22}O_6$	diterpenoid
32	45.087	1.21	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_3Si_3$	Organosilicon cyclic siloxane
33	48.901	12.95	N-ethyl-1,3-dithioisoindoline	207.32	$C_{10}H_9NS_2$	Heterocyclic sulfur compound

3.9. Antioxidant Activity

There are significant polyphenolic compounds in the resulting extracts, imparting antioxidant and antibacterial characteristics. To elucidate the antioxidant and antibacterial capabilities of the extracts, it is essential to examine the concentration of polyphenolic components. Polyphenolics are prominent categories of secondary metabolites acknowledged as natural antioxidants, indicative of the positive connections between antioxidant ability and phenolic concentration (Meral and Doğan İ, 2013). In this study, the SEs interact with DPPH radicals, resulting in the production of the yellow-colored DPPHH. The results demonstrate that extracts from all SEs exhibited radical-scavenging properties and significant antioxidant activities, and all SEs showed remarkable scavenging activities with IC50 as indicated in the Table 10 derived from the mean value and standard deviation. The antioxidant activity of BGSE, WGSE, ADSE, and BDSE at different concentrations as measured by DPPH model is presented in Figure 8.

It can be seen that both BGSE and WGSE at different concentrations exhibited various degrees of antioxidant activity with comparison to vitamin C as a positive control. Parallel to the increase in the extract concentration from (12.50 to 75.00 µg/mL), antioxidant activity of the extracts increased. This study determined the Pearson correlation coefficient (r) between the antioxidant activity of BGSE and vitamin C was 0.96, indicating a very strong positive linear correlation, and in WGSE was (r= 0.997) also indicated very strong positive linear correlation. The antioxidant activity of various grapes as determined by DPPH has been extensively researched in the literature. In the study by (Ahmed et al., 2022) it was discovered that the acetonic extract of grape seed had the highest DPPH scavenging potential and a significantly higher total phenolic content.

However, both ADSE and BDSE in this study exhibit antioxidant activity in correlation with an increase in extract concentration, the antioxidant activity of both grape seeds is higher than that of both date seeds. Additionally, (r) was calculated for the dates seed, and r = 0.999 for BDSE and r = 0.997 for ADSE showed a very significant positive linear association. Date seed extracts' antioxidant activity varies with different solvents, according to the current study by (Adheem et al., 2021), the extracts' superior DPPH free radical scavenging over butylated hydroxytoluene (BHT) as positive control is attributed to the presence of bioactive compounds with antioxidant qualities. In this study the ANOVA test performed on the antioxidant activity of the four extracts revealed a statistically significant difference (F > F critical, p < 0.05). This indicates that the extracts possess varying antioxidant capacities.

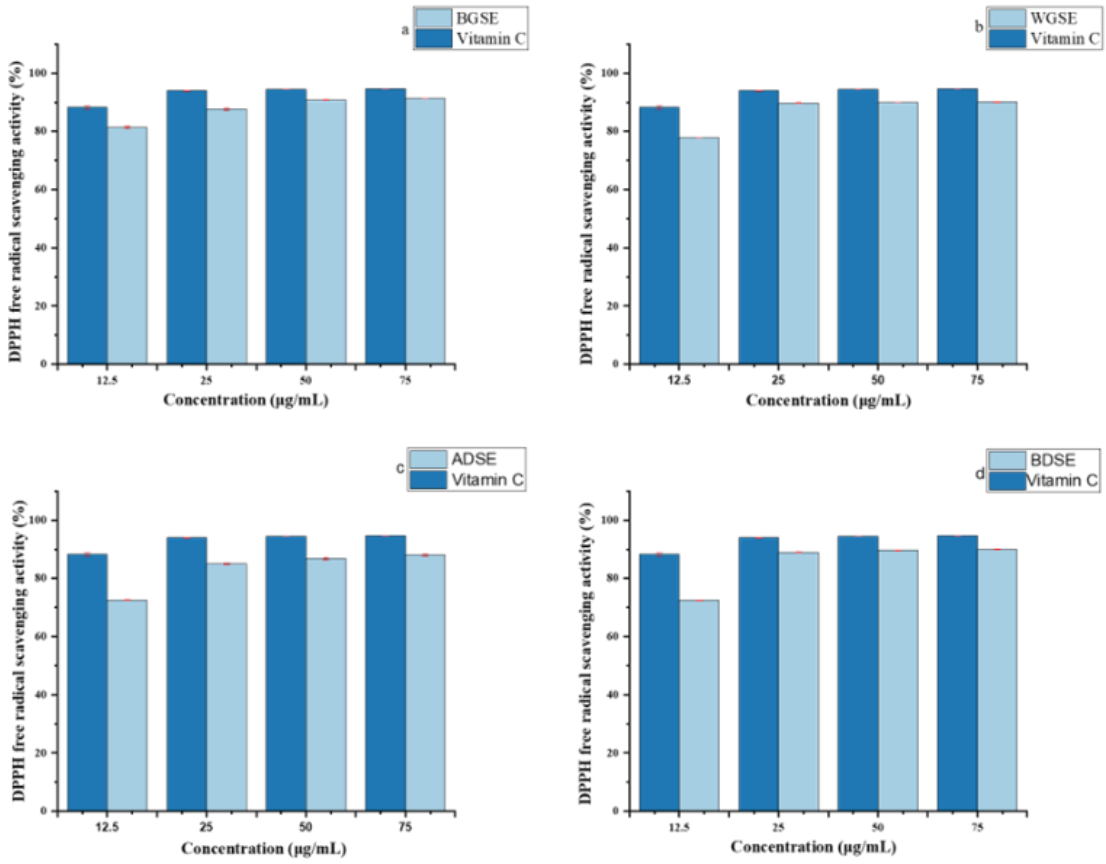

Figure 8: DPPH free radical scavenging capacity of a-BGSE, b-WGSE, C-ADSE, d-BDSE in comparison to Vitamin C.

Table 10: DPPH radical scavenging activity percentage with IC50 of SEs at different concentrations in comparison to Vitamin C.

Extracts	% RSA±SD				IC50 (µg/mL)
	Conc (µg/mL)				
	12.5	25	50	75	
BGSE	81.38±0.50	87.62±0.45	90.82±0.05	91.28±0.02	30.51±0.02
WGSE	77.86±0.05	89.78±0.03	89.95±0.03	90.02±0.04	30.78±0.04
ADSE	72.55±0.10	85.04±0.21	86.75±0.56	88.07±0.35	31.86±0.35
BDSE	72.43±0.13	88.95±0.09	89.65±0.06	89.995±0.04	31.01±0.04
Vit. C	88.21±0.57	93.91±0.04	94.41±0.01	94.60±0.04	29.17±0.04

3.10. Mineral Analysis

The mineral compositions of grape and date seeds were examined via ICP-MS, as presented in Table 11. The mineral elements exhibited significant variability based on the distinct contents of grape and date seeds, as indicated by the results (Ag, Al, As, B, Ba, Bi, Ca, Cd, Ce, Co, Cr, Cu, Er, Fe, Ga, Hg, K, La, Mg, Mn, Mo, Na, Ni, P, Pb, Pr, S, Sb, Se, Sn, Sr, Te, Th, Ti, U, V, W, Zn, Zr). White grape seeds have almost twice as much calcium (10209.26 mg/kg) as BGS, which have a maximum concentration of 5254.52 mg/kg in DW. Additionally, BGSs are rich in calcium, phosphorus, magnesium, potassium, sodium, and sulfur, whereas WGSs are abundant in calcium, potassium, phosphorus, sulfur, and magnesium. These results are comparable to the study described by (Özcan et al., 2017) who identified different concentration range of minerals measured in mg/kg (K, Ca, P, Mg, S, and Na), as analyzed by ICP atomic emission spectroscopy (ICP-AES). The current work by (Albakaa *et al.*, 2021) reported the mineral content of GSs, specifically potassium (K), magnesium (Mg), and calcium (Ca), in high concentrations (µg/g), utilizing atomic absorption spectrophotometry (AAS). But according to this study, ADSEs have the highest potassium concentration (5897.09 mg/kg of DW), while BDSEs have 3475.02 mg/kg of DW. Furthermore, ADSEs are high in calcium, magnesium, phosphorus, sulfur, and phosphorus, while BDSEs are high in potassium, sulfur, sodium, magnesium, and phosphorus. In one investigation, (Ahfaiter, Zeitoun and Abdallah, 2018), atomic absorption spectroscopy (AAS) was utilized to determine the minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn) in date seeds. (Perveen and Bokahri, 2020) also showed in another study that the potassium content of BDSEs is high, at 8171.24 mg/kg. Remarkably, it was discovered that the date seed types used in our study had the highest levels of K, Ca, Mg, and P (mg/kg of DW).

Table 11: Mineral contents (mg/kg of dry weight) of black and white grape and ajwa and barhi date seeds.

Minerals	Concentration (mg/kg) ±SD			
	BGSE	WGSE	ADSE	BDSE
Ag	0.03±0.00	4.46±0.03	4.71±0.02	0.04±0.00
Al	67.59±0.84	17.60±0.18	15.73±0.16	106.46±1.12
As	0.59±0.00	0.24±0.00	<0.1±0.00	1.05±0.01
B	19.81±0.17	13.19±0.13	6.10±0.04	31.66±0.18

Ba	3.43±0.05	10.01±0.14	9.90±0.14	3.61±0.06
Bi	51.47±1.09	14.39±0.13	2.44±0.03	60.53±1.08
Ca	5254.52±35.09	10209.26±15.99	627.17±7.6	765.96±7.9
Cd	0.51±0.00	<0.1±0.00	<0.1±0.00	0.76±0.00
Ce	<0.01±0.00	0.32±0.00	0.72±0.00	<0.01±0.00
Co	0.73±0.00	2.32±0.02	0.59±0.00	1.11±0.01
Cr	1.12±0.01	0.21±0.00	0.96±0.00	2.40±0.01
Cu	21.09±1.08	11.25±0.12	7.33±0.10	21.75±0.99
Er	<0.01±0.00	1.08±0.00	0.36±0.00	<0.01±0.00
Fe	210.40±10.00	242.49±10.07	57.03±1.06	300.75±6.68
Ga	0.16±0.00	<0.1±0.00	0.53±0.00	0.41±0.00
Hg	0.60±0.00	1.25±0.00	0.49±0.00	1.55±0.02
K	2451.57±5.17	5588.72±34.45	5897.09±33.07	3475.02±28.87
La	1.88±0.03	9.84±0.12	<0.1±0.00	2.64±0.05
Mg	2276.00±27.44	1011.50±25.32	530.88±16.8	1856.69±20.06
Mn	26.39±1.32	12.29±0.45	7.15±0.13	32.18±4.20
Mo	5.08±0.11	0.81±0.00	0.29±0.00	6.40±0.08
Na	2058.04±24.07	46.61±0.19	15.69±0.11	1878.64±18.32
Ni	2.85±0.08	1.81±0.03	14.29±0.16	14.59±0.14
P	2743.59±17.34	3376.76±27.20	1475.36±11.34	1717.72±11.07
Pb	14.50±0.13	6.38±0.09	7.88±0.05	11.73±0.10
Pr	2.80±0.05	3.12±0.01	<0.1±0.00	<0.01±0.00
S	1865.17±15.67	1634.78±20.10	1309.95±21.21	2433.38±19.12
Sb	0.82±0.00	<0.1±0.00	<0.1±0.00	2.00±0.01
Se	4.86±0.07	0.40±0.01	1.58±0.01	5.95±0.02
Sn	0.30±0.00	<0.1±0.00	<0.1±0.00	0.22±0.00
Sr	11.93±0.09	10.79±0.10	1.81±0.03	10.00±0.10
Te	1.97±0.01	1.19±0.01	3.57±0.01	0.73±0.00
Th	<0.01±0.00	2.46±0.01	<0.1±0.00	0.27±0.00
Ti	1.68±0.00	0.85±0.00	0.48±0.00	2.45±0.01
U	<0.01±0.00	1.95±0.01	2.08±0.00	<0.01±0.00
V	0.17±0.00	<0.1±0.00	<0.1±0.00	0.21±0.00
W	1.07±0.00	<0.1±0.00	<0.1±0.00	0.11±0.00
Zn	20.24±0.13	16.11±0.09	13.53±0.10	24.24±0.15
Zr	0.09±0.00	0.30±0.00	<0.1±0.00	0.11±0.00

3.11. Antibacterial Activities

The antimicrobial activity of the characterized seed extracts (SEs) was evaluated using the agar well diffusion method against five bacterial strains: two Gram-positive species (*Enterococcus faecalis (E. faecalis) and Staphylococcus aureus (S. aureus)*) and three Gram-negative species (*Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa*), *and Escherichia coli (E. coli)*). The effect of the SEs at different concentrations (1, 10, 50, and 100 mg/mL) on bacterial growth is illustrated in Figure 9, and the corresponding inhibition zone diameters are presented in Table 12.

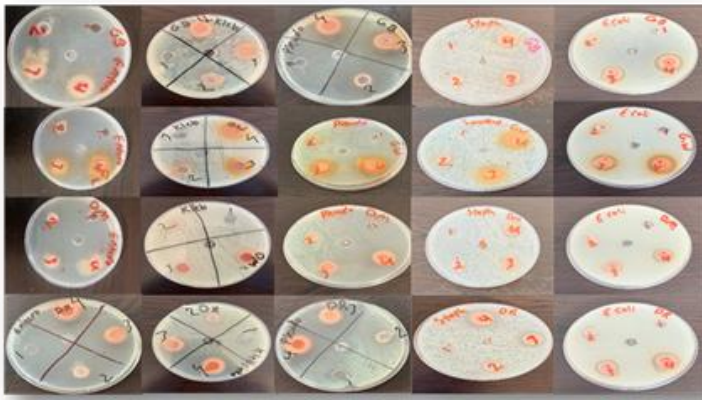

Figure 9: Antibacterial activity of ethanol+ water seeds extracts.

To ensure quality control and allow for comparative analysis, standard antibiotic discs were used as positive controls under the same conditions. Among these, imipenem (IPM) demonstrated strong antibacterial activity, with inhibition zones of 25 mm against *K. pneumoniae,* 24 mm against *P. aeruginosa* and 15 mm against *E. coli*. Gentamicin (GN) also showed significant effects, producing inhibition zones of 20 mm for *P. aeruginosa* and 15 mm for *E. coli* and for *K. pneumoniae*. In the case of gram-positive bacteria, tetracycline (TE) produced inhibition zones of 15 mm and 18 mm against *S. aureus* and *E. faecalis*, respectively. Conversely, amoxicillin clavulanic acid (AMC) showed no inhibitory effect (0 mm) against either *S. aureus* or *E. faecalis*, suggesting resistance under the experimental conditions, see Figure 10.

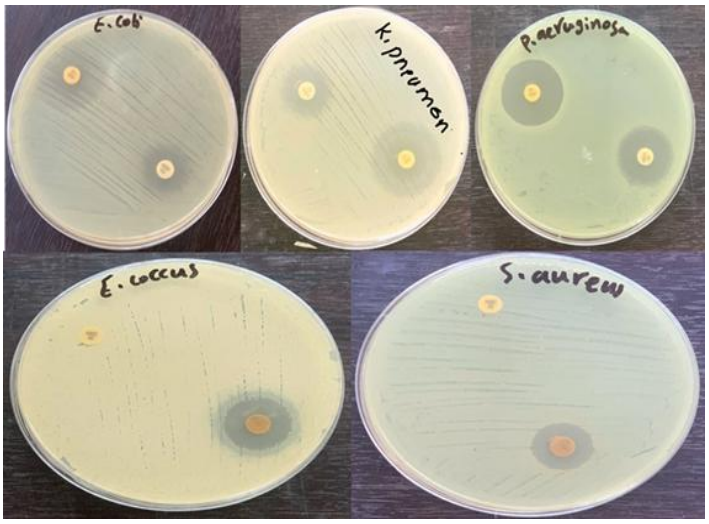

Figure 10: Zones of inhibition produced by the positive controls against different bacterial strains.

The SEs demonstrated notable antibacterial activity against four of the five tested bacteria: *E. faecalis, P. aeruginosa, S. aureus, and E. coli.* The diameter of inhibition zones ranged from 10 to 40 mm, depending on the extract type and concentration. All SEs exhibited little to no activity at the lowest tested concentration (1 mg/mL), with the exception of BGSE, which showed activity against *P. aeruginosa* even at this low concentration. Notably, BDSE exhibited the strongest antibacterial effect against *E. faecalis*, yielding the highest zone of inhibition among all tested combinations.

Table 12: Antibacterial activity of four seed extracts (BGSE, WGSE, ADSE, BDSE) against five bacterial strains.

Bacterial species	BGSE				WGSE				ADSE				BDSE			
	Concentration (mg/ml)															
	1	10	50	100	1	10	50	100	1	10	50	100	1	10	50	100

	Zone of inhibition (mm)															
E. faecalis	0	14	20	23	0	14	19	22	0	0	12	14	0	14	34	40
K. pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa	20	24	24	24	0	0	0	0	0	0	0	0	0	0	30	32
S. aureus	0	0	10	15	0	0	15	18	0	0	13	15	0	0	16	22
E. coli	0	10	18	19	0	11	16	18	0	0	15	15	0	13	18	18

The SEs demonstrated notable antibacterial activity against four of the five tested bacteria: *E. faecalis, P. aeruginosa, S. aureus, and E. coli.* The diameter of inhibition zones ranged from 10 to 40 mm, depending on the extract type and concentration. All SEs exhibited little to no activity at the lowest tested concentration (1 mg/mL), with the exception of BGSE, which showed activity against *P. aeruginosa* even at this low concentration. Notably, BDSE exhibited the strongest antibacterial effect against *E. faecalis*, yielding the highest zone of inhibition among all tested combinations.

However, none of the evaluated extracts demonstrated any inhibitory activity against *K. pneumoniae*. This finding contrasts with previous studies. For instance, (Felemban and Hamouda, 2024), reported that crude grape seed extract at 100% concentration produced an inhibition zone of 9.5 mm against *K. pneumoniae*. Similarly, (Kadhim et al., 2025), observed significant antibacterial activity from date seed extract at 100 mg/mL, with a corresponding inhibition zone of 16 mm. The lack of antibacterial activity in our extracts may be due to variations in extraction methods, phytochemical content, plant origin, or bacterial strain differences. Additionally, factors such as the concentration, solubility, and diffusion ability of active compounds in the agar medium could have affected the results, emphasizing the need for standardized protocols in antimicrobial testing.

Further support for the antimicrobial potential of grape seed and date seed extracts is provided by other literature. (Krishnan et al., 2016) reported that GSEs produced moderate antibacterial activity, with inhibition zones ranging from 11 to 15 mm at concentrations between 2 and 20 mg/mL. Additionally, (Swedan, Auzi and Lahmer, 2021) demonstrated that date seed extract (DSE) had antibacterial activity against *S. aureus*, with inhibition zones of 15.5, 13.3, 13, and 12 mm at concentrations of 100, 50, 25, and 12.5%, respectively, although no activity was observed against *E. coli*.

To further investigate the antibacterial effects of the extracts, minimum inhibitory concentration (MIC) values were determined for the four responsive bacterial strains: *E. faecalis, P. aeruginosa*, *S. aureus*, and *E. coli* (Table 13). BGSE showed the strongest MIC values, with an MIC of 1 mg/mL against P. aeruginosa, 10 mg/mL against both *E. faecalis* and *E. coli*, and 50 mg/mL against S. aureus. WGSE exhibited similar MICs for *E. faecalis*, *E. coli,* and *S. aureus*, but showed no detectable MIC against *P. aeruginosa*. ADSE displayed relatively weak activity, with a consistent MIC of 50 mg/mL for all strains except *P. aeruginosa*, where no inhibition was observed. In contrast, BDSE showed MIC values of 10 mg/mL

for *E. faecalis* and *E. coli*, and 50 mg/mL for *P. aeruginosa* and *S. aureus*.

Table 13: Minimum Inhibitory Concentration (MIC) values of extracts against tested bacterial strains. MIC values are expressed in mg/mL.

Bacterial species	MIC (mg/mL)			
	BGSE	WGSE	ADSE	BDSE
E. faecalis	10	10	50	10
K. pneumoniae	-	-	-	-
P. aeruginosa	1	-	-	50
S. aureus	50	50	50	50
E. coli	10	10	50	10

These findings collectively indicate that grape seed and date seed extracts possess variable antimicrobial activities depending on the bacterial strain and extract type. The relatively high efficacy of BGSE and BDSE against specific bacteria suggests their potential as sources of natural antimicrobial agents, although further investigation including compound isolation and mechanism studies is warranted.

3.12. The ATR-FTIR spectra of Seeds Extracts

The FTIR spectral analysis revealed significant functional group vibrations across the examined samples. The absorption band was observed at 3734.54 cm^{-1} for WGSE and at 3734.92 cm^{-1} for ADSE, corresponding to the stretching vibrations of free O–H groups involved in hydrogen bonding, in accordance with the interpretation provided by Pavia et al (Pavia et al., 2012). Also, the broad bands appearing at 3293.91 cm^{-1} for BGSE and ADSE, 3301.10 cm^{-1} for WGSE, and 3266.62 cm^{-1} for BDSE are signs of O–H stretching vibrations that are hydrogen-bonded.

A distinctive absorption at 3009.48 cm^{-1} was recorded in BGSE, which is attributed to the C–H stretching of cis-alkene (=CH) groups. Furthermore, strong bands identified at 2924.73 and 2854.34 cm^{-1} for BGSE, ADSE, and BDSE, and at 2926.17 cm^{-1} for WGSE, are ascribed to the C–H, N–H, and O–H stretching vibrations characteristic of alkanes, amine salts, and carboxylic acids, as reported by (Singh et al., 2022).). These absorptions also represent the asymmetric and symmetric stretching vibrations of CH_2 groups, particularly associated with hydrocarbon chains in lipids and lignins, as noted by (Lucarini et al., 2020). A peak observed at 2363.08 cm^{-1} in ADSE indicating N–H vibrations and implying the presence of amino acids or amine-containing compounds, consistent with findings by (Pharmawati and Wrasiati, 2020). Absorption bands at 1743.94 cm^{-1} for BGSE, and at 1741.05 cm^{-1} and 1743.92 cm^{-1} for ADSE and BDSE, respectively, are attributed to C=O stretching in ester functionalities. These are likely derived from fatty acids, glycerides, pectins, and the ester or acetyl groups of carboxylic acids such as ferulic and p-coumaric acids. In addition to the peak observed at 3009.48 cm^{-1}, which corresponds to aromatic C–H stretching vibrations, the presence of peaks near 1604 cm^{-1} and approximately 1400 cm^{-1} further supports the existence of aromatic ring structures within the extract. The peak around 1604 cm^{-1} is typically attributed to C=C stretching vibrations in aromatic rings, while the band near 1400 cm^{-1} is commonly associated with C–C stretching or in-plane bending vibrations of aromatic systems. Together, these spectral features provide complementary evidence for the presence of aromatic compounds in the analyzed sample. As absorption peak appear at 1604.58 cm^{-1} for BGSE and 1607.45 cm^{-1} for WGSE, which correspond to aromatic C=C stretching typically observed in phenolic compounds and pectins, as per (Günter and Popeyko, 2022).Similarly, the band at 1606.02 cm^{-1} observed in ADSE and BDSE may indicate either C=C or C=N stretching in aromatic systems (Nabili *et al.*, 2016). Additional aromatic skeletal vibrations are evident from peaks at 1516.95 cm^{-1} and 1440.82 cm^{-1} in ADSE, and 1522.70 cm^{-1} and 1440.82 cm^{-1} in BDSE, which are assigned to C=C stretching modes.

A band at 1371.87 cm^{-1} in BDSE is associated with C–H bending, while the spectral range between 1210 and 1320 cm^{-1} in both ADSE and

BDSE indicates C–O stretching, as corroborated by (Himanshu et al., 2024). In BGSE, a peak at 1519.83 cm^{-1} is indicative of aromatic C–C stretching within phenolic structures (Ricci et al., 2015; Lucarini et al., 2020). Peaks at 1442.25 cm^{-1} in BGSE and 1368.99 cm^{-1} in WGSE are also consistent with C–H bending vibrations (Himanshu et al., 2024). An out-of-plane CH_3 scissoring vibration is observed at 1318 cm^{-1} in BGSE, while C–O stretching vibrations near 1035.72 cm^{-1} in BGSE and 1027.10 cm^{-1} in WGSE are characteristic of polysaccharide structures (Heredia-Guerrero et al., 2014). Moreover, C–O–C stretching is observed at 1101.80 cm^{-1} in both ADSE and BDSE, while peaks at 1042.91 cm^{-1} (ADSE) and 1044.34 cm^{-1} (BDSE) further support C–O bond vibrations, as reported by (Nabili et al., 2016). The region between 700 and 900 cm^{-1} exhibits bands due to C–H out-of-plane bending in substituted aromatic rings (Pharmawati and Wrasiati, 2020). The absorptions in the 400–500 cm^{-1} region are attributed to metal–oxygen stretching vibrations (Pavia et al., 2012).

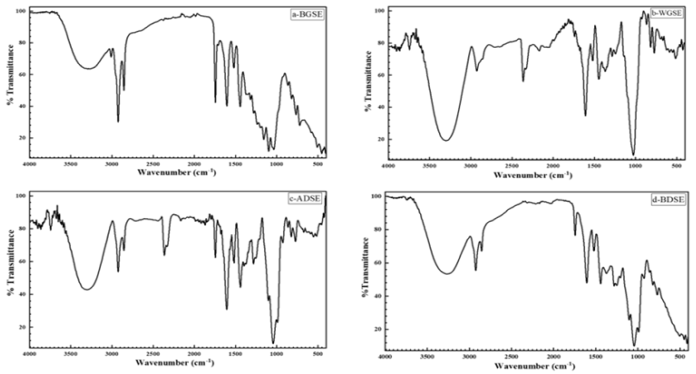

Figure 11: ATR-FT-IR spectra of seed extract powder.

4. Conclusions

This study comprehensively evaluated the bioactive composition, mineral content, antioxidant capacity, and antibacterial efficacy of seed extracts derived from black and white grapes, as well as Ajwa and Barhi dates. Among the most significant findings is the high concentration of phenolic compounds in all seed types, along with measurable levels of flavonoids, anthocyanins, betalains, carotenoids, and chlorophyll a and b, as determined by UV–VIS spectrophotometry. FTIR analysis further confirmed the presence of phenolic structures and other functional groups, while ICP-MS revealed distinct mineral profiles with notable variability across seed types, indicating their nutritional diversity. Consequently, these phytochemicals are shown to impart antibacterial and antioxidant properties. Interestingly, all seed extracts demonstrated substantial antioxidant activity against DPPH radicals, reinforcing their potential as natural antioxidant sources. Antibacterial assessments revealed varying degrees of efficacy against four clinically relevant bacterial strains with BGSE and BDSE exhibiting particularly promising antimicrobial activity. GC–MS analysis revealed unique and previously unreported phytochemicals, underscoring the novelty of this study.

This work advances the understanding of seed by-products as valuable sources of natural antioxidants, antimicrobials, minerals, and bioactive compounds. The identification of rare or unexpected metabolites, including alkyne alcohols, bile acid derivatives, and organosilicon compounds, represents a novel contribution to the field of plant phytochemistry. These findings open promising avenues for further exploration into unique plant metabolic pathways, compound isolation, and mechanism-of-action studies. Collectively, this study supports the valorization of grape and date seeds as economically and biologically significant resources with potential applications in nutraceuticals, pharmaceuticals, and sustainable functional food development.

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Potential conflicts of interest. The authors declare no competing interests.

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