Journal of Food and Dairy Sciences

Journal homepage & Available online at: www.jfds.journals.ekb.eg

Utilization of Green Extract Techniques for Preserving Bioactive Compounds in Pomegranate Peels

Amira K. Abdel-Daem; Sanaa A. Elshrif and Rokaia R. Abdelsalam*

Food Science Department, Faculty of Agriculture, Minia University, Minia 61519, Egypt

Article Information
Received / /
Accpted / /

ABSTRACT

Pomegranate (*Punica granatum* L.) peels a major agro-industrial byproduct, is a rich source of bioactive compounds with promising applications in food, pharmaceutical, and nutraceutical formulations. This study presents a detailed phytochemical and functional evaluation of pomegranate peel extract obtained through ultrasound-assisted extraction (UAE), a green and efficient technique. The pomegranate peel extract (PU) exhibited high concentrations of total phenolics (1169.4 ± 6.9 mg GAE/100 g), flavonoids (3326.1 ± 1.8 mg QE/100 g), and tannins (850.3 ± 2.4 mg/100 g). Correspondingly, it demonstrated strong antioxidant capacity, including total antioxidant activity (792.9 ± 5.8 mg AAE/100 g), high DPPH radical scavenging ability (93.5 ± 0.09%), and a low IC_{50} value (30.58 µg/mL). HPLC analysis revealed that punicalagin was the dominant ellagitannin (~ 46.03 µg/mL; 53.8% area), along with gallic acid, protocatechuic acid, ellagic acid, and quercetin. FTIR spectra results confirmed the presence of hydroxyl, carboxyl, and aromatic functional groups, indicating a polyphenol-rich structure. GC–MS analysis identified a low-molecular-weight compounds including 1H-Indene, 5-butyl-6-hexyloctahydro, [1,1'-Bicyclohexyl]-4-carboxylic acid ester, and furfural, which are infrequently mentioned in pomegranate peel and play an effect role as antioxidant and antimicrobial. The pomegranate extract (PU) demonstrated a significant antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. These obtained results support the use of pomegranate peel extract as a multifunctional natural agent for clean-label preservation, functional foods, and nutraceuticals, while promoting sustainable waste valorization through green extraction technologies.

Keywords: Pomegranate peel ; FTIR; GC-MS; Bioactive compounds; Waste valorization

INTRODUCTION

In the era of sustainable food systems, valorizing agro-industrial by-products has emerged as a promising strategy for health innovation and waste reduction. Pomegranate peel (Punica granatum L.), which accounts for up to 40–49% of the fruit's weight (*Magangana et al., 2020*). It is frequently discarded despite being rich in bioactive phenolics such as ellagic acid, gallic acid, punicalagin, and diverse tannins and flavonoids. These compounds exhibit antioxidant, antimicrobial, anti-inflammatory, cardioprotective, and anticancer properties (*Liu et al., 2022; Kim and Yoon, 2023*; *Huang et al., 2025*). Efficient recovery of these compounds is essential for converting peel waste into value-added products. Traditional extraction techniques, however, are often inefficient and environmentally burdensome. Ultrasound-assisted extraction (UAE) offers a green and scalable alternative, enhancing the release of bioactive through acoustic cavitation without high temperatures or toxic solvents (*Chemat et al., 2017*). UAE is particularly suitable for phenolics with high molecular weight and thermal sensitivity.

To maximize utilization, robust analytical techniques are required. High-Performance Liquid Chromatography (HPLC) enables quantification of polyphenols, Fourier Transform Infrared Spectroscopy (FTIR) provides insights into functional groups, and Gas Chromatography–Mass Spectrometry (GC-MS) identifies volatile and semi-volatile bioactive components with additional antimicrobial and antioxidant effects. Integrating these analyses with antibacterial assays helps link chemical composition to functional properties and industrial potential (*Kim and Yoon, 2023)*. Although prior studies on pomegranate peel have reported antioxidant potential and phenolic content, few have provided a detailed, multi-technique characterization that connects phytochemical composition with biological activity, particularly using UAE. Therefore, this study aims to comprehensively evaluate the phytochemical profile and functional groups of pomegranate peel extract via HPLC, FTIR, and GC-MS. In addition, it assesses the antibacterial efficacy of the extract against selected pathogens. The results will contribute to the sustainable valorization of pomegranate peel as a bioactive-rich resource for nutraceutical, pharmaceutical, and food preservation application

MATERIALS AND METHODS

Raw Material

Fresh pomegranate fruits (*Punica granatum* L.) were procured from the Horticulture Research Farm, Faculty of Agriculture, Minia University, Minia, Egypt

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. They were sourced from Sigma–Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany), and El-Naser Pharmaceutical Chemicals. Specific reagents included Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), which were obtained from Sigma–Aldrich and Merck.

Methods

Preparations of Pomegranate Peel

The pomegranate fruits approximately 4 kg were initially washed under running tap water to remove surface

* **Corresponding author.**
E-mail address: rokaia.guaid@minia.edu.eg
DOI: 10.21608/jfds.2025.410088.1207

impurities, followed by sanitization in a 0.1% NaCl solution for five minutes. They were thoroughly rinsed with distilled water and dried with sterile paper towels. The fruits were manually sectioned using a stainless-steel knife, and the peels were carefully separated, cut into small pieces (~1 cm^2), and then dried in a hot-air oven at 45 ± 2 °C for 72 hours or until a constant weight was achieved. This drying step aimed to inhibit microbial growth and preserve bioactive compounds. Once dried, the samples were finely ground using a laboratory mechanical grinder and then sifted through a 60-mesh sieve to ensure uniform particle size. The dried powdered peel was stored in amber glass dry containers at 4 °C until further steps.

Extraction for Pomegranate Peel Bioactive Components

Pomegranate peel extracts were prepared using a modified green extraction method incorporating ultrasound-assisted extraction, as described by *Chaudhary et al. (2022*). The optimized extraction conditions included a 30-minute sonication period using ethanol (70%) as the solvent, a solid-to-solvent ratio of 1:20 (w/v), and a temperature maintained at 35 ± 2 °C. An ultrasonic bath operating at 40 kHz was used to perform the extraction (Unique, USC-3300, Brazil). Following extraction, the mixtures were vacuum-filtered. The filtrates were concentrated using a rotary evaporator, and the concentrated extracts were subsequently dried in a vacuum oven at 40 ± 5 °C (WISD, WOV-30, Korea). Dried extracts were kept in amber bottles at 4 °C

Physicochemical and Phytochemical Profiling of Pomegranate Ultrasonic Extract (PU)

Total Anthocyanin

The total anthocyanin (TA) was quantified according to the *Myrtilli fructus* monograph described in, the Hungarian Pharmacopoeia, 5th edition with minor modifications. Anthocyanins, known for their pH-dependent chromatic properties, exhibit maximal absorbance at 528 nm in acidic conditions. Briefly, 5 g of pomegranate peel powder was extracted with 95 mL of analytical-grade methanol and shaken for 30 minutes at 25 °C. The resulting solution was diluted up to 50-fold using 0.1% (v/v) HCl in methanol, and further two-fold dilutions were prepared to ensure accurate spectrophotometric readings. Absorbance was recorded at 528 nm using HCl (0.1%) in methanol as the blank.

Total Tannin (TT)

The total tannins (TT) were determined using a modified colorimetric technique, based on the procedure described by Kavitha Chandran and Indira (2016). For the assay, a reaction mixture was prepared by combining 50 µL of diluted extract (8 mg/mL dissolved in water), 3.75 mL of distilled water, 0.25 mL of Folin–Ciocalteu reagent, and 0.5 mL of 35% Na_2CO_3. To achieve the target final volume, an extra 0.45 mL of distilled water was added. The blend was subsequently incubated in the absence of light at ambient temperature for 30 minutes before measuring the absorbance at 700 nm utilizing a UV–Vis spectrophotometer. A calibration curve was constructed by tannic acid (TA) standards ($y = 0.102x + 0.150$, $R^2 = 0.99$). The outcomes presented as (mg (TA) equivalents/g).

Total Flavonoids (TF)

The total flavonoids (TF) compounds were determined using a colorimetric method developed by *Abu Bakar et al. (2009*), with slight modifications. In this procedure, one mL of the extract was mixed with 4.5 mL of distilled water and 0.3 mL of $NaNO_2$ (5%). After allowing the mixture to react for 5 minutes, 0.6 mL of 10% aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) was added and left for six minutes. Subsequently, 2.0 mL of NaOH (1 M) was introduced, and the final volume of the solution was adjusted and thoroughly mixed, then absorbance was measured at 510 nm. Flavonoid concentrations were determined against a quercetin standard curve ($R^2 = 0.997$) and reported as (mg QE equivalents/ 100 g).

Total Phenolic (TP)

The total phenolics (TP) compounds of the PU extracts was determined using spectrophotometric method as described by *Musa et al. (2011*). The absorbance was measured at the respective optimal wavelength after reaction with the FC reagent. Results were derived using a gallic acid standard curve. Phenolic content was expressed as (mg GAE equivalents/g).

DPPH Radical Scavenging Activity

The antioxidant capacity of the PU extract was determined using the protocol of *Brand-Williams et al. (1995*) methodology. For the assay, 2 mL of the extract at different concentrations (1–64 µg/mL) was mixed with an equal volume of DPPH solution in methanol (25 mg/L). The mixtures were vortexed to ensure uniform blending and incubated for 30 min at room temperature in darkness to minimize photodegradation, after which absorbance was determined at 517 nm using a UV–Vis spectrophotometer. Methanol used as the blank reference. The radical scavenging activity (%) was determined using this formula:

$$[(A_0 - A_1) / A_0] \times 100,$$

Where A_0 represents the absorbance of the control and A_1 denotes the absorbance of the test sample. The half-maximal inhibitory concentration (IC_{50}), reflecting the extract concentration required to neutralize 50% of DPPH radicals, was also calculated to evaluate antioxidant potential.

Characterizations of Functional Groups

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra was utilized to characterize the functional groups of pomegranate peels before (PE) and after ultrasonic (PU) extraction. Approximately two mg of PU or PE was mixed with fifty mg of FTIR-grade KBr and compressed into pellets. A Shimadzu IR Affinity^{-1} spectrophotometer (Japan) was employed for spectral analysis, Following was covered a wavenumber range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}, as described by *Khalid et al. (2023).*

Gas Chromatography–Mass Spectrometry (GC–MS)

The GC–MS analysis was carried out on pomegranate peels before (PE) and after ultrasonic extraction (PU). The analysis was conducted using a Thermo Scientific system featuring a TG-5MS fused silica capillary column (30 m × 0.25 mm internal diameter, 0.1 µm film thickness) at the National Research Centre (NRC) in Dokki, Giza. Components were identified by comparing their retention times and mass spectral with those in the NIST and Wiley libraries (Adams, 2007). Quantification was expressed as relative percentage peak areas.

Quantitative Determination of Polyphenols by HPLC

The characterization and quantification of phenolic and flavonoid compounds were performed using HPLC, employing an Agilent 1260 Series system (USA) supplied with a multi-wavelength detector calibrated to 280 nm. Sample preparation involved dissolving the extract in one

milliliter of a methanol (50%), followed by clarified by passing through a 0.45 µm PTFE syringe filter. Subsequently, a ten-microliter aliquot of the prepared solution was introduced into the HPLC system for analysis. The separation of components was performed using an Inertsil C18 column with a particle size of 5 µm and dimensions of 4.6 × 250 mm, maintaining a constant temperature of 25 °C throughout the procedure. A gradient elution technique was employed at with a flow rate set to 0.9 mL/min, the mobile phase included two solvents: solvent A, containing 0.1% orthophosphoric acid in water, and solvent B, comprising 0.05% trifluoroacetic acid in acetonitrile. The total runtime of the analysis was 80 minutes. The following standard reference compounds were used for qualitative and quantitative comparison: gallic acid, rutin, chlorogenic acid, catechin, ellagic acid, quercetin, kaempferol, apigenin, hesperidin, and caffeic acid.

Antimicrobial Activity Evaluation for Pomegranate Extract

Microbial Strains and Inoculum Preparations

Antimicrobial evaluation was conducted against four bacterial strains, including *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive), and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative). Cultures were cultivated to the logarithmic phase on nutrient-dense media. Subsequently, they were harvested, washed, and resuspended in sterile saline. The inoculated samples were standardized to 0.5 McFarland standard. It was corresponding to approximately 1.5×10^8 CFU/mL through optical density measurement. Subsequently, inoculated into tryptic soy broth (TSB) and maintained at 37 °C for 24 hours. Neomycin (100 µg) was utilized as the positive control for both bacterial strains (*Gonelimali et al.,2018).*

Agar Diffusion Assays

Antibacterial activity of PU extract was determined using well and disc diffusion assays on Mueller-Hinton Agar (MHA). An inoculum of 100 µL was applied to the plates of fresh bacterial suspension. For the well diffusion, wells (6 mm) were bored into the agar and filled with 100 µL of extract solution (20 mg/mL). For the disc diffusion, sterile paper discs (6 mm) were impregnated with the extract and set on the inoculated agar surface. Incubation of the plates was carried out at 37 °C for 24 hours., after which the zones of inhibition (ZOI) were measured (mm) with the aid of Vernier caliper (*Gonelimali et al., 2018*).

Statistical Analysis

SPSS software version 26 was utilized to carry out the statistical analysis. Results are presented as mean ± standard deviation (SD). Duncan's multiple range test was applied to determine significant differences at $p < 0.05$. Moreover, identification and assignment of functional groups in FTIR spectra, according to their characteristic peak positions and relative intensities, were carried out using IR Analyzer Spectroscopic Solution software.

RESULTS AND DISCUSSION

The present study investigates the impact of ultrasonic-assisted extraction on the phytochemical composition and functional properties of pomegranate peel extracts. Key parameters including total phenolics, flavonoids, anthocyanins, and antioxidant capacity were assessed, alongside structural characterization via FTIR, compound identification by GC-MS, and antimicrobial potential. The findings are interpreted in light of previous reports to demonstrate the efficacy of green extraction in enhancing bioactive yield and biological activity.

Phytochemical Composition and Antioxidant Potential

Phytochemical characterizations and antioxidant evaluation of PU were evaluated (Table 1). The peel exhibited high level of flavonoids (3326.1 ± 1.8 mg QE/100 g), tannins (850.3 ± 2.4 mg/100 g), and total phenolic content (1169.4 ± 6.9 mg GAE/100 g). Correspondingly, it showed superior antioxidant properties, as evidenced by its elevated total antioxidant capacity (792.9 ± 5.8 mg AAE/100 g), strong DPPH radical scavenging activity (93.5 ± 0.09%), and low IC_{50} value (30.58 µg/mL).

Table 1. Phytochemical Compositions and Antioxidant Activity of Pomegranate Ultrasonic Peel Extract (PU)

Components	TP (mg GAE/100g)	TF (mg QE/100g)	TAC (mg AAE/100g)	DPPH (% Inhibition)	IC_{50} (µg/mL)	Tannins (mg/100g)	Anthocyanins (mg/100g)
PU	1169.4 ± 6.9	3326.1 ± 1.8	792.9 ± 5.8	93.5 ± 0.09	30.58	850.3 ± 2.4	4.62 ± 0.22

TP: Total phenolic content; TF: Total flavonoid content; TAC: Total antioxidant capacity; GAE: Gallic acid equivalent; QE: Quercetin equivalent; AAE: Ascorbic acid equivalent; IC_{50}: The concentration required to inhibit 50% of DPPH radicals

These results concur with earlier studies, which consistently report that pomegranate peel has enhanced bioactive compound content and antioxidant activity compared with other fruit components (*Abid et al., 2017; Siddiqui et al., 2024*). High-performance liquid chromatography (HPLC) profiling of PU revealed a rich spectrum of phenolics, underscoring its potential as a valuable rich reservoir of natural antioxidants. Eleven prominent peaks were observed in the chromatogram, shown in Figure1 &Table 2. Ellagic acid, confirmed at a retention time of 49.67 minutes, represented 2.43% of the total peak area with an estimated concentration of 2.08 µg/mL. The dominant phenolic compound was identified at RT = 21.19 min and attributed to punicalagin isomer, accounting for 53.8% of the total chromatographic area (46.03 µg/mL). This finding supports recent studies highlighting punicalagin as the principal ellagitannin and antioxidant in pomegranate peel extracts (*Yang et al., 2023; Giri et al., 2023; Noreen et al., 2025*).

Gallic acid (RT = 2.96 min) was also abundant (21.0%, 17.98 µg/mL), aligning with reports that gallic acid is a key hydroxybenzoic acid in pomegranate peel with strong antioxidant capacity and anti-inflammatory potential (*Singh et al., 2022 and Azmat et al., 2024*). Additional early-eluting peaks were tentatively identified as protocatechuic acid (~5.5 min), p-hydroxybenzoic acid (~6.9 min), and chlorogenic acid (~9.4 min), with estimated concentrations ranging from 1.80 to 2.74 µg/mL. These assignments are characteristic of the fingerprints reported in recent chromatographic investigations of pomegranate peel (*Man et al., 2022; Giri et al., 2023*). A mid-polar compound at ~11.6 min was more accurately identified as p-coumaric acid, rather than caffeic acid, based on retention behavior and comparative literature data (*Farouk et al., 2023; Man et al.,*

2022). Other minor peaks included vanillic acid (~13.8 min) and syringic acid (~15.0 min), known contributors to the antioxidant and antimicrobial capacity of the peel (*Azmat et al., 2024*). Ferulic acid (RT = 17.18 min) was detected at 4.20 µg/mL, while a late peak at RT = 40.490 min was assigned to tannic acid or related hydrolysable tannins (2.14 µg/mL). Thus, pomegranate peel's rich polyphenolic matrix and reinforcing its potential application in nutraceuticals, food preservation, and pharmaceutical development (*Yang et al., 2023; Noreen et al., 2025*).

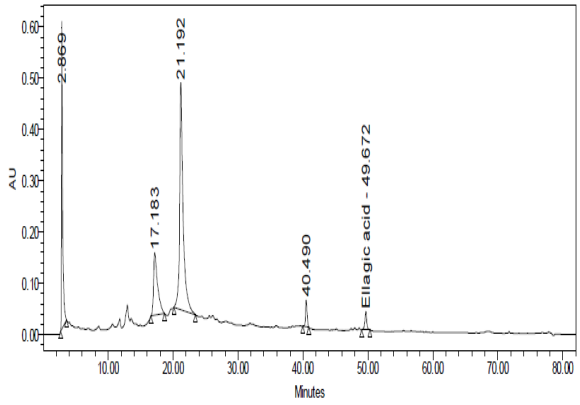

Figure1. Phenolic Composition of Pomegranate Peel Extract (PU) Revealed by HPLC

Fourier Transform Infra-Red

Infrared spectral analysis is a vital method for characterization of the functional group represent in extracts. The FTIR spectrum, shown in found in Figure 2, displays several important changes in absorption bands. Pomegranate peel is known to contain a variety of biologically active components. Prior to ultrasonic extraction, Key absorption bands were observed. Firstly, stretching of hydroxyl groups was peaked at 3416 cm^{-1}; aliphatic C–H stretching at 2929 cm^{-1} and C=O stretching of esterified carboxylic acids or flavonoid glycosides at 1732 cm^{-1} (*Chemat et al., 2017*). Additional, notable aromatic C=C stretching was peaked at 1617 cm^{-1}, aromatic ring vibrations of phenolics at 1516 cm^{-1}, C–H bending at 1442 cm^{-1} and O–H bending at 1354 cm^{-1}. Asymmetric stretching C–O–C in esters was peaked at 1231 cm^{-1}, C–O stretching in polysaccharides at 1054 cm^{-1} and finally at 875 cm^{-1} (C–H out-of-plane bending) in aromatic rings (*Giri et al., 2021*). Following ultrasonic extraction, several absorption bands showed increased intensity and resolution in the FTIR spectrum. A broad peak at 3384 cm^{-1} confirms the presence of alcohols or phenols. Alkanes were identified at 2936 cm^{-1} attributed to aliphatic C–H stretching. A sharp absorption peak at 1728 cm^{-1} is corresponds to carbonyl groups. It is implying the presence of aldehydes, ketones, carboxylic acids, or esters (*Wong et al., 2015*). A moderate band at 1613 cm^{-1} indicates the unsaturated compounds, while a peak at 1350 cm^{-1} is related to the amide III bond potentially associated with triple helical structures (*Gupta et al., 2023*). The presence of esters and ethers peak confirms at 1226 cm^{-1}. The band at 1054 cm^{-1} suggests the continued presence of polysaccharides, and a new peak at 918 cm^{-1} implies the formation or exposure of additional functional groups (*Phyo et al., 2025*). Due to the complex nature of the peel matrix, overlapping bands were observed; however, the most critical peaks are summarized in the accompanying Figure 2. Ultrasonic extraction resulted shift of the O–H stretching band from 3416 cm^{-1} to 3384 cm^{-1}, which indicating a change in the hydrogen bonding environment. This shift may be attributed to solvation effects during extraction, which could enhance the release of polyphenolic hydroxyl groups. Such modifications are directly linked to stronger antioxidant activity, as hydroxyl groups serve as primary hydrogen donors for free radical scavenging (Idris *et al., 2024*). Similarly, C–H stretching peak was shifted from 2929 cm^{-1} to 2936 cm^{-1} with accompanied by a 10% decrease in absorption intensity. It was possibly due to structural changes in the methylene chains. Shifting of the carbonyl band from 1732 cm^{-1} to 1728 cm^{-1} along with increased sharpness, suggests a modified extraction environment for esters, likely due to partial hydrolysis or free acid formation. The exposure of carbonyl and ester groups may enhance antibacterial activity by facilitating interactions with microbial cell membranes and proteins (*Singh et al., 2022*). Moreover, presence of a new peak at 918 cm^{-1} further supports formation or exposure of new functional groups. This novel peak could reflect structural transformations that contribute to improved bioavailability and functional bioactivity of the extract (*Tang et al., 2025*).

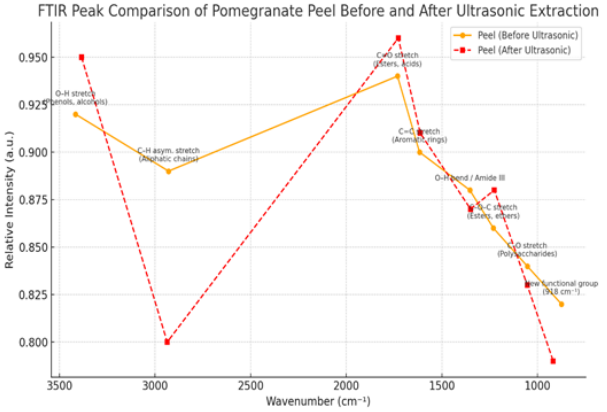

Figure 2. Comparative FTIR Analysis of Pomegranate Peel Before (PE) and After (PU) Ultrasonic Extraction.

Table 2. Phenolic Composition of Pomegranate Peel Extract (PU) Revealed by HPLC

RT (min)	Compound	Area (%)	Conc. (µg/mL)
2.96	Gallic acid	21.0%	17.98
5.5	Protocatechuic acid	3.2%	2.74
6.9	p-Hydroxybenzoic acid	2.9%	2.48
9.4	Chlorogenic acid	2.1%	1.80
11.6	p-Coumaric acid	2.0%	1.71
13.8	Vanillic acid	2.1%	1.80
15.0	Syringic acid	2.0%	1.71
17.18	Ferulic acid	4.9%	4.20
21.19	Punicalagin	53.8%	46.03
40.49	Tannic acid	2.5%	2.14
49.67	Ellagic acid	2.43%	2.08

GC-MS Profiling of PU Extract

GC-MS analysis revealed distinct compositional differences between conventionally extracted (PE) and ultrasound-assisted (PU) pomegranate peel extracts (Tables 3 and 4). In PE, the extract was primarily dominated by 5-(Hydroxymethyl)-2-furancarboxaldehyde (HMF) at 16.44%, a well-documented Maillard reaction product known for its

antioxidant, antimicrobial, and cytoprotective properties (*Choudhary et al., 2021; Utami et al., 2025*). The presence of HMF may, therefore, partly explain the measurable inhibition zones recorded against Gram-positive strains, as HMF has recently been reported to exhibit antibacterial activity at MIC values ranging between 40–160 µg/mL (*Noreen et al., 2025*). Additionally, high levels of 11,13-dimethyl-12-tetradecen-1-ol acetate (15.9%) and E-10,13,13-trimethyl-11-tetradecen-1-ol acetate (14.3%) were identified. These long-chain aliphatic esters belong to a structural class recognized for antimicrobial, anti-inflammatory, and insecticidal activities, likely contributing to the peel's natural defense mechanisms (*Alsufyani et al., 2023*). Fatty acid derivatives such as oleic acid (9.01%), cis-vaccenic acid (7.67%), and trans-13-octadecenoic acid (3.22%) were also prevalent. Importantly, unsaturated fatty acids like oleic and vaccenic acids have been previously linked to membrane-disruptive antibacterial mechanisms, providing additional support for the inhibitory patterns noted in this study *(Kupnik et al., 2021 and Gruznov et al., 2024*).

Table 3. GC-MS Profile of Pomegranate Peel Extract before Ultrasonic Extraction

Nr	Compound Name	Molecular Formula	MW (g/mol)	Area (%)
1	Furfural	$C_5H_4O_2$	96	1.85
2	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	$C_{13}H_{21}NO_4$	255	0.89
3	2,5-Furandione, 3-methyl-	$C_5H_4O_3$	112	0.66
4	4H-Pyran-4-one, 2,3-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.96
5	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	16.44
6	cis-9,10-Epoxyoctadecan-1-ol	$C18H_{36}O_2$	284	1.38
7	2-Dodecylcyclohexanone	$C_{18}H_{34}O$	266	1.72
8	S (+)-Z-13-Methyl-11-pentadecen-1-ol acetate	$C_{18}H_{34}O_2$	282	0.58
9	Erucic acid	$C_{22}H_{42}O_2$	338	0.56
10	[1,1'-Bicyclohexyl]-4-carboxylic acid, ester	$C_{22}H_{31}FO_2$	346	2.4
11	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280	0.68
12	Cyclohexane, 1,1'-dodecylidenebis [4-methyl-	$C_{26}H_{50}$	362	3.4
13	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	3.22
14	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	3.34
15	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282	15.93
16	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356	0.56
17	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	$C_{19}H_{36}O_2$	296	14.29
18	Oleic Acid	$C_{18}H_{34}O_2$	282	9.01
19	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	3.09
20	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	7.67
21	9-Octadecenoic acid, 1,2,3-propanetrinyl ester (E, E, E)	$C_{57}H_{104}O_6$	884	1.84
22	1-Monolinoleoylglycerol TMS ether	$C_{27}H_{54}O_4Si_2$	498	7.93
23	9,12,15-Octadecatrienoic acid,2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy] methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	0.57

MF: Molecular Formula;MW: Molecular weight

Table 4. GC-MS Profile of Pomegranate Peel Extract after Ultrasonic Extraction

Nr	Compound Name	MF	MW (g/mol)	Area (%)
1	Furfural	$C_5H_4O_2$	96	4.67
2	3,4-Dehydro-L-proline	$C_5H_7NO_2$	113	1.5
3	3-Furancarboxylic acid, methyl ester	$C_6H_6O_3$	126	1.67
4	1,3-Dioxane-4-methanol, 4,5-dimethyl	$C_7H_{14}O_3$	146	1.57
5	3,4-Altrosan	$C_6H_{10}O_5$	162	0.7
6	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	$C_{13}H_{21}N_4$	255	3.24
7	4-Chloro-3-n-butyltetrahydropyran	$C_9H_{17}ClO$	176	0.66
8	4H-Pyran-4-one, 2,3-dihydroxy-6-methyl-	$C_6H_8O_4$	144	5.6
9	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	48.57
15	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.79
12	Pentanoic acid, 10-undecenyl ester	$C16H_{30}O2$	254	1.36
13	1H-Indene, 5-butyl-6-hexyloctahydro-	$C_{19}H_{36}$	264	1.67
14	[1,1'-Bicyclohexyl]-4-carboxylic acid, ester	$C_{22}H_{31}FO_2$	346	2.45
16	Cyclohexane, 1,1'-dodecylidenebis [4-methyl-	$C_{26}H_{50}$	362	0.81
17	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	1.43
18	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	0.67
19	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282	10.8
21	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	$C_{19}H_{36}O_2$	296	3.14
22	Oleic Acid	$C_{18}H_{34}O_2$	282	2.53
23	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	2.21
24	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	2.09
26	1-Monolinoleoylglycerol TMS ether	$C_{27}H_{54}O_4Si_2$	498	0.85

After UAE, the chemical profile of PU became more diverse and enriched. HMF content surged to 48.57%, reflecting the ultrasound-induced breakdown of carbohydrate-bound structures and increased liberation of Maillard-derived antioxidants. Notably, esterified alcohols were remained significant, supporting the hypothesis that UAE facilitates the release of thermolabile and matrix-bound compounds. New bioactive peaks appeared post-ultrasound,

including 3,4-dehydro-L-proline, 3-furancarboxylic acid methyl ester, and 17-octadecynoic acid, which were not detected pre-treatment. These suggest the cavitation forces generated during UAE can dislodge or transform previously inaccessible compounds. Notably, 17-octadecynoic acid has been reported to exert inhibitory effects on microbial fatty acid synthesis, thereby reinforcing the biological plausibility of the enhanced antibacterial outcomes obtained after UAE (*Altaie et al., 2023*). Simultaneously, fatty acid esters and cyclohexane derivatives increased in abundance, further contributing to the peel's bioactive spectrum (*Sharayei et al.,2019; Yassin et al., 2021; Zhou et al., 2023*). Altogether, these results highlight the efficiency of UAE in intensifying the extraction of aromatic aldehydes, esters, fatty acids, and structurally diverse volatiles from pomegranate peel. The post-ultrasound profile not only confirms greater yield but also richer complexity of biologically active molecules, reinforcing UAE as a sustainable, green extraction method for valorizing agro-industrial byproducts (*Kim and Yoon, 2023)*. Importantly, the presence and enrichment of antimicrobial-related compounds such as HMF, unsaturated fatty acids, and bioactive esters directly align with the improved inhibition zones observed, providing a mechanistic link between chemical composition and antibacterial efficacy (*Kupnik et al., 2021, Abdelmegiud et al., 2024 and Noreen et al., 2025*).

Antibacterial Efficacy of PU Extract

The antibacterial potential of PU extract (PU) was assessed against four clinically relevant bacterial strains shown in (Figure 3).

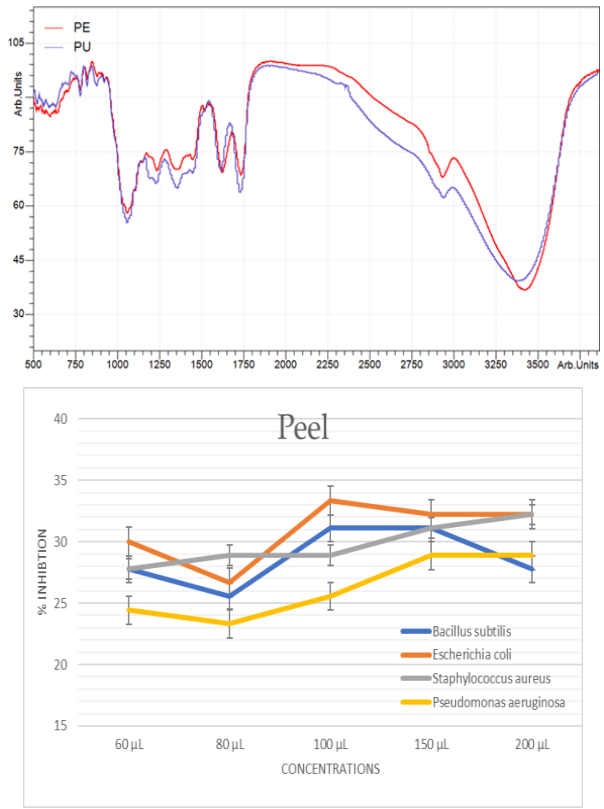

Figure 3. Antibacterial Efficacy of Pomegranate Extracts Against Gram-Positive and Gram-Negative Strains

It had exhibited an inhibitory effect proportional to the applied dose with the strongest activity observed against Gram-positive bacteria. Notably, B. subtilis inhibition reached 31.11% at concentrations of 100–150 µL, while S. aureus inhibition ranged from 27.78% to 32.22%. These effects are likely attributable to the high levels of phenolics and hydrolysable tannins in the extract, which are known to interfere with microbial membranes and inhibit enzymatic activity (*Abutayeh et al., 2024*). The extract also demonstrated activity against the Gram-negative bacterium P. aeruginosa, achieving up to 28.89% inhibition. Despite the known resistance mechanisms of P. aeruginosa, including efflux pumps and a low-permeability outer membrane (Poole, 2001), the observed activity in agreement with previous findings by *Chen et al. (2020)*. Compared to earlier studies (*Antonelli et al., 2021; Kupnik et al., 2021*), the current work highlights the advantages of ultrasonic extraction in enhancing both phytochemical content and antimicrobial properties. The emergence of unique bioactive compounds post-sonication likely contributes to the improved antibacterial performance.

CONCLUSION

This study highlights the potential of pomegranate peel as a valuable source of bioactive compounds, exhibiting significant antioxidant and antimicrobial properties. The use of ultrasound-assisted extraction proved to be an efficient and environmentally friendly technique, enhancing the yield and quality of phytochemicals, as verified by FTIR, GC-MS, and HPLC analyses. The FTIR spectra confirmed functional group modifications post-extraction, while GC-MS and HPLC profiling revealed a diverse array of phenolic and flavonoid compounds with known biological activities. The antimicrobial properties exhibited by the extracts against both Gram-positive and negative bacteria underscore their potential as natural substitutes for synthetic antimicrobial agents. These results hold promise for applications in nutraceuticals, natural preservatives, and antimicrobial agents, aligning with global efforts toward circular bioeconomy and clean-label ingredient sourcing. Future research should focus on scaling up extraction processes, elucidating molecular mechanisms of action, and conducting in vivo studies to fully validate the therapeutic and industrial potential of these extracts.

REFERENCES

Abdelmegiud, M. H., El-Soukkary, F. A. H., EL-Naggar, E. A., & Abdelsalam, R. R. (2024). Evaluation of some gluten-free biscuits formulations comparison to wheat flour biscuits. Egyptian Journal of Food Science, 52(2), 231-242.

Abid, M., Yaich, H., Cheikhrouhou, S., Khemakhem, I., Bouaziz, M., Attia, H., Ayadi, M. A. (2017). Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. Journal of food science and technology 54(9): 2890-2901.

Abu Bakar, M.F.; Mohamed, M.; Rahmat, A. Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan mangifera pajang and tarap Artocarpus odoratissimus. Food Chemistry 113(2): 479-483.

Abutayeh, R. F., Ayyash, M. A., Alwany, R. A., Abuodeh, A., Jaber, K., Al-Najjar, M. A. (2024). Exploring the antimicrobial potential of pomegranate peel extracts (PPEs): Extraction techniques and bacterial susceptibility. PloS one 19(12): e0315173.

Adams, R. P. (2007). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing 46-52.

Alsufyani, T., Al-Otaibi, N., Alotaibi, N. J., M'sakni, N. H., & Alghamdi, E. M. (2023). GC analysis, anticancer, and antibacterial activities of secondary bioactive compounds from endosymbiotic bacteria of pomegranate aphid and its predator and protector. Molecules 28(10): 4255.

Altaie, A. M., Mohammad, M. G., Madkour, M. I., Shakartalla, S. B., Jayakumar, M. N., KG, A. R., Soliman, S. S. (2023). The essential role of 17-octadecynoic acid in the pathogenesis of periapical abscesses. Journal of Endodontics, 49(2), 169-177.

Antonelli, G., Cappelli, L., Cinelli, P., Cuffaro, R., Manca, B., Nicchi, S., Schiavetti, F. (2021). Strategies to tackle antimicrobial resistance: The example of Escherichia coli and Pseudomonas aeruginosa. International journal of molecular sciences 22(9): 4943.

Azmat, F., Safdar, M., Ahmad, H., Khan, M. R. J., Abid, J., Naseer, M. S., Suleria, H. A. R. (2024). Phytochemical profile, nutritional composition of pomegranate peel and peel extract as a potential source of nutraceutical: A comprehensive review. Food Science & Nutrition 12(2): 661-674.

Brand-Williams, W., Cuvelier, M. E., Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 28(1): 25-30.

Chaudhry, F., Ahmad, M. L., Hayat, Z., Ranjha, M. M. A. N., Chaudhry, K., Elboughdiri, N., Uddin, J. (2022). Extraction and evaluation of the antimicrobial activity of polyphenols from banana peels employing different extraction techniques. Separations, 9(7), 165.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrasonics sonochemistry 34: 540-560.

Chen, J., Liao, C., Ouyang, X., Kahramanoğlu, I., Gan, Y., Li, M. (2020). Antimicrobial activity of pomegranate peel and its applications on food preservation. Journal of Food Quality (1): 8850339.

Choudhary, A., Kumar, V., Kumar, S., Majid, I., Aggarwal, P., Suri, S. (2021). 5-Hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: Recent developments. Toxin Reviews 40(4): 545-561.

Farouk, S. M., Abu-Hussien, S. H., Abd-Elhalim, B. T., Mohamed, R. M., Arabe, N. M., Hussain, A. A., Galal, A. (2023). Biosynthesis and characterization of silver nanoparticles from Punica granatum (pomegranate) peel waste and its application to inhibit foodborne pathogens. Scientific Reports 13(1): 19469.

Giri, N. A., Gaikwad, N. N., Raigond, P., Damale, R., Marathe, R. A. (2023). Exploring the potential of pomegranate peel extract as a natural food additive: a review. Current nutrition reports 12(2): 270-289.

Giri, R., Kumari, N., Behera, M., Sharma, A., Kumar, S., Kumar, N., Singh, R. (2021). Adsorption of hexavalent chromium from aqueous solution using pomegranate peel as low-cost biosorbent. Environmental Sustainability 4(2): 401-417.

Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in microbiology 9: 1639.

Gruznov, D. V., Gruznova, O. A., Lobanov, A. V., Sokhlikov, A. B., Shcherbakova, G. S., Stepanova, S. P., Popov, N. I. (2024). Influence of Different Heat Treatment Regimes on the Change of the Chemical Composition and Antibacterial Activity of Bee Honey. Russian Journal of Physical Chemistry B, 18(1), 258-265.

Gupta, Preeti, S., Das S., Singh, N. B. (2023). "Infrared spectroscopy." In Spectroscopy, pp. 151-211. Jenny Stanford Publishing. https:// doi.org /10.12 01/9 781003412588.

Huang, L., Zhang, J., Li, Y., Chen, X. (2025). Pomegranate peel polyphenols: Characterization, antioxidant activity and potential applications. Bioresources and Bioprocessing 12(1): 38.

Idris, S., Jan, F., Waheed, M., Alam, A., Ibrahim, M., Alasmari, A. F., Khan, M. (2024). Multifaceted bioactivity of thiosemicarbazide derivatives: synthesis, characterization, and DFT investigations on inhibition of α-amylase, hydroxyl radical scavenging, and iron chelating activities with molecular docking insights. Journal of Molecular Structure, 1304, 137669.

Kavitha Chandran, C. I., Indira, G. (2016). Wound healing activity of ethanolic extract of roots of Morinda pubescens JE Smith. Journal of Pharmacognosy and Phytochemistry 5(3): 43-46.

Khalid, S., Arshad, M., Mahmood, S., Siddique, F., Roobab, U., Ranjha, M. M. A. N., Lorenzo, J. M. (2023). Extraction and quantification of Moringa oleifera leaf powder extracts by HPLC and FTIR. Food Analytical Methods 16(4): 787-797.

Kim, H. J., Yoon, K. Y. (2023). Optimization of ultrasound-assisted deep eutectic solvent extraction of bioactive compounds from pomegranate peel using response surface methodology. Food Science and Biotechnology 32(13): 1851-1860.

Kupnik, K., Primožič, M., Vasić, K., Knez, Ž., Leitgeb, M. (2021). A comprehensive study of the antibacterial activity of bioactive juice and extracts from pomegranate (Punica granatum L.) peels and seeds. Plants 10(8): 1554.

Liu, X., Renard, C. M., Bureau, S., Le Bourvellec, C. (2021). Revisiting the contribution of ATR-FTIR spectroscopy to characterize plant cell wall polysaccharides. Carbohydrate Polymers 262: 117935.

Liu, Y., Kong, K. W., Wu, D. T., Liu, H. Y., Li, H. B., Zhang, J. R., Gan, R. Y. (2022). Pomegranate peel-derived punicalagin: Ultrasonic-assisted extraction, purification, and its α-glucosidase inhibitory mechanism. Food Chemistry 374: 131635.

Magangana, T. P., Makunga, N. P., Fawole, O. A., Opara, U. L. (2020). Processing factors affecting the phytochemical and nutritional properties of pomegranate (Punica granatum L.) peel waste: A review. Molecules 25(20): 4690.

Man, G., Xu, L., Wang, Y., Liao, X., Xu, Z. (2022). Profiling phenolic composition in pomegranate peel from nine selected cultivars using UHPLC-QTOF-MS and UPLC-QQQ-MS. Frontiers in Nutrition 8: 807447.

Mphahlele, R. R., Fawole, O. A., Mokwena, L. M., Opara, U. L. (2016). Effect of extraction method on chemical, volatile composition and antioxidant properties of pomegranate juice. South African Journal of Botany 103: 135-144.

Musa, K. H., Abdullah, A., Jusoh, K., Subramaniam, V. (2011). Antioxidant activity of pink-flesh guava (Psidium guajava L.): effect of extraction techniques and solvents. Food Analytical Methods 4(1): 100-107.

Noreen, S., Hashmi, B., Aja, P. M., Atoki, A. V. (2025). Phytochemicals and pharmacology of pomegranate (Punica granatum L.): nutraceutical benefits and industrial applications: a review. Frontiers in Nutrition 12: 1528897.

Phyo, H. M., Al-Maqtari, Q. A., Othman, N., Hussain, A., Yuan, S., Yu, H., Yao, W. (2025). Ultrasonication-enhanced nanoencapsulation of thymol and cinnamaldehyde with chitosan and Hydroxypropyl Methylcellulose: a novel approach to improving physicochemical properties, stability, and bioactive content. Food and Bioprocess Technology 18(3), 2925-2948.

Poole, K. (2001). Multidrug resistance in Gram-negative bacteria. Current Opinion in Microbiology 4(5): 500-508.

Sharayei, P., Azarpazhooh, E., Zomorodi, S., Ramaswamy, H. S. (2019). Ultrasound assisted extraction of bioactive compounds from pomegranate (Punica granatum L.) peel. LWT 101: 342-350.

Siddiqui, S. A., Singh, S., Nayik, G. A. (2024). Bioactive compounds from pomegranate peels-Biological properties, structure–function relationships, health benefits and food applications–A comprehensive review. Journal of Functional Foods 116: 106132.

Singh, B., Singh, J. P., Kaur, A., Singh, N. (2022). Phenolic composition and antioxidant activity of pomegranate peel and seed extract. Journal of Functional Foods 91: 105023.

Tang, S., Wang, B., Liu, X., Xi, W., Yue, Y., Tan, X., Huang, L. (2025). Structural insights and biological activities of flavonoids: Implications for novel applications. Food Frontiers, 6(1), 218-247.

Utami, M. M. D., Dewi, A. C., Tri Hertamawati, R., Jasmine, D., Wafia, N., Saputri, J., Septiyanto, K. H. (2025). Determination of chemical, bioactive compounds and volatile composition in different extracts and the parts of pomegranate (*Punica granatum* L.) as a natural feed additive. Advances in Animal and Veterinary Sciences 13(3): 648-657.

Viuda-Martos, M., Fernández-López, J., Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: a review. Comprehensive reviews in food science and food safety 9(6): 635-654.

Yang, X., Niu, Z., Wang, X., Lu, X., Sun, J., Carpena, M., Li, N. (2023). The nutritional and bioactive components, potential health function and comprehensive utilization of pomegranate: a review. Food Reviews International 39(9): 6420-6446.

Yassin, M. T., Mostafa, A. A. F., Al Askar, A. A. (2021). In vitro evaluation of biological activities and phytochemical analysis of different solvent extracts of Punica granatum L. (Pomegranate) peels. Plants 10(12):2742.

Zhou, M., Fakayode, O. A., Li, H. (2023). Green extraction of polyphenols via deep eutectic solvents and assisted technologies from agri-food by-products. Molecules 28(19): 6852.

الاستفاده من تقنيات الفصل الامنه للحفاظ على المركبات الحيويه فى قشور الرمان

اميره خلف عبد الدايم ، سناء احمد الشريف و رقيه رمضان عبد السلام

قسم علوم الاغذيه - كليه الزراعه - جامعه المنيا

الملخص

يُعد قشر الرمان (*Punica granatum L.*) أحد المنتجات الثانوية الرئيسية للصناعات الغذائيه، فهو مصدرًا غنيًا للمركبات النشطة بيولوجيًا، لذلك له تطبيقات واعدة في مجالات الأغذية والصيدلة والمستحضرات الغذائية الصحية. تهدف هذه الدراسة إلى إجراء تقييم كيميائي نباتي ووظيفي شامل لمستخلص قشر الرمان المُستخلص باستخدام تقنية الموجات فوق الصوتية (UAE) ، والتي تُعد تقنية خضراء وفعّالة. أظهرت النتائج احتواء القشور على تركيزات مرتفعة من المركبات الفينولية الكلية (١١٦٩,٤ ± ٦,٩ ملجم مكافئ حمض الجاليك/١٠٠جم)، والفلافونويدات (٣٣٢٦,١ ± ١,٨ ملجم مكافئ كيرسيتين/١٠٠جم)، والتانينات (٨٥٠,٣ ± ٢,٤ ملجم/١٠٠جم). كما أظهرت نشاطًا مرتفعا مضادًا للأكسدة، بما في ذلك القدرة الكلية المضادة للأكسدة (٧٩٢,٩ ± ٥,٨ ملجم مكافئ حمض الأسكوربيك/١٠٠جم)، ونشاطًا عاليًا في تثبيط جذور DPPH (0.09 ± 93.5%) ، بالإضافة إلى قيمة IC_{50} منخفضة (٣٠,٥٨ ميكروجرام/مل). كشفت نتائج تحليل ال HPLC عن وجود البونيكالاجين كمركب إيلاجيتانين سائد (~٤٦,٠٣ ميكروجرام/مل) الى جانب حمض الجاليك، حمض البروتوكاتيكويك، حمض الإيلاجيك، والكيرسيتين. كما أكدت أطياف FTIR وجود مجموعات وظيفية مثل الهيدروكسيل، الكربوكسيل، والبنزينات العطرية، مما يشير إلى ارتفاع نسبه البوليفينولات. وحدد تحليل GC–MS وجود مركبات ذات وزن جزيئي منخفض تشمل إندين-H١، ٥-بيوتيل-٦-هيكسي أوكتاهيدرو، إستر حمض البيسيكلوهيكسيل-٤-كربوكسيليك، والفورفورال، وهي مركبات لم يسبق الحصول عليها في قشر الرمان سابقا وتُساهم في النشاطين سواء المضاد للأكسدة والمضاد للميكروبات. كما أظهرت نتائج مستخلص القشور نشاطًا مضادًا للبكتيريا ضد *Staphylococcus aureus* و *Escherichia coli*. لذلك تُعزز هذه النتائج إمكانيات الاستفاده من مستخلص قشر الرمان كمكون طبيعي في حفظ الأغذية و الأغذية الوظيفية، والمستحضرات الغذائية الصحية، مع زياده الاستغلال الامثل للنفايات باستخدام تقنيات الاستخلاص الخضراء.

الكلمات الداله: قشر الرمان؛ المركبات الحيويه الفعاله ؛ FTIR ؛ GC-MS ؛ تثمين النفايات.