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Ultrasound-Assisted Extraction and HPLC Quantitative Profiling of Phytochemical Compounds in Yemeni Red Onion Peels (*Allium cepa* L.)

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Abstract

This study reports the ultrasound-assisted extraction (UAE) and quantitative profiling of antioxidants in methanolic extracts of Yemeni red onion peels (Allium cepa L.) using high-performance liquid chromatography (HPLC). The HPLC profiling identified a chemically diverse composition comprising 15 Phenolic compounds classified into four major groups: Flavonoids (61.85 %), Phenolic Acids (33.07 %), Isoflavones (0.61 %), and Phenolic Derivatives (2.26 %). Quercetin was the predominant compound with a concentration of 52289.14 µg/g DW, followed by Chlorogenic acid 18,761.89 µg/g DW, alongside significant amounts of Gallic acid, Ellagic acid, and Daidzein. The results indicate a high Phenolic content and strong antioxidant potential in Yemeni red onion peels, confirming their suitability as a natural source of bioactive compounds for nutraceutical and pharmaceutical applications.

Article Info

Keywords:
Yemeni Red Onion Peels, Ultrasound-Assisted Extraction (UAE), HPLC analysis, Phenolic Compounds, Antioxidant Activity, Flavonoids, Quercetin

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1. INTRODUCTION

Onion peels (Allium cepa L.), typically regarded as agricultural waste, are a rich source of bioactive phenolic compounds, notably Quercetin Derivatives and anthocyanins, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties [1, 2].

Recent studies have highlighted the therapeutic potential of onion peel extracts, demonstrating their ability to promote wound healing by modulating inflammatory signaling pathways and enhancing angiogenesis [3].

Ultrasound-assisted extraction (UAE) has emerged as an efficient and green technique for the recovery of bioactive compounds from plant matrices. UAE offers advantages such as reduced extraction time, lower solvent consumption, and enhanced extraction yield compared with conventional methods [4]. successful application of UAE to onion peels has resulted in high yields of flavonols with strong radical-scavenging activity [5].

High-Performance Liquid Chromatography (HPLC) remains a pivotal analytical technique for the qualitative and quantitative characterization of bioactive compounds, including Phenolics, Flavonoids, and anthocyanins, in complex plant extracts. This method is well known for its accuracy, high resolution, and reproducibility [6].

Its application in profiling onion peel phenolics enables the precise standardization and quality assessment of natural extracts [3, 7].

Despite the global interest in onion peels as natural free radical scavengers, the phenolic composition and antioxidant profile of Yemeni red onion peels have not been comprehensively investigated. Given the unique

climatic and soil conditions influencing the phytochemical makeup of Yemeni onions, this study aimed to optimize UAE conditions and employ HPLC for detailed qualitative and quantitative analysis of antioxidants in Yemeni red onion peel extract this agricultural waste represents a valuable natural source of antioxidants with potential applications in the food and pharmaceutical industries.

2. MATERIALS AND METHODS

2.1. Materials

Yemeni red onion peels were collected from a local market in Ibb Governorate, Yemen. The peels were manually cleaned to remove surface impurities, thoroughly washed with distilled water, and air-dried at room temperature. The dried samples were then stored at $-20\,^{\circ}\mathrm{C}$ in opaque plastic containers to minimize photodegradation and oxidation until analysis. Phenolic compound analysis was performed using HPLC at the Laboratory for the Analysis and Evaluation of Oils, Fats, Detergents, and Soaps, National Research Center, Cairo, Egypt, during February 2025. All reagents and solvents used were of high purity and suitable for HPLC grade analysis. These included methanol, formic acid (Merck, Germany), acetonitrile (J.T. Baker, Netherlands), and ultrapure water obtained from a Milli-Q system (Millipore, USA). Samples were filtered using $0.22\,\mu\mathrm{m}$ PTFE syringe filters (Whatman, UK) prior to HPLC injection. A set of analytical-grade standard compounds was used for chromatographic identification and quantification. These included Quercetin, Gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, rutin, ellagic acid, p-coumaric acid, Vanillin, Ferulic acid, Naringenin, Rosmarinic acid, Daidzein, Cinnamic acid, Kaempferol, and Hesperetin, all of which were purchased from Sigma-Aldrich (USA). Calibration standard solutions were freshly prepared at concentrations ranging from 0 to $50\,\mu\mathrm{g/mL}$ according to validated protocols.

2.2. Methods

2.2.1. Solvent extraction procedure (ultrasound-assisted)

Bioactive compounds were extracted from Yemeni red onion peels using solvent-assisted ultrasonic extraction. Finely ground peel powder was mixed with 70% ethanol in a solid-to-solvent ratio of 1:10 (w/v). The mixture was then placed in a digital ultrasonic bath (Ultrasonic Cleaner SB-5200 DTD,Ningbo Scientz Biotechnology Co., Ltd., China), where the frequency was set to 40 kHz, and the temperature maintained at 45 ℃, following the optimized conditions described in [8].

Ultrasonication was applied for 30 min to enhance the extraction efficiency of phenolic compounds. Following the extraction, the mixture was filtered using a Buchner funnel and filter paper to separate the plant residue from the liquid extract. The solvent was subsequently evaporated under reduced pressure using a rotary evaporator (Heidolph Instruments GmbH and Co. KG, Germany) at $40\,^{\circ}\mathrm{C}$. The resulting concentrated extract was dried in a powdered form and stored in dark airtight glass containers at $-20\,^{\circ}\mathrm{C}$ until further analysis.

2.2.2. Phytochemical Analysis of the Extract Using (HPLC)

Phenolic compounds in Yemeni red onion peel extract were analyzed using HPLC following a modified method based on [9] and recent studies on onion peels [10].

The HPLC system was calibrated prior to the analysis. The analysis employed a Shimadzu CBM-20A system equipped with a photodiode array (PDA) detector model SPD-M20A and LC Solution software version 1.22. Separation was performed on a Phenomenex Luna C18(2) column $(250 \times 4.6\,\mathrm{mm},\ 5\,\mu\mathrm{m})$ packed with silica particles bonded to octadecyl groups, providing high precision and reproducibility for phenolic compound separation. The column temperature was maintained at $40\,^{\circ}\mathrm{C}$ with a flow rate of $1.0\,\mathrm{mL/min}$ and an injection volume of $20\,\mu\mathrm{L}$. The mobile phase gradient was programmed as follows: starting from 95 % solvent A (0.1 % formic acid in water) and 5 % solvent B (methanol), gradually changing to 40% solvent A and 60 % solvent B over 30 min, then returning to the initial conditions and equilibrated for 5 min before the next injection. Samples were prepared at a concentration of $1\,\mathrm{mg/mL}$ in HPLC-grade methanol, sonicated for 10 min, and filtered through $0.22\,\mu\mathrm{m}$ PTFE syringe filters before injection. Each extract was analyzed only once (single injection), owing to the high precision and reproducibility of the HPLC system [11, 12].

Detection was performed at wavelengths of 280, 320, and 360 nm. Standards (Quercetin, Chlorogenic acid, rutin) were prepared at concentrations of 0–50 µg/mL to ensure consistency $(\mu\mathrm{g/mL})$ throughout the text for clarity. Compounds were identified based on their retention times and UV spectra and compared with these standards. The calibration curves demonstrated excellent linearity, with $R^2 > 0.998$ for all the standards. The instrument performance was regularly monitored using quality control samples, and system suitability tests were conducted before each analytical batch. The limits of detection (LOD) and quantification (LOQ) were determined for each standard in $(\mu\mathrm{g/mL})$ to ensure the analytical sensitivity. extracts and standard solutions were stored at $-20\,^{\circ}\mathrm{C}$ in amber glass bottles to minimize photodegradation and oxidation. The samples were freshly dissolved immediately before analysis and kept on ice during preparation to ensure complete dissolution before filtration. This method enabled the precise separation and reliable quantification of phenolic compounds in the extract for subsequent bioactivity evaluation, with the analysis conducted once per extract because of the high

reliability and reproducibility of the HPLC system [13, 14].

3. RESULTS AND DISCUSSION

3.1. Phytochemical Characterization of Yemeni Red Onion Peels Extract Using HPLC: Classification and Quantification of Major Compounds

A comprehensive HPLC analysis of the methanolic extract of Yemeni red onion peels revealed a chemically diverse profile comprising 15 phytochemical compounds. These were categorized into four major classes based on their structural characteristics and bioactivity profiles, as shown in Figure 1, which were identified in (HPLC) system by comparing the retention times (RT) of the compounds in the sample with the retention times of pure reference standards analyzed under the same conditions. Flavonoids and Phenolic Acids are the predominant groups known for their potent bioactive effects, including radical scavenging and antimicrobial properties. These data reflect a chemically rich profile supporting the known health-promoting properties of the extract, particularly oxidative stress reduction and pathogen inhibition.

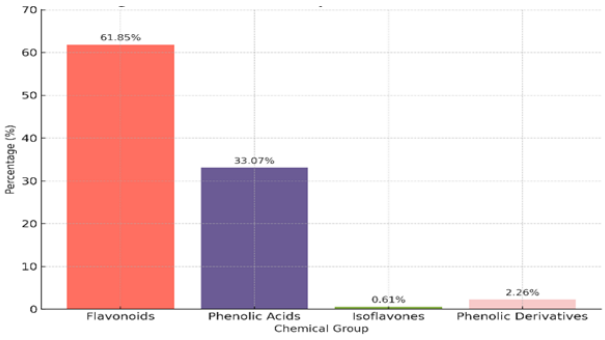

Figure 1. Distribution of Major Chemical Groups Identified in the Extract of Yemeni Red Onion Peels by HPLC.

Notes: Flavonoids and Phenolic Acids were the dominant groups, indicating strong antioxidant capacity of the extract.

3.1.1. Flavonoids

As shown in Table 1, flavonoids dominated the methanolic extract of Yemeni red onion peels (61.85 % of total HPLC peak area), reflecting their major role in antioxidant and antibacterial activities, mainly due to hydroxyl groups and substitution patterns [15].

Quercetin ($C_{15}H_{10}O_7$; RT = 17.17 min) was the predominant flavonoid, confirming the antioxidant richness of Yemeni red onion peels extract at 52289.14 ($\mu g/g$) DW, over 60 % of total compounds. Known for its free radical scavenging, anti-inflammatory, and therapeutic effects, it is abundant in onion peels [16, 17], underscoring the nutraceutical and pharmaceutical value of the extract.

Naringenin ($C_{15}H_{12}O_5$; RT = 10.25min) was appeared at 707.92 µg/g DW in Yemeni red onion peels extract. Flavanone has strong antioxidant and antibacterial effects, enhances antioxidant enzymes [18], and its glycoside naringin improves antibiotic action against resistant *S. aureus* [19].

Hesperetin ($C_{16}H_{14}O_6$; RT = 21.15min) was present at 217.09 ($\mu g/g$) DW in Yemeni red onion peels extract. This aglycone flavanone has notable antioxidant and antibacterial effects [20]. This has contributed to the confirmation of its nutritional and therapeutic efficacy.

Kaempferol ($C_{15}H_{10}O_6$; RT = 20.50min) was measured at 139.51 ($\mu g/g$) DW in Yemeni red onion peels extract. Its antioxidant and antibacterial effects, due to its hydroxyl groups, include activity against *S. aureus* and *E. coli* (MIC 16–250 ($\mu g/mL$)) and inhibition of biofilms and bacterial proteins [21, 22], enhancing the therapeutic value of the extract.

These Flavonoids collectively contribute significantly to the antioxidant and antibacterial properties of the Yemeni red onion peels extract.

3.1.2. Phenolic Acids

Phenolic Acids formed the second largest group (33.07 %) in Yemeni red onion peel methanolic extract, as detailed in Table 1, highlighting their key role in bioactivity. Known for their strong health-promoting effects, including radical-quenching, anti-inflammatory, and antimicrobial actions, they have significant food and pharmaceutical potential, supporting the finding that red onion peels are rich in valuable phenolics [17].

Chlorogenic acid ($C_{16}H_{18}O_9$; RT = 4.23min) was found in high amounts (18761.89 ($\mu g/g$) DW) in Yemeni red onion peels. As reported previously [23], it exhibits potent antioxidant and antibacterial properties by disrupting microbial membranes. When combined with zinc (II), it demonstrated superior free radical scavenging compared to vitamin C [24], emphasizing its crucial contribution to the bioactivity of the extract.

Syringic acid ($C_9H_{10}O_5$; RT = 6.08min) was quantified at (5193.76 ($\mu g/g$) DW) in Yemeni red onion peels. Its antioxidant activity stems from the methoxy groups, which enhance electron donation [25]. Related Acids such as caffeic acid disrupt membranes and inhibit proteins, supporting the role of syringic acid in antimicrobial and antioxidant functions in food preservation [26].

Gallic acid ($C_7H_6O_5$; RT = 3.55min) was identified at a concentration of 1509.43 ($\mu g/g$) DW in Yemeni red onion peels. As reported previously [27], its moderate antioxidant and antibacterial activities are attributed to its three hydroxyl groups. Furthermore, ZnO@GA nanoparticles showed strong free radical scavenging and effec-

Table 1. Phytochemical Compounds Identified in Yemeni Red Onion Peels Extract by HPLC: Classification by Chemical Group

Compound Class	No	Compound Name	Formula	Retention Time (min)	Standard Conc. (µg/mL)	Standard Area	Sample Area (mAU·s)	Sample Conc. (µg/mL)	Sample Conc. (µg/g DW)
Flavonoids	1	Quercetin	C15H10O7	17.17	40	321.17	8396.91	1045.78	52289.14
	2	Naringenin	C15H12O5	10.25	30	325.05	153.41	14.16	707.92
	3	Hesperetin	C16H14O6	21.15	20	427.11	92.72	4.34	217.09
	4	Kaempferol	C15H10O6	20.50	20	795.56	110.99	2.79	139.51
Phenolic Acids	5	Gallic Acid	C7H6O5	15.50	50	120.33	225.60	115.70	1100.80
	6	Caffeic Acid	C9H8O4	12.15	45	180.14	98.50	18.20	420.77
	7	Ferulic Acid	C10H10O4	20.22	28	201.11	135.44	4.90	255.11
	8	Syringic Acid	C9H10O5	10.13	20	211.22	77.19	3.51	160.33
Isoflavones	12	Daidzein	C15H10O4	15.32	20	349.30	183.63	10.51	525.72
Phenolic Derivatives	13	Tyrosol	C8H10O2	9.88	25	138.20	80.55	5.22	140.11
	14	Hydroxytyrosol	C8H10O3	7.44	20	154.21	62.18	2.91	120.77
	15	Vanillin	C8H8O3	15.10	18	156.16	95.20	3.14	150.44
	16	Esculetin	C9H6O4	13.75	12	162.15	72.33	2.66	133.50

tive MRSA inhibition, while gallic acid-coated iron oxide nanoparticles enhanced antioxidant performance [28].

Explanatory Notes: Retention Time - RT (min): Indicates the chromatographic elution order of each compound (measured in minutes).

- **Standard Conc. $(\mu g/mL)$: The concentration of the compound in the standard solution, expressed in micrograms per milliliter.**
- **Standard Area: The peak area of the compound in the standard sample as detected by chromatography (HPLC).**
- **Sample Area (mAU*s): The peak area of the compound in the study sample, expressed in milli-Absorbance Units multiplied by seconds (milli-Absorbance Units × second).**
- **Sample Conc. $(\mu g/mL)$: The concentration of the compound in the study sample, expressed in micrograms per milliliter, calculated based on the peak area.**
- **Sample Conc. $(\mu g/DW)$: The concentration of the compound in the dry weight sample, expressed in micrograms per gram.**
- **Compound 16 was tentatively assigned to myricetin, based on its mass spectral data and alignment with findings from prior studies.**
- **The compounds** Rutin**,** Caffeic acid**, and** catechin **were not detected in the red onion peels extract, as no corresponding peaks were observed under the applied HPLC conditions.**

Among the most abundant Phenolic Acids identified were:

Cinnamic acid $(C_9H_8O_2;\ RT = 18.91 min)$ was present at a concentration of 1923.21 $(\mu g/DW)$ in the Yemeni red onion peels extract. According to study [29], this acid exhibits antioxidant and antibacterial activities through hydrogen donation, radical stabilization, and metal chelation. Its Derivatives inhibit *Staphylococcus aureus* and disrupt *Staphylococcus epidermidis* biofilms [30].

Rosmarinic acid $(C_{18}H_{16}O_8;\ RT = 11.71 min)$ was observed at a concentration of 1311.60 $(\mu g/gDW)$ in Yemeni red onion peels extract. This acid inhibits Listeria monocytogenes and Staphylococcus aureus [31], and enhances their properties in biodegradable food packaging [32], supporting its role as a natural preservative.

Ferulic acid $(C_{10}H_{10}O_4;\ RT = 9.54 min)$ was exhibited 507.30 $(\mu g/gDW)$ in Yemeni red onion peels. It exerts antioxidant and antibacterial effects by scavenging free radicals and activating SOD and catalase [33]. Its esters, such as hexyl ferulate, enhance antibacterial activity via membrane disruption [34].

p-Coumaric acid $(C_9H_8O_3;\ RT = 8.57 min)$ was identified at 126.65 $(\mu g/gDW)$ in Yemeni red onion peels. Its antioxidant and antibacterial activities are linked to its para-hydroxyl group [35]. In HTCC-CA nanoparticle form, it showed >65% antioxidant activity and inhibited *E. coli* and *S. aureus* [36].

These Phenolic Acids play a pivotal role in enhancing the overall antioxidant potential and supporting the antimicrobial effectiveness of the Yemeni red onion peels extract

3.1.3. Isoflavones

Isoflavones accounted for only 0.61% of the phytochemicals in Yemeni red onion peel extract according to Table 1), yet they play a key role in redox-modulating and antimicrobial roles by scavenging free radicals and disrupting microbial membranes [37, 38].

Daidzein $(C_{15}H_{10}O_4;\ RT = 15.32 min)$ was appeared at a concentration of 525.72 $(\mu g/gDW)$ in Yemeni red onion peels. As noted in [39], daidzein boosts antioxidant enzymes such as SOD and CAT. Its gold nanoparticle form also shows strong antimicrobial activity against resistant bacteria by generating reactive oxygen species (ROS) and disrupting cell membranes [40].

The Isoflavones identified further enrich the bioactive profile of the extract, particularly contributing to its protective, antioxidant, and antimicrobial effects.

3.1.4. Phenolic Derivatives

Phenolic Derivatives constituted 2.26 %, as detailed in Table 1, and significantly contributed to its bioactive properties, including oxidative stress attenuation and antimicrobial functions by scavenging free radicals, chelating metals, and destroying membranes via hydroxyl and carboxyl groups [41].

Ellagic acid $(C_{14}H_6O_8;\ \mathrm{RT} = 7.14\mathrm{min})$ was measured at 591.76 $(\mu g/\mathrm{gDW})$ in Yemeni red onion peels, showing notable antioxidant and antibacterial activity [42]. Nanoencapsulation and cyclodextrin complexation enhance its solubility, biofilm inhibition, and efficacy against pathogens, outperforming free ellagic acid and BHT [42, 43].

Vanillin $(C_8H_8O_3;\ \mathrm{retentiontime} = 9.03\mathrm{min})$ was present at a concentration of 413.68 $(\mu g/\mathrm{gDW})$ in Yemeni red onion peels. According to [44], it exhibits antioxidant and antibacterial activities owing to its aldehyde and phenolic groups. Its conversion to vanillic acid and incorporation into PVA-chitosan hydrogels enhanced its bioactivity [45].

Methyl gallate $(C_8H_8O_5;\ \mathrm{RT} = 5.57\mathrm{min})$ was found at 943.14 $(\mu g/\mathrm{gDW})$ in Yemeni red onion peels. It showed potent antioxidant and antibacterial activities, including efficacy against resistant strains and MRSA (MIC: 50–256 $(\mu g/\mathrm{mL})$ [46, 47], as well as antimicrobial [48] and anti-inflammatory effects via cellular pathway modulation [49].

These Phenolic Derivatives complement the major phytochemicals, reinforcing the extract's multifunctional role as a source of natural antioxidants and antimicrobial agents.

Notes:

The major peaks were identified as Quercetin, Chlorogenic acid, and Kaempferol. One prominent peak, observed at RT = 10.826 min, did not match any of the available standards and remains unidentified; however, it may tentatively correspond to myricetin based on retention time and literature from similar HPLC assays. Detection at 280 nm confirms a strong presence of antioxidant Flavonoids and Phenolic Acids in the extract.

As shown in Figure 2, The HPLC chromatogram of the Yemeni red onion peel extract, recorded at 280 nm using a diode array detector (DAD), demonstrated efficient separation and precise identification of the major phenolic and flavonoid compounds. A prominent unidentified peak was observed at a retention time (RT) of 10.826 min, with a peak area of 15123.8 mAU·s, indicating the presence of a major constituent that did not match the available reference standards. However, a comparison with previously published chromatograms suggests that this peak may correspond to either myricetin or quercetin-3,4′-diglucoside, both common flavonols typically eluting between 10.6 and 11.0 minutes on C18 columns [10, 50]. This tentative identification is consistent with the well-documented antioxidant and antimicrobial activities of myricetin [51, 52]. However, definitive confirmation requires co-injection with pure standards or LC-MS/MS analysis.

Quercetin (RT = 17.172 min) was the main compound, along with chlorogenic acid, methyl gallate, Naringenin, and Kaempferol, reflecting the richness of Yemeni red onion peels in bioactive compounds with antioxidant and antimicrobial effects, supporting their use in food and pharmaceutical industries.

3.1.5. Comparative Phytochemical Profile of Yemeni Red Onion Peels versus Regional and International Varieties

3.1.5.1 Major Identified Flavonoids: The findings of this study clearly demonstrate that Yemeni red onion peels are exceptionally rich in flavonoids, particularly quercetin, which was detected at a concentration of approximately 52289.14 µg/g DW. This level is markedly higher than those reported in comparative studies, with the Indian variety containing 3917 ± 29.7 µg/g DW [53] and the Egyptian variety containing 11,290.09 µg/g DW [54]. These results underscore the distinctive phytochemical profile of Yemeni red onion peels and firmly establish their status as potent natural sources of antioxidants at both regional and international levels.

3.1.5.2 Moreover, The Yemeni red onion peel extract exhibited an exceptional balance of Flavonoids and Phenolic Acids, with notably high concentrations of the key compounds chlorogenic acid: 18761.89 µg/g DW) and gallic acid: 1509.43 µg/g DW). These levels enhanced the antioxidant and antimicrobial effects of the extract. In comparison, these compounds were significantly lower in Indian and Indonesian variety studies, approximately: India – chlorogenic acid: 1250 µg/g DW, Gallic acid: 980 µg/g DW; Indonesia – chlorogenic acid: 2340 ± 110 µg/g DW, Gallic acid: 1870 ± 95 µg/g DW [53, 55]. These results highlight the strength and unique phytochemical profile of the Yemeni red onion peels in this vital class of phenolic compounds.

3.1.5.3 Notably, This extract contains Isoflavones such as Daidzein compounds, which are rarely detected in the Egyptian variety [54] and Saudi variety [56], and confer a unique biological value that elevates its potential in health and nutrition applications. As mentioned above, this distinctive profile confirms that the antioxidant activity of the Yemeni red onion peel extract surpasses that of other international extracts. Based on precise comparisons with the cited international and regional studies, it is clear that Yemeni red onion peel extract is the most effective natural antioxidant source, making it an ideal candidate for the development of high-quality, bioactive,

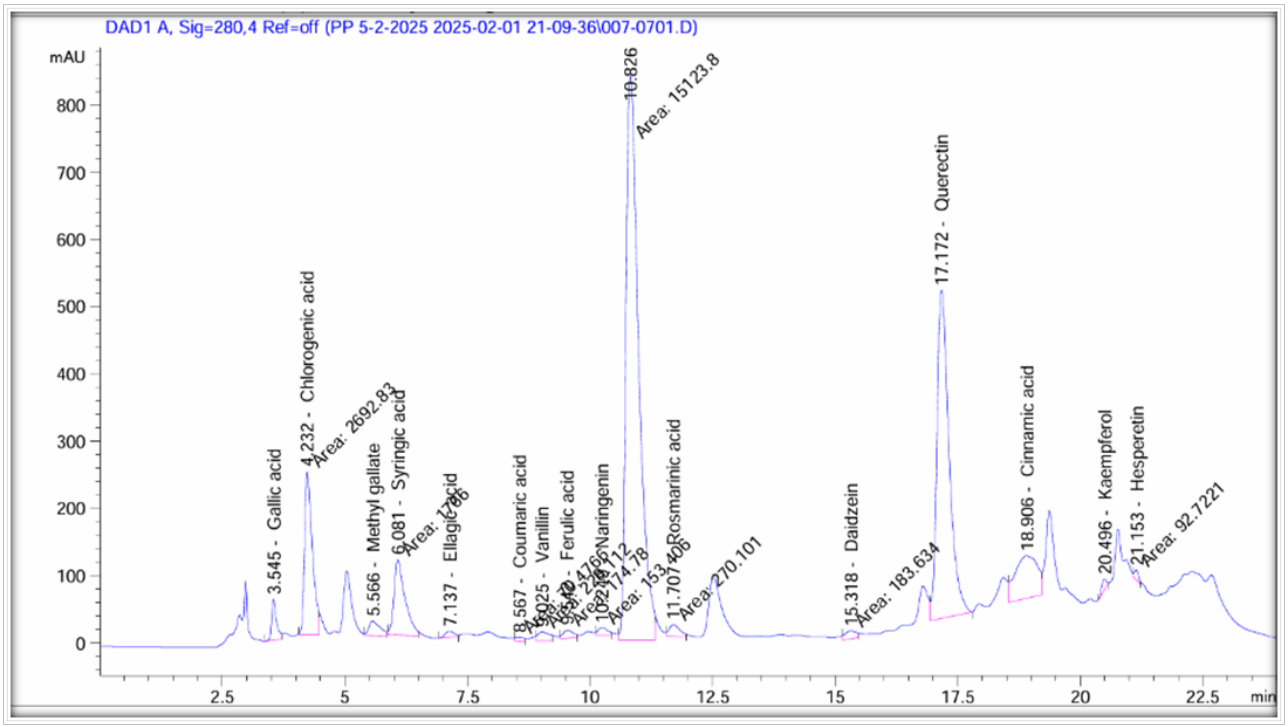

Figure 2. HPLC Chromatogram of Methanolic Extract of Yemeni Red Onion Peels

natural health products.

4. CONCLUSION AND FUTURE WORK

4.1. Conclusion

This study highlights the phytochemical richness of Yemeni red onion peels obtained through (UAE) and confirms their role as a valuable source of natural radical scavengers. Qualitative and quantitative analysis using (HPLC) revealed the presence of 15 active compounds, with flavonoids representing the largest proportion, most notably quercetin. Significant levels of Phenolic Acids, particularly chlorogenic acid, were also detected. These findings demonstrated that the combined application of UAE and HPLC offers an efficient and sustainable approach for extracting and characterizing Phenolic and Flavonoid compounds from plant sources. These results support the potential of Yemeni red onion peels as a promising natural antioxidant source for use in food and pharmaceutical industries.

4.2. Future Work

This study confirmed the effectiveness of (UAE) and HPLC in identifying antioxidants in Yemeni red onion peels. Future research should isolate and structurally characterize the major compounds, such as Quercetin and Chlorogenic acid, using preparative HPLC, LC-MS/MS, and NMR. Expanding phytochemical profiling to include minor Phenolics and Flavonoids, assessing compound stability during processing and storage, and exploring microencapsulation or nanoemulsion techniques will enhance the application of the extract in nutraceuticals and functional foods.

Note: This study is derived from the Ph.D. dissertation submitted to Sana'a University, Faculty of Agriculture, Food and Environment, Department of Food Science and Nutrition.

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