

Article

Valorisation of Agri-Food Residues: Comparative Analysis of Phenolic Composition, Antioxidant Activity and Extraction Methods

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Abstract

Phenolic compounds are recognized for their antioxidant properties and potential application in sustainable functional materials. In this study, the phenolic composition and antioxidant activity of four agro-industrial residues—rice husk, spent hops, and oregano and thyme stems—were evaluated to examine the influence of the extraction method and plant matrix on bioactive compound recovery. Extracts obtained by ultrasound-assisted extraction (UAE) and conventional water bath (WB) extraction were characterized using spectrophotometric assays for total phenolic content (TPC) and antioxidant activity (DPPH and ABTS), as well as HPLC analysis for phenolic profiling. Oregano and thyme stem extracts exhibited the highest phenolic content and antioxidant activity, whereas rice husk extracts showed the lowest concentrations of detectable phenolics. Hydroxycinnamic acids, particularly *p*-coumaric and ferulic acids, predominated across the studied matrices. Multivariate analysis indicated that matrix composition had a greater influence on phenolic profile and antioxidant behaviour than extraction method, with UAE providing comparable results to those obtained by WB extraction while requiring a substantially shorter extraction time. Pearson correlation analysis further suggested that antioxidant activity results from the combined interaction of multiple phenolic compounds rather than isolated constituents. Overall, these residues show potential as sustainable sources of phenolic-rich extracts for functional material applications.

Keywords: antioxidant activity; oregano stems; phenolic compounds; principal component analysis; rice husk; spent hops; thyme stems



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1. Introduction

Phenolic compounds represent a diverse group of secondary metabolites widely distributed in plants, aromatic herbs, and agricultural by-products. To date, more than 8000 distinct structures have been identified and categorized into several classes [1]. Among these, phenolic acids and flavonoids are the most prevalent [2–4]. Phenolic compounds act as antioxidants by donating hydrogen atoms and/or electrons to free radicals, thereby interrupting oxidative chain reactions [2]. This activity is strongly dependent on their chemical structure, particularly the number and position of the hydroxyl groups, with *ortho* and *para* positions enhancing the antioxidant activity [5]. Beyond their well-established antioxidant activity, phenolic compounds have also demonstrated other bioactive properties, including anti-inflammatory [6], antidiabetic [7], cardioprotective [1], and anticarcinogenic [8] effects. More recently, increasing attention has been given to their potential application in

functional materials, particularly in the textile sector, where they can provide added-value properties such as UV protection [4] and anti-odour [9].

At the same time, the growing generation of agro-industrial residues has intensified interest in their valorisation as renewable sources of bioactive compounds. In Portugal, residues such as rice husk, spent hops, and aromatic plant by-products including oregano and thyme stems are produced in significant quantities but remain largely underutilized in high-value applications, being mainly directed to composting, animal feed, or energy recovery [4,10].

Rice husk, produced during rice milling, is a lignocellulosic residue with high lignin and silica content. Despite its limited high-value applications, it has been reported to contain bound phenolic structures, particularly hydroxycinnamic acids such as ferulic acid, which are associated with antioxidant potential [11–13]. However, their extraction is challenging due to strong interactions with the cell wall matrix [13]. Spent hops are generated by the brewing industry after wort boiling under hot aqueous conditions. This processing step leads to substantial leaching and transformation of water-soluble phenolic compounds into the beer matrix, resulting in a modified and generally depleted phenolic profile compared to fresh hops [14–16]. Nevertheless, residual flavonoids and phenolic acids may still be present, depending on brewing conditions and hop variety, which supports their potential valorisation. Similarly, oregano and thyme stems, derived from *Lamiaceae* plants, are typically discarded in favour of leaves despite being part of the same biosynthetic system responsible for phenolic accumulation. While leaves have been extensively studied, stems remain underexplored even though they may contain phenolic acids such as caffeic, *p*-coumaric and ferulic acids, associated with plant defence mechanisms [10,17,18].

Although individual studies have reported phenolic compounds in these matrices, systematic comparisons under identical extraction and analytical conditions remain limited. In particular, the influence of industrial processing (brewing, milling, harvesting) and structural matrix differences on phenolic recovery and antioxidant activity is still not fully understood, limiting the rational valorisation of these residues in high-value applications. In addition, natural phenolic extracts are increasingly being explored for incorporation into functional textile finishes as a strategy to reduce the use of synthetic chemical additives [4,9]. However, a detailed understanding of phenolic composition and extraction behaviour is required as a preliminary step to support their effective application.

The extraction and characterisation of phenolic compounds rely on a combination of analytical techniques. The Folin–Ciocalteu (FC) method is the most widely used technique to quantify the total phenolic content in natural matrices, due to its simplicity and reproducibility, although it is not specific to phenolics and may respond to other reducing agents, such as sugars, ascorbic acid, and aromatic amines, which can lead to an overestimation of the phenolic content [19,20]. Antioxidant activity is commonly evaluated using colorimetric assays such as DPPH and ABTS, which differ in their sensitivity to hydrophilic and lipophilic antioxidants. For a more detailed characterisation, high-performance liquid chromatography (HPLC) is widely used for the chemical profiling of non-volatile phenolic compounds [21–23]. Given their different sensitivities and limitations, a combined application of DPPH and ABTS assays provides a more reliable assessment of the antioxidant activity. Reversed-phase HPLC, typically employing C18 columns and diode array detectors (DADs) or mass spectrometry (MS), allows the efficient separation and quantification of phenolic compounds [24–26].

Extraction conditions also play a crucial role in phenolic recovery. Ultrasound-assisted extraction (UAE) and conventional water bath (WB) extraction are widely used green extraction approaches with different mechanisms of action. UAE enhances mass transfer through cavitation effects and generally enables shorter extraction times and reduced

solvent consumption, whereas WB extraction provides milder thermal conditions that may favour the preservation of thermolabile compounds [27,28]. However, heat- or ultrasound-sensitive phenolic compounds may degrade depending on the operational conditions [29]. For example, in UAE, sonication has shown to degrade phenolics into reactive by-products, which can react with the FC reagent, compromising the accuracy of total phenolic content (TPC) [28,30]. UAE often produces higher total phenolic content due to the enhanced cell wall disruption and diffusion, but flavonoid levels may decline if the process reaches temperatures above 35 °C, and compounds like caffeic acid are also prone to thermal degradation, potentially compromising analytical reliability [31,32]. The conventional water bath (WB) extraction, although more time-consuming, is more suitable to preserve thermolabile compounds, such as certain flavonoids.

The aim of the work was to evaluate and compare the phenolic composition and antioxidant activity of rice husk, spent hops, oregano stems, and thyme stems, and to investigate how extraction method and residue type influence the recovery of bioactive compounds. To achieve this, complementary spectrophotometric and chromatographic approaches were applied to characterise the extracts and provide a comprehensive assessment of their phenolic composition and bioactivity. This study contributes to the understanding of the phenolic composition and antioxidant behaviour of these residues and supports their future application as sustainable sources of bioactive compounds for textile functionalisation.

2. Materials and Methods

2.1. Materials

2.1.1. Residues

The thyme and oregano stems were supplied by Ervital (Mezio, Portugal). The rice husk was provided by Nova Arroz (Oliveira de Azeméis, Portugal). These residues were used as received, without prior drying, and milled at 1500 rpm with a sieve of 1 mm, using a cutting mill SM 300 by Retsch (Haan, Germany). The processed biomass was stored in a dark and dry environment until further use. Spent hops were supplied by Fermentum (Vila Verde, Portugal) and underwent a freezing process for preservation until further use. As no drying step was performed prior to or after extraction, all results are expressed on an as-received residue basis.

2.1.2. Chemicals

HPLC grade methanol, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt > 98% (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) ≥ 95%, and gallic acid ≥ 98% were purchased from ThermoFisher (Geel, Belgium). HPLC grade glacial acetic acid, Folin–Ciocalteu reagent for clinical diagnosis, and potassium dihydrogen phosphate (KH₂PO₄) for analysis ACS (American Chemical Society reagent grade) were purchased from PanReac (Barcelona, Spain). Commercial standards (purity > 99%) of protocatechuic acid, caffeic acid, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, *trans*-resveratrol, rutin and quercetin, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid > 98% (Trolox) were obtained from TCI Europe (Zwijndrecht, Belgium). Anhydrous sodium carbonate ≥ 99.9%, potassium peroxydisulfate for analysis ACS, and anhydrous dipotassium hydrogen phosphate (K₂HPO₄) PA (pro analysis, analytical grade) were acquired from Merck (Darmstadt, Germany). Ethanol absolute anhydrous was purchased from CARLO ERBA Reagents (Milan, Italy).

2.2. Methods

2.2.1. Extraction by Water Bath

The residues were suspended in distilled water at a concentration of 5% (*w/v*) and subjected to water bath (WB) extraction in a thermostatic shaking water bath (OLS26 Aqua Pro by Grant Instruments, Royston, United Kingdom) at 50 °C and 100 rpm for 90 min. The extraction temperature was selected based on previous studies reporting that moderate temperatures (40–60 °C) improve phenolic recovery by enhancing solubility and mass transfer while limiting extensive thermal degradation of most phenolic acids and flavonoids [3,33,34]. The extracts were filtered using a vacuum pump and stored at 4 °C until further analysis.

2.2.2. Extraction by Ultrasound

The residues were suspended in distilled water at a concentration of 5% (*w/v*) and submitted to ultrasound-assisted extraction (UAE) using a sonicator (Q700 by QSonica, Newtown, Connecticut, USA). The samples were subjected to pulses of 40 W (1 min ON/1 min OFF) for a total of 15 min, while immersed in an ice bath. Pulse-mode operation, together with external cooling, was employed to reduce excessive heat generation during sonication while maintaining cavitation effects and mass transfer [28,32]. The extraction time and sonication conditions were selected within the range commonly reported for the recovery of phenolic compounds from plant matrices using probe ultrasound, limiting excessive degradation of sensitive compounds during prolonged sonication [27,28,30]. The extracts were filtered using a vacuum pump and stored at 4 °C until further analysis.

2.2.3. HPLC Analysis

Phenolic compounds were analysed by reversed-phase high-performance liquid chromatography (RP-HPLC), adapted from previously methods for plant-derived phenolic compounds [26,35], with minor modifications. Analyses were carried out using a LC-2060C 3D chromatograph by Shimadzu (Kyoto, Japan), equipped with a DAD and a reversed-phase MEDITERRANEA SEA18 column (4.6 × 250 mm, 5 µm) by Teknokroma (Barcelona, Spain). Chromatograms were recorded for 55 min, with the DAD set to scan between 190 and 800 nm at a constant temperature of 40 °C. The mobile phase consisted of two solvents: 2% acetic acid in water (solvent A) and methanol (solvent B). The gradient was as follows: 0–3.5 min 10% B; 3.5–37.00 min 10–90% B; 37.00–41.00 min 90% B; 41.01–55 min 90–10% B. The flow rate was programmed as follows: 0–25.0 min, 0.55 mL/min; 25.01–30.00 min, 0.95 mL/min; 30.01–40.00 min, 1.20 mL/min; 40.01–55 min, 0.55 mL/min. The initial lower flow rate was applied to improve the resolution of early-eluting compounds, while the subsequent increase allowed a reduction in analysis time without compromising separation efficiency. The qualitative identification of phenolic compounds was based on comparison with reference standards, using retention times and UV-Vis spectra for confirmation. For quantitative analysis, calibration curves were constructed using ten standard solutions prepared in methanol ranging from 0.10 to 5.00 mg/L for gallic acid, protocatechuic acid, caffeic acid, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, *trans*-resveratrol, and rutin. For quercetin, a concentration range of 0.015 to 0.801 mg/L was used. Calibration curves were based on the peak area *versus* concentration relationship. All calibration curves exhibited satisfactory linearity ($R^2 > 0.990$). The calibration parameters, including linearity ranges and coefficients of determination (R^2), are presented in Supplementary Materials Table S1. Intra-day precision (%RSD, $n = 3$) was evaluated using intermediate calibration standards at 2.5 mg/L for all phenolic compounds, except quercetin, which was evaluated at 0.4 mg/L according to its calibration range.

2.2.4. Determination of Total Phenolic Content

The total phenolic content (TPC) of the extracts was determined by the Folin–Ciocalteu method adapted from previous studies [36,37]. Prior to analysis, the extracts were diluted 10-fold with distilled water. The reaction mixture was prepared by mixing 111 μL of the extract with 111 μL of aqueous Folin–Ciocalteu reagent (1:1 (*v/v*)) and 778 μL of sodium carbonate solution (4.29% (*w/v*)). The samples were incubated for 1 h at room temperature in the dark, after which the absorbance was determined at 760 nm, using a spectrophotometer (UV-2600i ISR-2600Plus by Shimadzu, Kyoto, Japan). The control was prepared under the same conditions using distilled water instead of the extract. TPC was quantified using a gallic acid standard curve (10–100 mg/L; $y = 0.0113x + 0.0412$; $R^2 = 0.998$). All measurements were performed in triplicate, and results were expressed in mg equivalent of gallic acid (GAE) per gram of residue (as-received basis).

2.2.5. Determination of Antioxidant Activity—DPPH Method

Antioxidant activity was determined using the DPPH radical scavenging assay adapted from Brand-Williams et al. [38]. Prior to analysis, the extracts were diluted 5-fold with distilled water. Briefly, 1.5 mL of a 0.06 mM DPPH solution in ethanol was added to 37.5 μL of each extract. The reaction mixtures were incubated in the dark for 30 min at room temperature, after which the absorbance was measured at 517 nm, using a spectrophotometer. The control was prepared under the same conditions using ethanol instead of the extract. A calibration curve was constructed using Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a standard with concentrations ranging from 50 to 1.81 mM. The analytical reliability was confirmed by the high linearity of the calibration curve ($R^2 = 0.995$, $y = -0.0005x + 0.9559$). All measurements were performed in triplicate and results were expressed in μmol Trolox Equivalent Antioxidant Capacity (TEAC) per gram of residue (as-received basis).

2.2.6. Determination of Antioxidant Activity—ABTS Method

Antioxidant activity was determined using the ABTS radical cation decolorization assay adapted from Re et al. [39]. Prior to analysis, the extracts were diluted 5-fold with distilled water. The ABTS radical cation was generated by dissolving ABTS and potassium persulfate in distilled water to final concentrations of 7 mM and 2.45 mM, respectively. Prior to analysis, the ABTS working solution was diluted in phosphate buffer (0.1 M, pH 7.4) until an absorbance of 0.700 ± 0.025 at 734 nm was obtained. Phosphate buffer (0.1 M, pH 7.4) was previously prepared using 80.2 mL of dipotassium hydrogen phosphate and 19.8 mL of potassium dihydrogen phosphate 1 M solutions. For the assay, 30 μL of each extract were mixed with 2970 μL of ABTS solution. After an incubation period of 6 min at room temperature in the dark, the absorbance of the reaction mixture was measured at 734 nm using a spectrophotometer. The control was prepared under the same conditions using phosphate buffer (0.1 M at pH 7.4) instead of the extract ($y = -0.0002x + 0.6087$, $R^2 = 0.999$). Trolox was also the standard used in the calibration curve of this method with concentrations ranging from 0 and 2 mM. All measurements were performed in triplicate, and results were expressed in μmol TEAC per gram of residue (as-received basis).

2.2.7. Statistical Analysis

All extractions were performed once for each residue and extraction condition, while analytical determinations (HPLC, Folin–Ciocalteu, DPPH, and ABTS assays) were carried out in triplicate using the same extract. Results are presented as the mean $\pm$ standard deviation (SD) of the analytical determinations. Principal component analysis (PCA) was performed using the quantified phenolic compounds together with TPC and antioxidant activity data to evaluate relationships between extract composition and bioactivity. The

PCA dataset consisted of eight samples (corresponding to the eight extracts) and the quantified analytical variables obtained from HPLC and spectrophotometric analyses. Pearson correlation coefficients were also calculated to investigate relationships between phenolic composition and antioxidant activity.

All statistical analyses were conducted using Python 3.11.5 (Python Software Foundation, Beaverton, OR, USA).

3. Results and Discussion

3.1. Phenolic Compounds Identification

HPLC analysis was conducted to investigate the phenolic profiles of the eight natural extracts. The quantified compounds are summarized in Table 1. The selected ten phenolic standards were chosen based on their frequent occurrence in plant-based matrices reported in the literature [13,17,18,35,40], allowing a representative evaluation of the major phenolic classes typically present in agricultural and aromatic residues.

The chromatograms shown in Figures S2 and S3 indicate that the extracts are mainly composed of phenolic acids, particularly benzoic and cinnamic derivatives, and flavonoids. Among the various phenolic acids studied, the hydroxycinnamic acids *p*-coumaric acid (1.97–23.85 mg/g residue) and ferulic acid (2.11–3.68 mg/g residue) were predominant across the extracts, together with the hydroxybenzoic acid vanillic acid (2.63–5.57 mg/g residue). This distribution is consistent with the known abundance of hydroxycinnamic acids in plant cell walls, where these compounds are commonly associated with lignin and hemicellulosic structures, strongly influencing their extractability depending on matrix composition and extraction conditions [41].

Oregano and thyme stem extracts were characterized by high concentrations of *p*-coumaric acid, followed by vanillic acid, which may reflect the accumulation of structural and defence-related phenolics typically associated with *Lamiaceae* species. In rice husk extracts, ferulic acid was the predominant compound, followed by *p*-coumaric acid, which agrees with previous reports describing ferulic acid as one of the major bound phenolics in cereal by-products due to its esterification to arabinoxylans and lignin within lignocellulosic matrices. In contrast, spent hop extracts exhibited a more variable phenolic distribution, with ferulic, vanillic, and *p*-coumaric acids differing according to the extraction method. This behaviour is likely associated with brewing-related processing, where hot aqueous extraction and oxidative conditions promote the partial leaching, degradation, or transformation of more labile phenolic constituents into the beer matrix before residue collection. Overall, the predominant phenolic compound varied according to the residue type.

Certain compounds exhibited a more selective distribution among the studied matrices. Vanillin (2.72–2.84 mg/g residue) was detected exclusively in thyme stem extracts, suggesting either species-specific accumulation or release from bound precursors under the applied extraction conditions. Similarly, caffeic acid was only quantified in spent hop extracts obtained by water bath extraction (3.01 ± 1.19 mg/g residue), indicating that milder extraction conditions may favour its preservation, whereas ultrasound-assisted extraction may promote degradation or alter its release dynamics.

In contrast, *trans*-resveratrol and protocatechuic acid were not detected in any extract, suggesting either their absence in the studied matrices or concentrations below the detection limits of the chromatographic method. Interestingly, gallic acid, the phenol used for the calibration curve in the Folin–Ciocalteu method [42], was detected only on spent hops when extracted under ultrasound or on rice husk extracts (0.96–1.40 mg/g residue). This selective occurrence may reflect the release of low-molecular-weight phenolics from more complex bound structures in these matrices, particularly under ultrasound-assisted extraction conditions, which enhance cell wall disruption and mass transfer. Overall, the

occurrence and distribution of the identified phenolic compounds agree with previous studies in comparable plant-derived residues [43].

Flavonoids such as rutin (2.58–4.59 mg/g residue) and quercetin (0.27–0.31 mg/g residue) were mainly associated with oregano and thyme extracts, reinforcing the flavonoid-rich nature of aromatic residues from *Lamiaceae* species [17].

In contrast, rice husk exhibited the lowest diversity and abundance of detectable free phenolic compounds. This behaviour is likely related to its high lignin and silica contents, together with the strong incorporation of phenolics into the lignocellulosic matrix, which restricts solvent accessibility and limits the extraction of low-molecular-weight phenolics under mild conditions [44].

The chromatograms shown in Figures S2 and S3 also revealed the presence of several well-resolved peaks that remained unidentified, indicating a potentially more complex phenolic composition than the one identified in the present study. Additional flavonoid derivatives, including glycosylated forms, may be present in the extracts but remain unidentified within the scope of the analysis. This possibility is especially relevant for thyme and oregano stem extracts, which are known to contain a wide range of flavonoid conjugates [17,18]. Therefore, further studies employing complementary techniques such as LC-MS would be valuable for a more comprehensive characterization.

3.2. Quantitative Estimation of TPC

Regarding the total phenolic content, assessed by the Folin–Ciocalteu method, higher concentrations of phenolic compounds were observed in oregano and thyme stems, with the maximum concentration obtained for thyme stems (Figure 1). This observation is consistent with the well-documented accumulation of phenolic acids and flavonoids in aromatic plants of the *Lamiaceae* family [17,18]. Nevertheless, quantitative differences between studies are expected because TPC values are strongly influenced by extraction and analytical conditions.

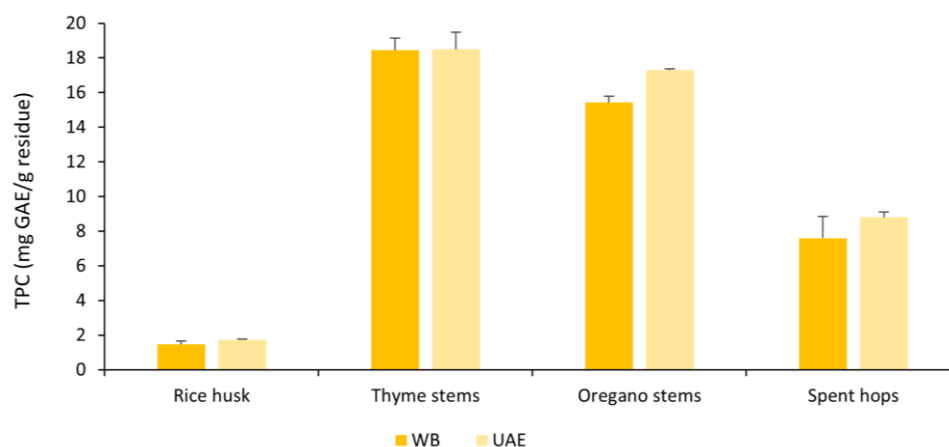


Figure 1. Total phenolic content, assessed by the Folin–Ciocalteu method, of different natural residue extracts, obtained by water bath or sonication, expressed in mg GAE/g residue. Data are presented as mean $\pm$ standard deviation ($n = 3$ analytical determinations).

In comparison, spent hop extracts exhibited lower TPC values, which can be explained by the brewing process. During wort production, conducted in a hot aqueous medium, a significant fraction of water-soluble phenolic compounds is extracted into the liquid phase and transferred to the final beer. Additionally, thermal and oxidative conditions may promote the degradation or transformation of phenolic compounds. As a result, the residual spent hops present a depleted and chemically altered phenolic profile compared to the original raw material. These observations are consistent with the HPLC results (Table 1),

where lower concentrations of major phenolic compounds, namely vanillic and *p*-coumaric acids, were detected in spent hop extracts.

Although rice husk is typically rich in phenolic compounds such as *p*-coumaric and vanillic acid [13,45], as well as, in this case, ferulic acid (Table 1), the relatively low TPC observed in this study may be due to the strong binding of phenolic compounds to cell wall components such as lignin and cellulose [45,46]. Consequently, more intensive extraction techniques may be required to release them effectively. These conclusions are consistent with the observations made by liquid chromatography, in which rice husk extracts gave the lowest sum of phenolic components (Table 1).

When comparing extraction techniques, both water bath and ultrasound-assisted extraction resulted in similar TPC values, particularly for rice husk and thyme stems, indicating comparable phenolic recovery under the applied conditions. However, extraction time should also be considered when evaluating process efficiency. While water bath extraction required 90 min, ultrasound-assisted extraction achieved similar results in only 15 min, suggesting that UAE provides a considerable advantage in terms of extraction time and process intensification. This improved performance is likely related to cavitation effects, which enhance solvent penetration and mass transfer, accelerating the release of intracellular compounds [28–30]. Nevertheless, spent hops represented an exception, as differences between extraction methods were observed for this matrix. In particular, UAE may promote the structural modification or degradation of more sensitive phenolic compounds remaining in the spent hop matrix [31,32], contributing to the differences observed between WB and UAE extracts.

Nevertheless, it should be noted that the Folin–Ciocalteu assay provides only an estimate of total phenolic content, as it is not specific to phenolic compounds and may also respond to other reducing substances present in the extracts [19,36,37]. Under ultrasound-assisted conditions, cavitation may promote not only the release of phenolics but also structural modifications or the formation of additional reducing species, potentially contributing to an overestimation of TPC values [31,32]. This may explain why similar TPC values were obtained for both extraction methods despite the compound-specific differences observed by HPLC analysis.

3.3. Quantitative Estimation of the Antioxidant Activity

In this study, both DPPH and ABTS assays were employed, to determine the antioxidant activity of the extracts and the results are presented in Figure 2. For both methods, the thyme and oregano stems extracts obtained through either water bath or ultrasound extraction exhibited the highest antioxidant concentrations, followed by spent hops and rice husk. These findings are consistent with the TPC results, reinforcing the strong correlation between phenolic concentration and antioxidant potential.

Table 1. Concentrations of the phenolic compounds identified in the eight natural extracts (mg/g residue; mean value $\pm$ SD, $n = 3$ analytical determinations performed on the same extract). ND = not detected; TR < LOD.

Class	Subclass	Phenolic Compound	Spent Hops		Thyme Stems		Oregano Stems		Rice Husk	
		Treatment	WB	UAE	WB	UAE	WB	UAE	WB	UAE
Phenolic acid	Hydroxybenzoic acid	Gallic Acid	TR	1.15 $\pm$ 0.55	ND	TR	TR	TR	0.96 $\pm$ 0.07	1.40 $\pm$ 0.09
		Protocatechuic Acid	ND	TR	ND	ND	ND	ND	ND	ND
		Vanillic Acid	2.75 $\pm$ 0.92	2.63 $\pm$ 0.35	4.69 $\pm$ 0.27	3.38 $\pm$ 0.26	4.74 $\pm$ 0.59	5.57 $\pm$ 0.70	ND	2.67 $\pm$ 0.53
	Hydroxycinnamic acid	Caffeic Acid	3.01 $\pm$ 1.19	TR	ND	ND	ND	ND	ND	ND
		<i>p</i> -Coumaric Acid	2.11 $\pm$ 1.69	2.89 $\pm$ 0.83	3.56 $\pm$ 0.46	8.73 $\pm$ 0.32	23.85 $\pm$ 2.62	16.05 $\pm$ 0.03	1.97 $\pm$ 0.35	3.04 $\pm$ 0.29
		Ferulic Acid	2.11 $\pm$ 1.03	3.68 $\pm$ 0.81	ND	2.31 $\pm$ 1.37	ND	ND	2.29 $\pm$ 0.24	3.34 $\pm$ 0.35
Phenolic aldehyde	Methoxyphenol	Vanillin	ND	ND	2.84 $\pm$ 0.76	2.72 $\pm$ 0.27	ND	ND	ND	ND
Stilbene	--	<i>trans</i> -resveratrol	ND	ND	ND	TR	ND	ND	ND	ND
Flavonoid	Flavonol glycoside	Rutin	ND	ND	2.58 $\pm$ 0.93	2.61 $\pm$ 0.71	2.65 $\pm$ 1.06	4.59 $\pm$ 2.11	ND	ND
	Flavonol	Quercetin	0.31 $\pm$ 0.03	0.27 $\pm$ 0.05	ND	ND	ND	ND	ND	ND
	TOTAL phenolics		10.29 $\pm$ 2.49	10.62 $\pm$ 1.33	13.67 $\pm$ 1.31	19.75 $\pm$ 1.64	31.24 $\pm$ 2.89	26.21 $\pm$ 2.22	5.22 $\pm$ 0.43	10.45 $\pm$ 0.70

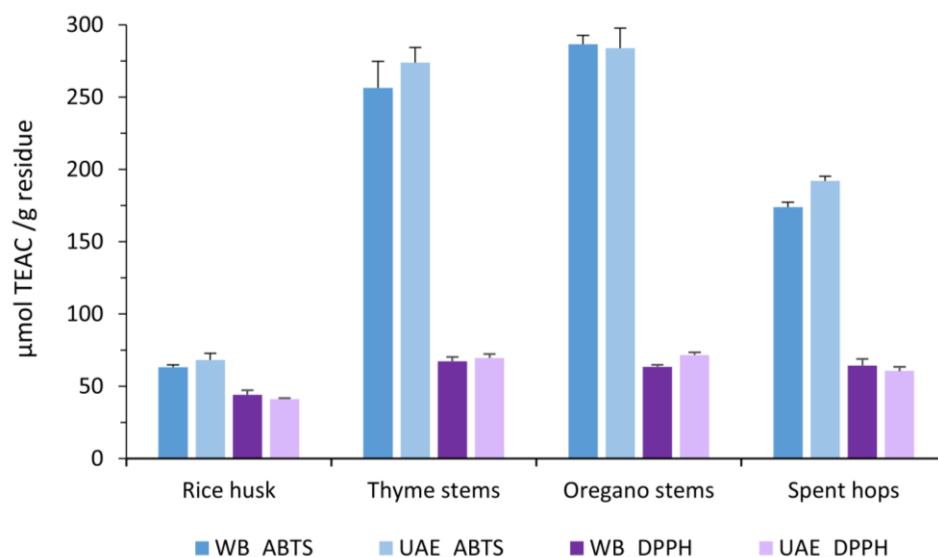


Figure 2. Antioxidant activity assessed by the DPPH (purple) and ABTS (blue) methods of different natural residue extracts, obtained by WB or UAE, expressed in TEAC. Data are presented as mean $\pm$ standard deviation ($n = 3$ analytical determinations).

Overall, the antioxidant capacity determined by the ABTS assay was consistently higher than the one obtained by the DPPH assay [47]. This difference was expected, as not all antioxidant molecules react equally with the different radicals, and the effect of the solvent used in the method must also be considered [47]. As the ABTS radical, generated in an aqueous medium in the assay, is soluble both in hydro- and lipophilic media, it reacts both with hydrophilic and lipophilic antioxidants. In contrast, the DPPH radical is only soluble in an organic solvent, making it more appropriate for hydrophobic systems [21,47–49]. Moreover, the colour interference between DPPH and pigmented compounds can lead to the underestimation of antioxidant activity. These results also highlight the importance of employing multiple assays to obtain a more comprehensive assessment of the antioxidant capacity of plant extracts. Regarding the different extraction methods, both water bath and ultrasound-assisted extractions produced comparable antioxidant activity values, indicating a similar performance of both extraction methods for antioxidant recovery.

3.4. Principal Component Analysis

PCA was applied to explore the relationship between phenolic composition and antioxidant activity among the extracts (Figure 3). Compounds not detected by chromatography were excluded from the analysis, and non-detected values were treated as zero, assuming concentrations below the detection limit. The first two principal components explained 71.0% of the total variance (PC1 = 50.3%; PC2 = 20.7%), providing a representative overview of the chemical variability among the studied matrices. A clear separation was observed between thyme/oregano extracts and rice husk/spent hop extracts, reflecting substantial differences in phenolic composition and antioxidant behaviour. Thyme and oregano extracts clustered closely together and were associated with higher antioxidant activity, likely due to their richer and more homogeneous phenolic composition, particularly regarding *p*-coumaric and vanillic acids. The tight clustering observed for these matrices suggests similar chemical behaviour and comparable phenolic profiles, which is consistent with their common botanical origin within the *Lamiaceae* family. In contrast, rice husk extracts were mainly associated with ferulic acid, in agreement with previous reports describing the abundance of this compound in lignocellulosic cereal residues [29,34]. However, the interpretation of individual compounds as specific chemical makers should be approached

cautiously, since PCA reflects covariance patterns between variables rather than direct quantitative contributions. Spent hop extracts exhibited a distinct distribution in the PCA model, which may be attributed to the combined effects of brewing and extraction. During brewing, water-soluble phenolic compounds are partially transferred to the wort, while thermal and oxidative conditions may further modify the native phenolic composition of the material. Consequently, spent hops presented a more differentiated phenolic profile compared to the remaining matrices. In particular, water bath extracts showed a stronger association with caffeic acid, suggesting that this compound may be better preserved or more efficiently extracted under milder extraction conditions. The confidence ellipses further confirmed the distinct grouping of samples according to matrix type, indicating that each residue exhibits a characteristic phenolic profile. In contrast, the limited separation observed between UAE and WB extracts suggests that, under the applied conditions, extraction method had a comparatively smaller influence on the overall phenolic composition and antioxidant behaviour than the intrinsic characteristics of the raw material itself. This observation is consistent with the TPC and antioxidant activity results obtained by spectrophotometric methods. Overall, the PCA results demonstrate that matrix composition plays a dominant role in defining the chemical and antioxidant behaviour of the extracts, while extraction method mainly influences the recovery of specific compounds rather than the overall compositional profile.

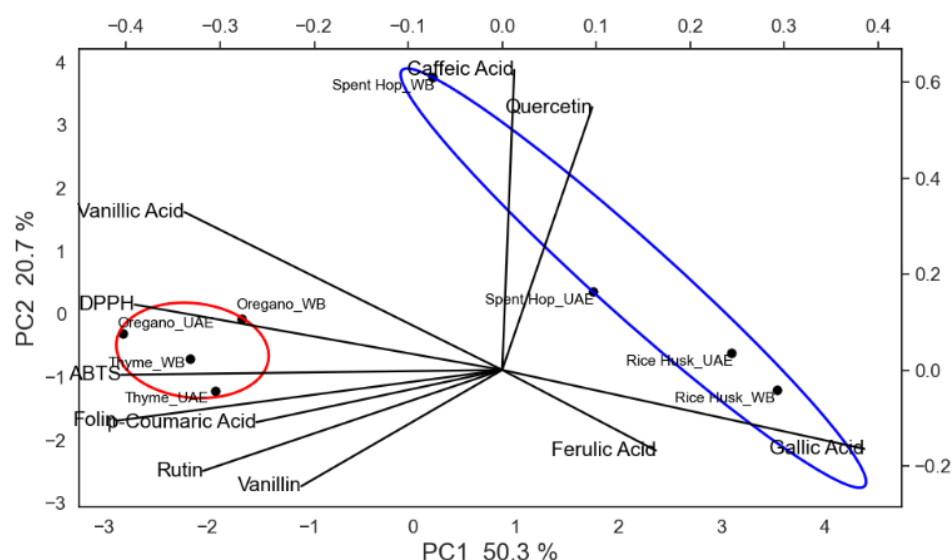


Figure 3. Principal component analysis of phenolic compounds and antioxidant potential of different natural extracts. Each line represents one of the variables included in the study.

3.5. Pearson Correlation Analysis

Pearson correlation analysis was performed after excluding phenolic compounds that were not detected by chromatography. The correlation matrix (Figure 4) revealed distinct relationships between individual phenolic compounds and the spectrophotometric assays used to estimate antioxidant activity.

Vanillic acid showed strong positive correlations with the DPPH ($r = 0.76$), Folin–Ciocalteu ($r = 0.66$), and ABTS ($r = 0.72$) assays, suggesting that this compound may contribute substantially to the antioxidant response of the studied extracts. Similar trends were observed for *p*-coumaric acid, rutin, and vanillin. Although rutin and vanillin were not detected in all samples, their concentrations covaried consistently with antioxidant activity when present, which explains the relatively high correlation coefficients despite their limited occurrence. In contrast, gallic acid exhibited strong negative correlations with the DPPH ($r = -0.88$), Folin–Ciocalteu ($r = -0.85$), and ABTS ($r = -0.84$) assays.

Similarly, ferulic acid also showed negative correlations with the antioxidant assays. These results indicate that the antioxidant activity of the extracts cannot be attributed solely to the concentration of individual phenolic compounds. Instead, the overall antioxidant response likely reflects synergistic or antagonistic interactions between multiple phenolic subclasses and other reducing constituents present in the extracts. Understanding these interactions is therefore essential for a reliable interpretation of antioxidant behaviour in heterogeneous plant matrices.

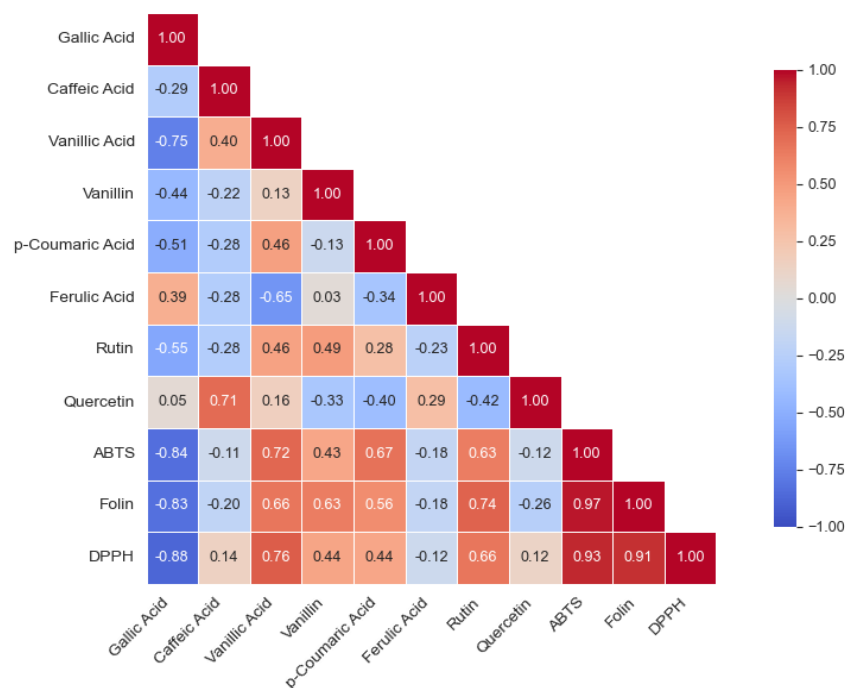


Figure 4. Pearson's correlation matrix of phenolic compounds and antioxidant activity of natural extracts. Correlation coefficients (r) range from -1 to 1 , with red colours indicating positive correlations and blue colours indicating negative correlations. The intensity of the colour reflects the magnitude of the correlation.

The weak association between gallic acid and the spectrophotometric assays also highlights the limitations of colorimetric methods when applied to complex plant matrices. Although gallic acid is commonly used as a reference standard in the Folin–Ciocalteu assay, its concentration did not parallel the overall antioxidant response of the extracts. This behaviour reinforces that spectrophotometric assays should be interpreted as indicators of global reducing capacity rather than direct measurements of specific phenolic compounds, since they may also respond to non-phenolic reducing substances.

Strong positive correlations were observed between the Folin–Ciocalteu, DPPH, and ABTS assays, indicating that these methods consistently reflect the overall antioxidant behaviour of the extracts despite their different sensitivities and mechanisms of action. Therefore, the combined use of chromatographic and spectrophotometric techniques provides a more comprehensive understanding of antioxidant behaviour in heterogeneous plant matrices than either approach alone.

4. Conclusions

This study demonstrated the feasibility of recovering and characterizing phenolic compounds from different agro-industrial and plant-derived residues, namely oregano and thyme stems, spent hops, and rice husk, using complementary analytical approaches including spectrophotometric assays and HPLC. The results highlight that the phenolic

profile and antioxidant potential are strongly dependent on the nature of the raw material, with aromatic herb residues showing the highest levels of bioactive compounds, while rice husk and spent hops exhibited more limited but still compositionally distinct phenolic profiles. Although both methods used for the antioxidant activity—DPPH and ABTS—gave the same trend among the extracts, the value of the activity achieved was quite different, which is already reported in the literature [50,51].

Overall, a consistent relationship was observed between total phenolic content and antioxidant activity, although the correlation varied depending on the analytical method applied, reflecting the known limitations and non-specificity of colorimetric assays. Multivariate analysis further confirmed clear chemical differentiation between the studied matrices, while also indicating that extraction method (UAE vs. WB) had a limited influence on overall extract composition under the conditions tested.

Although both extraction methods produced broadly comparable results under the conditions assessed, ultrasound-assisted extraction may represent a promising alternative for future studies and scale-up applications due to its shorter extraction time while maintaining similar phenolic recovery and antioxidant activity.

Importantly, the study also highlights that individual phenolic compounds should not be interpreted in isolation as direct predictors of antioxidant activity, since the observed bioactivity likely results from synergistic or antagonistic interactions between multiple phenolic subclasses.

From a broader perspective, this work contributes to the valorisation of underexploited agro-industrial residues by providing a comparative framework for understanding their phenolic composition and antioxidant behaviour under standardized extraction conditions. These findings support the potential future use of such extracts in sustainable functional materials, including textile applications, as alternatives to synthetic additives.

However, the study is limited to *in vitro* chemical characterization, and no direct performance testing in target applications was conducted. Therefore, future work should focus on evaluating the stability, durability, and functional performance of these extracts when incorporated into real substrates, as well as expanding chemical characterization using high-resolution techniques such as LC–MS to better resolve unidentified compounds.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/appliedchem6030052/s1>. Figure S1: Chromatogram of mixed standard solution of 10 phenolic compounds at 254 nm (peak numbers correspond to the respective phenolic compounds listed in Table S1); Figure S2: Chemical profile of phenolic compounds extracts at 254 nm using the chromatographic method: (A) Thyme WB, (B) Thyme UAE, (C) Oregano WB, and (D) Oregano UAE; Figure S3: Chemical profile of phenolic compounds extracts at 254 nm using the chromatographic method: (A) Spent hops WB, (B) Spent hops UAE, (C) Rice husk WB, and (D) Rice husk UAE; Table S1: Analytical performance parameters of the developed HPLC-DAD method for phenolic compound analysis. Intra-day precision (%RSD, $n = 3$) was evaluated using intermediate calibration standards at 2.5 mg/L for all phenolic compounds, except quercetin, which was evaluated at 0.4 mg/L according to its calibration range.

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Abbreviations

The following abbreviations are used in this manuscript:

DAD	Diode Array Detector
FC	Folin–Ciocalteu
GAE	Gallic Acid Equivalents
HPLC	High-Performance Liquid Chromatography
LC	Liquid Chromatography
MS	Mass Spectrometry
PCA	Principal Component Analysis
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenolic Content
UAE	Ultrasound-assisted extraction
WB	Water Bath

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