ORIGINAL ARTICLE

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Polyvinyl Alcohol Films with Algerian *Eruca vesicaria* Extract as Natural Antioxidants for Food Packaging

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ABSTRACT

Background: The increasing interest in active packaging films stems from their potential to reduce reliance on synthetic chemical additives for food preservation, thereby enhancing food quality and extending shelf life.

Aims: This study aimed to develop, characterize, and evaluate the functional properties of polyvinyl alcohol (PVA)-based solvent cast films loaded with a hydroethanolic extract derived from *Eruca vesicaria* (arugula), intending to produce novel active packaging materials.

Methods: Initially, the total phenolic content of the *Eruca vesicaria* extract was quantified, and its precise chemical profile was determined through chromatography coupled with tandem mass spectrometry (LC-MS/MS). The resulting flexible and active PVA films were subjected to comprehensive analysis using Fourier-transform infrared spectroscopy (FTIR) to identify molecular interactions, along with assessments of water contact angle, opacity, transparency, and antioxidant activity using the DPPH and the phosphomolybdenum assay.

Results: The LC-MS/MS analysis successfully identified eight distinct phenolic compounds within the hydroethanolic extract. FTIR spectroscopy confirmed effective molecular interactions between the incorporated extract and the PVA polymer matrix. Furthermore, the inclusion of the extract significantly altered the surface wettability (water contact angle) of the films. Critically, the films demonstrated substantially improved functional properties. The antioxidant activity assessment demonstrated that films incorporated with the *Eruca vesicaria* extract exhibited significantly higher antioxidant activity (IC_{50} = 0.75 ± 0.01, EC_{50} = 1.8 ± 0. 1 mg/mL), compared to pure PVA films (IC_{50} = 1.53 ± 0.03 mg/mL, EC_{50} = 10.69 ± 0.5mg/mL).

Conclusions: The findings demonstrate that integrating *Eruca vesicaria* antioxidants into PVA films is a viable and highly effective strategy for producing active packaging materials with enhanced functional and barrier properties.

Keywords: Arugula; Active Packaging; Bioactive Additive; *Eruca vesicaria*; Polyvinyl Alcohol Films.

Article Information

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1 INTRODUCTION

Packaging constitutes a crucial component of the food production and distribution chain, fulfilling a critical function in preserving food quality and safety of food products. Given the increasing global awareness of the environmental impact caused by the widespread reliance on petroleum-derived polymers, scientific investigation has witnessed a notable transition toward developing sustainable and eco-friendly packaging alternatives (Siracusa *et al.*, 2008).

Consequently, published research focusing on the synthesis and practical application of biodegradable films has expanded significantly, reflecting a growing interest in reducing the environmental footprint associated with conventional plastics. Polyvinyl alcohol (PVA) serves as a noteworthy example of a synthetic polymer that exhibits inherent biodegradability, thereby contributing substantially to the overall sustainability of packaging processes. PVA holds regulatory approval for use in packaging applications, specifically for meat and poultry products, granted by the US Department of Agriculture (DeMerlis & Schoneker, 2003).

In this context, active films represent an additional innovative strategy aimed at enhancing food safety and quality attributes. This approach entails the functional incorporation of active substances, such as various plant extracts (Sanches-Silva *et al.,* 2014), including peppermint extract and pomegranate peel extract (Kanatt *et al.* 2007; Kanatt *et al.*, 2010). These naturally derived extracts possess antioxidant properties and can effectively contribute to maintaining the quality of food products and protecting them against oxidation decay (Parveen *et al.,* 2025).

Phenolic compounds, sourced from diverse botanical origins, agro-industrial byproducts, and waste streams, offer a compelling functional solution for active packaging development. These compounds, characterized by varying molecular weights and structural complexities, possess hydroxyl groups capable to establish hydrogen bonds with PVA, offering potential enhancements to film flexibility and bioactive properties (Arcan & Yemenicioğlu, 2011). The integration of phenolic compounds thus introduces a new dimension to the fabrication of bio-functional packaging materials.

The plant *Eruca vesicaria*, commonly recognized as arugula or rocket, is a prominent member of the Brassicaceae family. This species is characterized by its lobed leaves and oval-shaped flowers, and it is prevalent as a ruderal species in corn and flax fields, uncultivated areas, and along roadsides. Historically originating in the Mediterranean basin, its cultivation and natural distribution have spread globally (Bell *et al.,* 2022). Rocket is a versatile plant that finds widespread usage in culinary applications, particularly as a staple ingredient in salads and as a flavorful garnish atop pizzas (Acemi, 2022). Recent studies have suggested that rockets contain effective compounds, particularly phenols and flavonoids, which act as antioxidants (Grami *et al.*, 2024).

Building upon this foundational knowledge, the present investigation focuses on the development of bioactive PVA-based films incorporating a hydroethanolic extract of *Eruca vesicaria.* To the best of our knowledge, this work represents the first attempt within the Algerian context to valorize this species for use in active food packaging. This study adopts a multidisciplinary approach that integrates phytochemical analysis, antioxidant activity evaluation, and polymer-based application, highlighting its novelty and relevance in the field of sustainable food packaging. The total phenolic and flavonoid contents, as well as the antioxidant activity of the extract, were evaluated. In addition, LC-MS analysis was performed to identify the major phenolic compounds present in the extract. Its incorporation into PVA films was assessed at varying concentrations (0.25–0.75% w/v) to characterize its impact on ultraviolet (UV) barrier capacity, structural characteristics, and antioxidant properties.

2 Material and Methods

2.1 Chemicals and Materials

Poly(vinylalcohol) (PVA), characterized by an 87.16% hydrolysis degree, 95.4% purity, was obtained from Zag Kimya (Turkey). The standard reagents employed for phytochemical analysis, specifically Folin–Ciocalteu reagent, gallic acid, ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH°) were provided by Sigma-Aldrich. Ammonium molybdate, sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), and sodium dihydrogen phosphate (NaH_2PO_4) were supplied by (BIOCHEM Chemopharma). All other chemicals and solvents utilized throughout this investigation were of analytical grade.

2.2 Preparation of Plant Extracts

The plant material *Eruca vesicaria* was collected as a wild species during its blossoming stage in March 2023 from El Assafia, Laghouat, Southern Algeria (geographic coordinates: 33.7507° N, 2.8650° E). The aerial components, comprising the flowers, leaves, and stems, were air-dried in the shade at room temperature. Following drying, the plant parts were finely pulverized using an electric grinder. A mass of 50 g of the resulting powder were then placed in a 1000 mL flask with a mixture of 500 mL ethanol and water (Sultana *et al.,* 2009) in a ratio of 7:3. The flask was then placed in a water bath at 70°C while stirring for 40 minutes (Ghasemzadeh & Jaafar, 2014; Vuong *et al.*, 2013). Following this, the resulting liquid was filtered using a Büchner funnel, and the filtrate was concentrated using a rotary evaporator at 40°C. Finally, the extract was dried through freeze-drying until it became a powder, which was stored in the refrigerator at 4°C until required for subsequent analyses.

2.3 Preparation of Active Films

Four PVA-based polymeric films loaded incorporating the *Eruca vesicaria* extract were prepared using the conventional solvent-casting technique, adapted from Terzioğlu *et al.* (2021). As summarized in Table 1, the polymer solution was prepared by dissolving 6% PVA (w/v) in distilled water under magnetic stirring at 500 rpm at 90°C for 3 hours. Subsequently, the solution was cooled to 50°C, and arugula

Table 1. Formulations of Initial Film Forming Solutions

Sample Code	PVA (w/v %)	*Eruca vesicaria* extract (w/v %)	Glycerol (v/v%)
L0	6	0	0.66
L1	6	0.25	0.66
L2	6	0.50	0.66
L3	6	0.75	0.66

extract was added at concentrations of 0.25, 0.50, and 0.75% (w/v) with magnetic stirring for 1 hour. Then, 0.2 mL of glycerol was added, and stirring continued for 30 minutes. Finally, the solution was poured into a glass Petri dish with a diameter of 11 cm and allowed to dry at 50°C for 18 hours.

2.4 Characterization of the Plant Extract

2.4.1 Total Phenolic Contents (TPC) Determination

The total phenolic content in the ethanol extract of the plant *Eruca vesicaria* was determined using the Folin-Ciocalteu method as previously described by Scalbert, Monties, & Janin, (1989). A working solution of the dried extract of 1 mg/mL was prepared in distilled water. Subsequently, 0.1 mL of the sample was mixed with 0.5 mL of the Folin-Ciocalteu reagent, diluted 10 times with distilled water, and 2 mL of 7.5 mg/mL sodium carbonate solution. The reaction mixture was incubated in the dark at ambient temperature for two hours. Following incubation, the absorbance was recorded at a wavelength of 760 nm against a blank. Gallic acid was employed to prepare the calibration curve, and the results were expressed in units of gallic acid equivalents (mg) per gram of dry extract (mg GAE/g DE).

2.4.2 Total Flavonoid Contents (TFC) Determination

The total flavonoid content (TFC) of *Eruca vesicaria* extract was calculated the method of Chang *et al.* (2002), which was predicated on the complex formation of flavonoid molecules with aluminum trichloride ($AlCl_3$). 1.5 mL of $AlCl_3$ (2% ethanol solution) was added to 1.5 mL of *Eruca vesicaria* extract. The mixture was subsequently incubated in the dark at room temperature for 30 minutes, after which the absorbance was measured at 430 nm. Quercetin was used as the reference standard to generate the calibration curve, and the TFC was expressed as milligrams of Quercetin Equivalents (mg QE) per gram of dry plant weight (mg QE/g DW).

2.4.3 UPLC-ESI-MS-MS Phytochemical Profiling

The chemical profile of the *Eruca vesicaria* hydroethanolic extract was analyzed using a Shimadzu 8040 Ultra-High Sensitivity Ultra-Performance Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (UPLC-ESI-MS-MS) system, equipped with UFMS technology and a Nexera XR LC-20AD binary pump. The electrospray ionization (ESI) conditions were set as follows: collision-induced dissociation (CID) gas pressure at 230 KPs, conversion dynode at −6.00 kV, and desolvation line (DL) temperature at 250°C. Nebulizing gas flow was maintained at 3.00 L/min, while the heat block temperature was 400°C, with a drying gas flow rate of 10 L/min. The mobile phase consisted of solvent A (water with 0.1% formic acid) and solvent B (methanol). The flow rate was set at 0.2 mL/min, and an injection volume of 5 µL was used. Chromatographic separation was achieved using a Restek Ultra C18 column (150 mm × 4.6 mm, 3 µm particle size).

2.5 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The molecular structures of the *Eruca vesicaria* extract and the resulting bioactive films were investigated employing Fourier Transform Infrared Spectroscopy (FT-IR). Spectra were recorded on a Thermo Scientific Nicolet i550 model spectrometer equipped with a Smart Orbit-Diamond Attenuated Total Reflectance (ATR) accessory. Measurements were conducted in transmittance mode across the spectral range of 4000–400 cm^{-1}, with a spectral resolution of 4 cm^{-1}.

2.6 Light Transmission

The optical properties, specifically the absorbance and transmittance spectrum were measured at selected wavelengths for each sample on a UV–visible spectrophotometer (Shimadzu UV-3600, Japan) according to the procedure described by Terzioğlu *et al.* (2021). Rectangular film specimens were positioned directly within the spectrophotometer's test cell for analysis at selected wavelengths across the UV Vis spectrum, allowing for the evaluation of the films' barrier capacity against light.

2.7 Water Contact Angle (WCA) Measurements

The surface wettability of the bioactive films was assessed by measuring the static water contact angle (WCA). Measurements were performed using an Attension Theta Lite model optical tensiometer (Finland) equipped with an automatic dispenser system. A standardized droplet volume of 4 μL of distilled water droplet was carefully dispensed onto the film surface. The reported WCA results represent the average of three independent determinations per sample.

2.8 Determination of Film Antioxidant Activity

2.8.1 DPPH Free Radical Scavenging Assay

The antiradical activity of the active films was assessed using the DPPH method, modified from Wang *et al.* (1998). Polymer solutions loaded with *Eruca vesicaria* extract were prepared at concentrations of 12.5, 25, 50, 75, and 100 ppm. Equal volumes were mixed with a methanolic solution of the 2,2-diphenyl-1-picryl hydrazyl radical (DPPH°) at a concentration of 200 µM. After incubating the mixture in the dark at room temperature for 30 minutes, the absorbance was measured at 517 nm against a methanol blank. The free radical scavenging percentage was calculated using the following equation:

$$I\% = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \; x \; 100$$

Where: Abs $_{blank}$ = Control Absorbance, Abs $_{samùple}$ = Sample Absorbance

For comparative assessment, ascorbic acid was utilized as a positive reference standard. Subsequently, the half-maximal inhibitory concentration (IC_{50}), corresponding to the concentration required to achieve 50% free radical inhibition, was determined and presented.

2.8.2 Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the plant extract-loaded polymer solution at 25, 50, and 100 ppm concentrations was determined using the phosphomolybdenum assay, following a method described by Prieto, Pineda, & Aguilar, (1999) with minor modifications. Briefly, 0.3 mL of the active polymer solutions loaded with purslane extract was mixed with 3 mL of the phosphomolybdenum reagent, which comprised 28 mM sodium molybdate and 4 mM ammonium molybdate in 0.6 M sulfuric acid. The sealed reaction tubes were then incubated in a water bath at 95°C for 90 minutes. Upon cooling the mixture to room temperature, the absorbance was measured at 695 nm using a spectrometer against a reagent blank. Ascorbic acid served as the positive reference standard. The results were expressed as a concentration of EC_{50} at an absorbance of 0.5.

2.8.3 Statistical analysis

To facilitate the robust statistical interpretation of the experimental data, a one-way Analysis of Variance (ANOVA) was performed using SPSS software to assess the correlation between variables and factors, as well as to determine the efficacy of the studied extract and the resulting bioactive films in comparison to both positive and negative control groups.

3 Results and Discussion

3.1 Quantification of TPC and TFC

As displayed in Table 2, the total phenolic content (TPC) of *Eruca vesicaria* extract was quantified at 74.49 ± 0.68 mg GAE/g, while the total flavonoid content (TFC) reached 13.77 ± 0.10 mg QE/g. These values highlight the richness of the extract in phenolic constituents. The selection of *Eruca vesicaria* for this investigation was established on its favorable phytochemical profile and established antioxidant potential, which is strongly corroborated by the high measured TPC and TFC values. Furthermore, *E. vesicaria* is widely recognized as a safe and non-toxic edible plant with a history of traditional consumption across various regions, supporting its suitability for incorporation into bio-based films. Phenolic chemicals are especially valuable in this application due to their capacity to form hydrogen bonds with the polymer matrix to improve the films' mechanical strength, barrier properties, and antioxidant activity (Siddiqui *et al.,* 2023). These physiochemical qualities are necessary for developing effective active packaging materials.

Table 2. Results of quantification of total phenolic and total flavonoid contents.

Test	*Eruca vesicaria* extract
TPC mg GAE/g ext.	74.49 ± 0.68
TFC mg QE/g ext.	13.77 ± 0.1

3.2 UPLC-ESI-MS/MS Profiling of *Eruca vesicaria* Extract

The chromatographic analysis of the hydroethanolic extract of *Eruca vesicaria* using UPLC-ESI-MS-MS successfully enabled the identification of several key phytochemical constituents. The optimized analytical conditions allowed for efficient separation and detection of various compounds within the extract matrix. The specific compounds identified, along with their retention times and molecular weights, are summarized in Table 3, Figure 1 and Figure 2.

The LC-MS/MS analysis of the hydroethanolic extract of *Eruca vesicaria* revealed eight major phytochemicals, selected based on their prominent peak areas and identified by comparing their retention times with reference standards. The results confirm that the extract is rich in phenolic compounds, including phenolic acids such as 2-methoxybenzoic acid and sinapic acid, as well as the triterpenoid oleanolic acid. Additionally, three distinct flavonoids —luteolin, rutin, and quercetin—were identified, alongside the polyphenol oleuropein and the carotenoid beta-carotene. These results align with previous studies that have also reported the presence of flavonoids such as rutin and quercetin in related *Eruca* species (Villatoro-Pulido *et al.,* 2013). These compounds are recognized as common antioxidant agents within the Brassicaceae family (Favela-González, Hernández-Almanza, & De la Fuente-Salcido, 2020; Li *et al.*, 2018). Similarly, other phenolic compounds, such as sinapic acid, have been previously detected in various species belonging to this family (Olsen, Aaby, & Borge, 2009). Significantly, compounds such as 2-methoxybenzoic acid, luteolin, and oleuropein have been tentatively identified in *Eruca vesicaria* for the first time in this study. Variations in the plant's phytochemical composition are likely attributable to a

Table 3. Results of Phytochemical Profiling of *Eruca vesicaria* by UPLC-ESI-MS-MS Analysis

ID#	Compound	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Ret. Time
1	Epicatechin	$C_{30}H_{48}O_3$	456.7	(+)	457.3000 > 411.4000	3.255
2	Quercetin	$C_{15}H_{10}O_7$	302.23	(+)	303.0500>153.1000	4.532
3	Resveratrol	$C_{14}H_{12}O_3$	228.24	(+)	No peak is detected	4.970
4	Luteolin	$C_{15}H_{10}O_6$	286.24	(+)	286.7500>153.0000	4.740
5	Oleuropein	$C_{25}H_{32}O_{13}$	540.5	(-)	540.5000>88.9500	6.202
6	Rutin	$C_{27}H_{30}O_{16}$	610.5	(+)	611.0000>303.1000	5.121
7	Beta carotene	$C_{40}H_{56}$	536.87	(+)	537.1000>280.9500	8.541
8	2-mythoxybenzoic Acid	$C_8H_8O_3$	152.15	(+)	153.0500>135.0000	4.399
9	Kojic acid	$C_6H_6O_4$	142.11	(+)	No peak is detected	1.493
10	Oleanolic Acid	$C_{15}H_{14}O_6$	290.27	(+)	No peak is detected	4.130
11	Sinapic Acid	$C_{11}H_{12}O_5$	224.21	(+)	225.0000>207.1500	4.709
12	p-Coumaric Acid	$C_9H_8O_3$	164.16	(-)	No peak is detected	3.862
13	Vanillic Acid	$C_8H_8O_4$	168.15	(-)	No peak is detected	4.595

Note: Ret. Time: Retention time; ESI: Electrospray Ionization

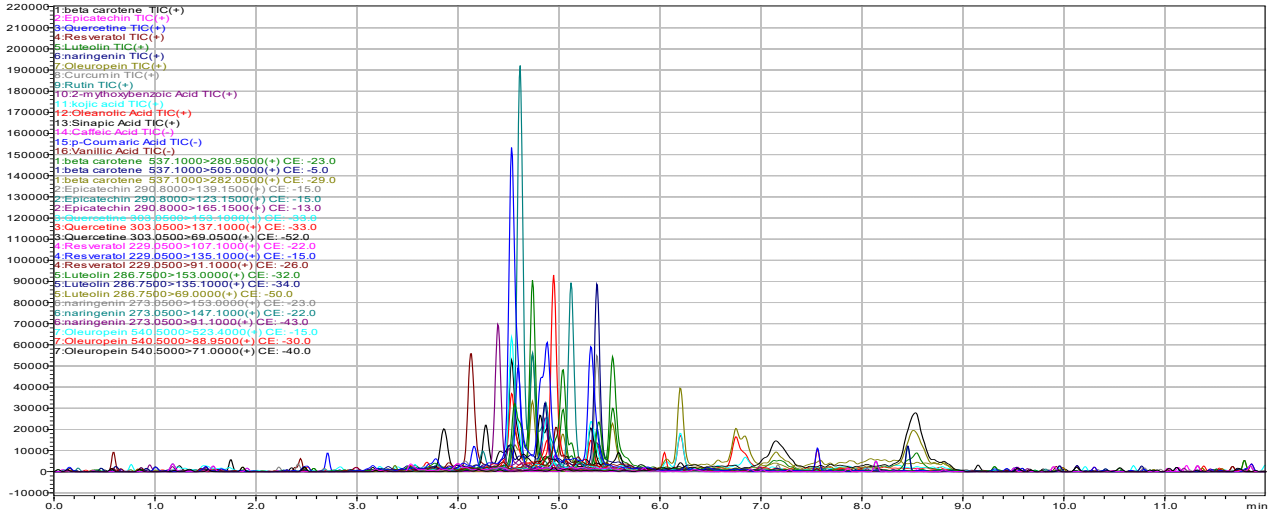

Figure 1. The LC–MS/MS Chromatogram Profile of the Extract of *Eruca vesicaria*

confluence of factors, including seasonal harvest, specific habitat, or environmental conditions. Indeed, it is well established that environmental and agronomic factors —such as species and cultivar, soil type, cultivation practices, plant age, temperature, light intensity, and water availability—exert a significant impact on the chemical makeup of plants of the Brassicaceae family. This variability among different studies and geographical areas affects both the overall quantity and the specific profiles of bioactive chemicals, including glucosinolates and phenolics (Björkman *et al.,* 2011).

3.3 Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

The chemical and potential interactions between the extract and the polymeric films were investigated using FT-IR analysis. Figure 3 displays the FT-IR spectra corresponding to the *Eruca vesicaria* extract, neat PVA film, and the bioactive films containing the extract.

The FT-IR analysis of the extract of *Eruca vesicaria* revealed characteristic absorption peaks corresponding to known phytochemical groups. The band centered at 3175 cm^{-1} was assigned to the O-H stretching vibration, typical of phenolic and alcoholic hydroxyl groups, while the peak at 2924 cm^{-1} was attributed to CH_2 asymmetric stretching vibration (Rizwana *et al.*, 2016). The other important peaks at 1511 cm^{-1}, 1397 cm^{-1} and 1033 cm^{-1} belong to C = C stretching vibration of the aromatic ring, CH_3 of aromatic rings, and aliphatic alcohol esters, respectively. The detection of these key signals provides confirmation of the presence of phenolic compounds (García-Gurrola *et al.*, 2021).

The spectra of the active films exhibited multiple distinctive peaks corresponding to stretching and bending vibrations associated with PVA (Kavas, Terzioğlu, & Sıcak,

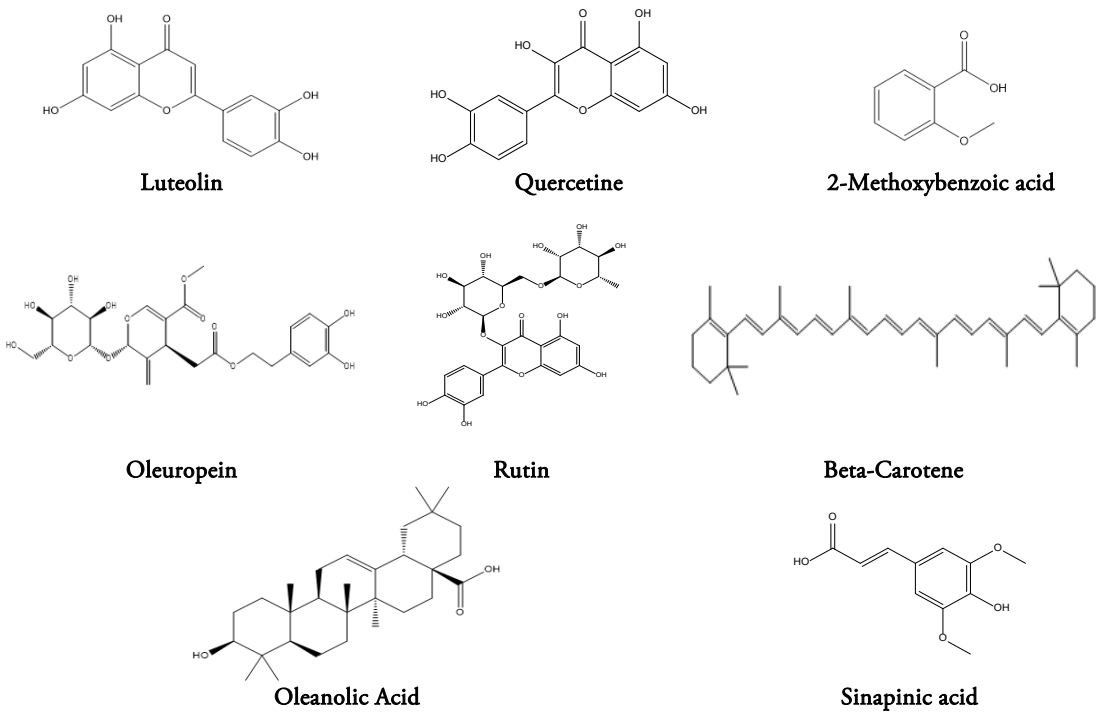

Figure 2. Chemical Structures of Molecules Detected in *Eruca vesicaria* Extract

2023). A comparison between the neat PVA spectrum and the extract-loaded films indicated subtle shifts and variations in peak intensity across the spectra. These observed changes provide evidence for intermolecular interactions, likely involving hydrogen bonding, occurring between the functional groups of the Eruca vesicaria extract and the PVA polymer matrix.

3.4 Light Transmission of the Films

Table 4 illustrates the influence of the *Eruca vesicaria* extract concentration on the optical transparency of the films. The neat PVA film (L0) demonstrated the highest level of transparency. Conversely, the incorporation of extract resulted in a reduction in transparency values across the 280nm to 600nm range proportional to the extract concentration. The neat PVA film transmitted 91.66% of the light at 400nm. However, UV-Vis transmission decreased significantly to 55.47%, 36.07%, and 17.84% for the L1, L2, and L3 films, respectively (Table 4). The extract-loaded films were found to be effective at filtering out the most detrimental UV radiation UV-C light (100–280 nm). The enhanced UV-blocking performance of the active films is directly correlated with the high phenolic content of the extract. Previous research has demonstrated that phenolic-rich plant extracts, including those derived from *Uncaria gambir*, increased the UV-blocking effectiveness of PVA-based films (Abral *et al.*, 2022). This effectiveness is attributed to the inherent absorbance properties of polyphenolic compounds, such as tannins (Zhai *et al.*, 2018).

Table 4. Light transmission values of films at different wavelengths

Sample	T_{280} (%)	T_{400}(%)	T_{600}(%)
L0	77.30	91.66	92.35
L1	4.89	55.47	92.47
L2	2.32	36.07	89.52
L3	2.08	17.84	85.58

3.5 Water Contact Angle (WCA) Measurements

The water contact angle (WCA) serves as a critical surface parameter for assessing both wettability and hydrophilicity. Surface-wetting studies analyses were conducted to determine variations in the WCA among the neat PVA and PVA-based bioactive films (Figure 4). All synthesized films exhibited WCA values below 90°, confirming their overall hydrophilic nature as summarized in Table 5. A noticeable trend was observed where an increased ratio of the plant extract within the PVA matrix led to a corresponding reduction in the WCA.

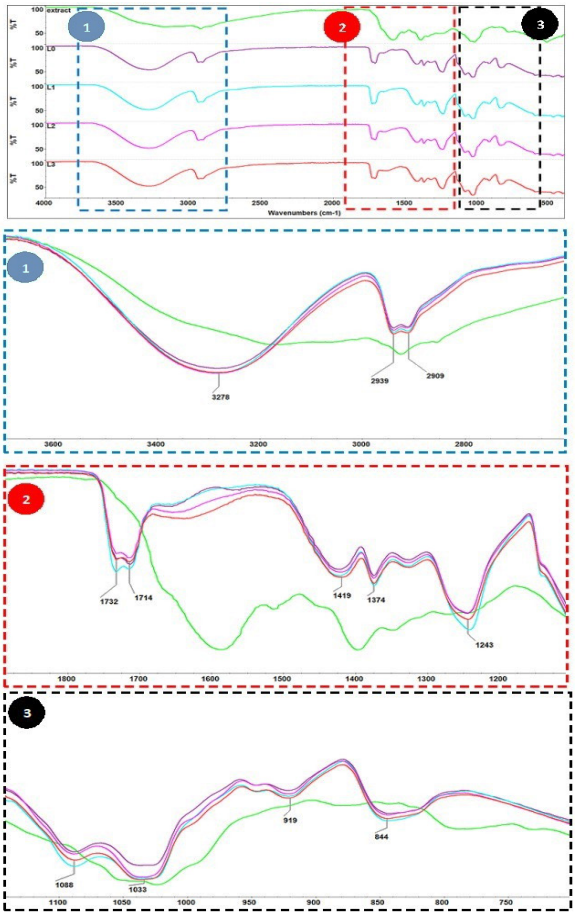

Figure 3. Infrared Spectrum of Plant Extract, PVA film (L_0), Composite bioactive films (L_1, L_2, L_3)

(1) The expanded range of FTIR spectra of bioactive films between 3700–2750 cm^{-1} (2) 1900–1150 cm^{-1}, and (3) 1150–550 cm^{-1}

The observed increase in hydrophilicity is attributed to the chemical composition of the *Eruca vesicaria* extract, which is rich in phenolic compounds containing highly polar or hydrophilic functional groups, such as hydroxyl and carboxyl fractions (Luciano *et al.*, 2021). These groups enhance the film surface polarity, facilitating interaction with water molecules and consequently reducing the contact angle. Moreover, the films' wettability might experience a boost owing to the raised mobility of the polymeric matrix chains induced by the incorporation of the extract (Luciano *et al.*, 2021). Identical findings have been reported in the literature for gelatin films loaded with hydroethanolic extract from *Pitanga* leaves (Luciano *et al.*, 2021).

Table 5. Hydrophilicity of PVA Based Films

	L_0	L_1	L_2	L_3
Contact angle (deg.)	67.25 ± 4.12	25.78 ± 4.1	18.07 ± 2.53	19.38 ± 1.83

3.6 Evaluation of Antioxidant Activity

Oxidation represents a primary factor that compromises the quality of food products, leading to reduced shelf life and the deterioration of sensory attributes, including color, odor, and flavor. To preserve the food's quality and safety, there is a substantial scientific interest in developing packaging films that interact with the food or packaged product. Table 6 presents the results of antioxidant activity of *Eruca vesicaria* extract, and the extract-loaded films, evaluated using two distinct methods: DPPH and TAC.

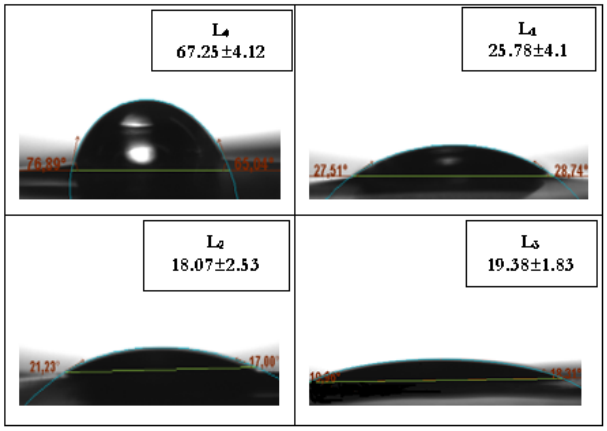

Figure 4. Surface Wetting Properties

Table 6. Antioxidant Potential of PVA Films Containing Various Amounts of *Eruca vesicaria* Extract

Antioxidant Activity Sample	DPPH· Scavenging (IC_{50} mg/mL)	TAC (EC_{50} mg/mL)
L_0	1.53 ± 0.03 [a]	10.69 ± 0.5 [a]
L_1	1.31 ± 0.06 [b]	5.83 ± 0.2 [b]
L_2	1.14 ± 0.005 [c]	2.32 ± 0.05 [c]
L_3	0.75 ± 0.01 [d]	1.8 ± 0. 1 [d]
Extract	0.61 ± 0.01 [e]	1.58 ± 0.01 [e]
Ascorbic acid	0.05 ± 0.0005	0.107 ± 0.01

Note: Results are expressed in terms of mean ± standard deviation (n = 3). In statistical analyses by ANOVA One way the mean difference is significant at the 0.05 level, n = 5. Dunnett t-tests treat one group as a control, and compare all other groups against it. The values with different superscripts (a, b, c, d, and e) in the same columns are significantly different ($p < 0.05$).

The results related to the antioxidant activity demonstrate that the ethanol extract of the *Eruca vesicaria* and the bioactive films exhibit potent antioxidant activity against the free radical DPPH. The highest antioxidant capacity was recorded in the ethanol extract, yielding an IC_{50} value equal to 0.61 ± 0.01 mg/mL. Critically, increasing the concentration of the extract within the bioactive films led to a significant enhancement in their antioxidant performance. This activity

is primarily ascribed to the electron and proton donating capability of the phenolic compounds identified in the extract. It is noteworthy that, despite the activity of the extract, the antioxidant capacity of the active films (L1, L2, L3) is dependent upon the controlled release of the incorporated antioxidants, represented by phenols and flavonoids contained in the extract of the plant *Eruca vesicaria*, into the solution that consumes free radicals. As displayed in Table 5, the L0 film presents weak DPPH radical scavenging activity and remains less active compared to the ascorbic acid standard. Analogous results, found by Chen *et al.* (2018), reported significant improvements in the antioxidant capacity of polyvinyl alcohol films active with green tea extract, and this packaging system was applied to the packaging of dried eel. Furthermore, Annu *et al.* (2021) reported improvements in films composed of two components, chitosan (CS) and polyvinyl alcohol (PVA), by incorporating a natural extract from Ocimum Tenuiflorum, which enhanced DPPH radical scavenging properties (~41.1% antioxidant activity) for CS/PVA films.

The phosphomolybdenum test, which relies on the reduction of molybdenum (VI) to molybdenum (V) by sample antioxidants resulting in the formation of green phosphate compounds, also yielded significant results. The *Eruca vesicaria* exhibited a higher TAC that the active films, demonstrating an antioxidant capacity closely comparable to the ascorbic acid standard. The EC_{50} were determined as 0.103 ± 0.011 for the extract and 1.22 ± 0.02 mg/mL for ascorbic acid (Note: The EC_{50} value for the standard appears unusually high compared to the extract and may require verification in the original data). The antioxidant capacity of active films improved proportionally with the concentration of the incorporated extract; however, it remained lower than that of ascorbic acid. When comparing our results to previous studies, we found that a study conducted by Silva *et al.* (2021) indicated that incorporating active films with plant extracts rich in phenolics improved the total antioxidant capacity properties of these films intended for packaging and preserving food products.

These antioxidant compounds possess considerable potential in active food packaging applications. When integrated into bio-based films, they can effectively reduce oxidative degradation of food products by neutralizing free radicals, thus preserving product quality and extending shelf life. Recent studies have confirmed the pivotal role of phenolic compounds in food packaging, demonstrating their efficacy in retarding lipid oxidation and delaying spoilage (Albuquerque *et al.*, 2021; Singh, Kim, & Lee, 2022). Phenolic acids such as p-coumaric acid and oleanolic acid have been demonstrated to enhance the antioxidant capacity of packaging films, contributing to increased food stability and safety (Ordoñez, Atarés, & Chiralt, 2022). Additionally, the incorporation of resveratrol and oleuropein into biodegradable films has demonstrated promising results in extending shelf life by improving barrier properties and reducing microbial growth (Figueroa-Enríquez *et al.,* 2024). Given their antioxidant properties, PVA-based bioactive films incorporating *Eruca vesicaria* may compounds are highly appropriate for packaging oxidation-sensitive food products such as meat, cheese, and fresh-cut fruits, where they can extend shelf life and reduce the reliance on synthetic preservatives (López-de-Dicastillo *et al.*, 2012).

The one-way ANOVA statistical analysis performed on antioxidant activity data (obtained via both DPPH and phosphomolybdate assays) indicated a lack of significant difference between the groups treated with varying concentrations of plant extracts and the positive control (ascorbic acid). However, a highly significant difference was observed when comparing the extract-treated groups to the polymer control group (L0, without extract). This control group demonstrated a highly significant difference compared to both ascorbic acid and the extract-loaded groups. Furthermore, the results of the homogeneous subsets statistical test for the phosphomolybdate TAC method suggest that the 0.50 mg/mL and 0.75 mg/mL extract concentrations display statistically equivalent antioxidant effects.

In addition to their enhanced antioxidant capacity, the fabricated PVA-based films incorporating *Eruca vesicaria* extract present further critical advantage for sustainable packaging: biodegradability. Blending polyvinyl alcohol with natural additives or plant extracts can accelerate its rate of disintegration, which is known to be biodegradable under certain environmental conditions, particularly in the presence of microbial activity (Chiellini *et al.*, 2003). In addition to enhancing the films' functional qualities, the incorporation of bioactive plant-based substances contributes to their overall environmental compatibility. These novel biofilms offer an attractive alternative to conventional petroleum-based synthetic films, positing them as appealing substitutes for environmentally conscious food packaging systems due to their reduced ecological footprint and limited environmental persistence (Siracusa *et al.,* 2008; Tokiwa *et al.*, 2009).

4 Conclusions

This study successfully demonstrated the development and comprehensive characterization of polyvinyl alcohol (PVA) films incorporated with varying concentrations of *Eruca vesicaria* extract. The incorporation of the extract significantly enhanced key functional properties, including antioxidant capacity and UV protection. LC-MS/MS analysis identifies eight major phytochemicals in the extract, selected based on their prominent peak areas—including

phenolic acids, flavonoids, a polyphenol, a triterpenoid, and a carotenoid, all contributing to the films' improved functionality. These extract-loaded PVA films demonstrate promise as environmentally friendly materials for food packaging, as they can extend product shelf life by preventing oxidation and reducing the need for chemical additives. However, further studies are required to fully assess their potential for specific food products, focusing on oxygen and water vapor transmission rates. Additionally, future research should explore the interaction between these films and various food types to optimize their practical applications in real-world scenarios.

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