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Abstract: This study employed High-Performance Liquid Chromatography coupled with Diode Array Detector and Mass Spectrometry (HPLC-DAD-MS) to characterize bioactive compounds in olive leaf extracts. The analysis revealed a diverse metabolic profile, predominantly composed of tyrosol, flavonoids, and hydroxycinnamic acid. The antioxidant activity of the extracts demonstrated their efficacy, with variations observed between neutral and acidic pH conditions. Encapsulation was explored as a preservation method, successfully maintaining the integrity of phenolic compounds in both liquid and powdered forms. All four extracts have an undeniable antimicrobial effect.Moreover, the antibacterial activity tested on*E. coli* ATCC 25922 and *S. aureus* ATCC 25923 strains significantly improved post-encapsulation, particularly for the acidic pH extract. The findings suggest that encapsulation can potentially conserve and/or enhance the antimicrobial activity of olive leaf extract, paving the way for developing functional products with improved shelf life and nutritional properties. Further investigations are recommended to elucidate underlying mechanisms and optimize encapsulation processes to maximize the benefits of olive leaf extract in diverse applications.

Keywords: encapsulation; olive leaf extract; spray drying; maltodextrin

1. Introduction

Oliveleaf, a by-product of olive tree cultivation, have been recognized for their rich content of bioactive phenolic compounds, with oleuropein and hydroxytyrosol as prominent examples. These phenolic compounds have gained attention for their numerous potential health benefits, including antioxidant and antimicrobial properties [1]. However, the practical utilization of these compounds often faces challenges due to their sensitivity to environmental factors, such as heat, light, and oxygen, which can lead to degradation and reduced efficacy. In response to these challenges, encapsulation has emerged as a promising technique to protect and preserve the bioactive components of olive leaf[2]. Encapsulation involves the entrapment of these compounds within a protective matrix, often in the form of microcapsules or nanoparticles. This encapsulation process shields the compounds from external factors and offers several other advantages, including controlled release, improved stability, and enhanced bioacessibility[3].

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The encapsulation of olive leaf extracts has garnered considerable attention in the food, pharmaceutical, and nutraceutical industries. Encapsulated olive leaf extracts can be incorporated into various products, from functional foods and dietary supplements to pharmaceutical formulations and cosmetic products. The encapsulation process allows for the controlled release of the phenolic compounds, ensuring their efficacy over time and expanding their potential applications.Various methods are accessible for encapsulating food compounds, focusing on techniques suitable for liquid forms. Many of these technologies involve drying processes. Among the available are spray drying, spray-bed-drying, fluid-bed coating, spray-chilling, spray-cooling, and melt injection to encapsulate active agents [3,4]. Spray drying is one of the classicand most extensively employed techniques in the food industry for encapsulation. This method is valued for its flexibility, continuous operation, and economic efficiency. It consistently generates high-quality particles, typically smaller than 40 μm, aligning with the final product's desired sensory and textural attributes. Despite its prevalence, spray drying has drawbacks, including equipment complexity, non-uniform drying chamber conditions, and challenges in particle size control. Approximately 80–90% of encapsulates in use are produced through spray drying [5]

The aim of the present study was to explore the extraction and encapsulation techniques of phenolic compounds from olive leaf, focusing on their efficiency, yield, and the stability of bioactive compounds during the encapsulation process.

2. Materials and Methods

2.1. Plant Material

Fresh leaves of the Chemlali olive tree, a variety grown in Tunisia, were harvested from February to April in the Sidi Bouzid region. This collection method was defined byBouaziz&Sayadi, [6]because of the high concentration of oleuropein in olive leaf. The leafwere harvested in the morning, immediately after the dew had evaporated. After harvesting, the leafwere transported to the laboratory and air-dried at room temperature for a week.

2.2. The Extraction Procedure: Autoclave Extraction Method of Phenolic Compounds

The extraction procedure was assisted by the autoclaving method defined by Bouaziz et al. [7]with some modifications. Briefly, the samples (20 g) were finely ground in a mortar, placed in a vial, and 1 L of neutral (pH 7) and acid (pH 2) solvent (water) was added. The vials were placed in an autoclave for 1 h at 110°C and filtered after cooling. The supernatant was collected, and the extraction procedure was repeated twice with the same amount of solvent. The extraction of phenolic compounds with acidified water (olive leaf extract obtained at acidic pH: OLEA) and with neutral water (olive leaf extract obtained at neutral pH: OLEN) were furtherexamined.

2.3. Preparation of Olive Leaf Extract Microparticles

The olive leaf extract (OLE) encapsulation process consisted of spray drying, using food-grade maltodextrin (DE 17.00) as the encapsulating agent. The maltodextrin was first mixed with the extract in a 1:10 (w:v) ratio. Once the mixtures were prepared, they were introduced into a laboratory-scale spray-drying system (Buchi B290, Switzerland), following the methodology described in previous research [8]. Spray drying began with the immediate introduction of the mixtureinto the system at a feed rate of 5 mL/min, facilitated by an adjustable peristaltic pump. Drying air parameters were controlled, with the airflow rate maintained at 140 ± 2°C and inlet and outlet temperatures at 70 ± 2°C. The extract feed rate was set at 25 mL/min, while the spray gas flow rate was regulated at 536 L/h. Once the spray drying process was complete, the resulting microcapsules were carefully collected and sealed in plastic bags. These bags were then securely sealed and stored in a dark environment at 25 ± 2°C until further characterization and analysis.

The encapsulation process was carried out for both extracts (olive leaf extract obtained at an acid pH: OLEA) and (olive leaf extract obtained at a neutral pH: OLEN).

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2.4. Characterization of Olive Leaf Extracts before and after encapsulationhplc-DAD-MS-ESI+ Analysis

Analysis was carried out using an HP-1200 liquid chromatograph equipped with a quaternary pump, autosampler, DAD detector, and MS-6110 single quadrupole API-electrospray detector (Agilent-Technologies, USA). The positive ionization mode was applied to detect the phenolic compounds; different fragmentor, in the range 50-100 V, was applied. The column was a Kinetex XB-C18 (5 μm; 4.5x150 mm id.) from Phenomenex, USA. The mobile phase was (A) water acidified by formic acid 0.1 % and (B) acetonitrile acidified by formic acid 0.1 %. The following multistep linear gradient was applied: start with 5% B for 2 min; from 5% to 90% of B in 20 min, hold for 4 min at 90% B, then 6 min to arrive at 5% B. The total analysis time was 30 min, flow rate 0.5 ml/min, and oven temperature 25±0.5 0C. Mass spectrometric detection of positively charged ions was performed using the Scan mode. The applied experimental conditions were: gas temperature 3500 C, nitrogen flow 7 l/min, nebulizer pressure 35 psi, capillary voltage 3000 V, fragmentor 100 V, and m/z 120-1500. Chromatograms were recorded at wavelength λ=280 nm, λ=350 nm, and data acquisition was done with the Agilent Chem Station software.

2.4. Determination of Total Phenolic Content (TPC)

The amount of TPC in olive leaf extracts (OLEA, OLEN) was determined using the Folin-Ciocalteu method, as described in detail by Szydłowska-Czerniak&Tułodziecka, [9]. Samples (0.2 - 1.0 mL) were transferred to a 10 mL volumetric flask, then 0.5 mL of Folin-Ciocalteu reagent was added and stirred for 3 min. Next, 1 mL of saturated sodium carbonate solution was added, and the volume was made up to the mark with redistilled water. After 1 hour, the solutions were centrifuged for 15 minutes, and the absorbance at 765 nm was measured against a reagent blank. The total content of phenolic compounds, determined by the Folin-Ciocalteu method, is expressed as mg of gallic acid per gram of olive leaf.

2.5. Determination of Antioxidant Activity

The DPPH scavenging effect was evaluated following the method described byBouaziz et al.[10].Briefly, 2 ml of methanolic solution of varying sample concentration (25, 50, 100, and 150 μg/mL) were added to 5 mL of DPPH methanol solution.At 517 nm, the optical density was measured using a Shimadzu UV-160 spectrophotometer after mixing the two solutions gently and leaving them for 30 min at room temperature. The test samples were tested in the same concentration range. The test samples and the positive control (BHT) were tested at different concentrations. The test samples were experimented over the same concentration range.The test samples and the positive control (BHT) were tested at various concentrations.The antioxidant activity of each extract (OLEA and OLEN) and BHT was expressed in terms of concentration needed to impede 50 DPPH radical formation IC 50 μg/mL and calculated from the log-dose inhibition curve.

2.6. Determination of Antimicrobial Activity

2.6.1. Standard Strains

Gram-positive (Gram (+)) and Gram-negative (Gram (−)) bacterial and yeast strains (Table 1) were utilized in the current study. Furthermore, thesestrains were incorporated to assess the antibacterial and antifungal properties of the extracts. The following standard strains were examined: *Escherichia coli ATCC 25922, Salmonella enteritidis ATCC 13076, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 8739, Pseudomonas aeruginosa, and Candida albicans ATCC 10231.*

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Table 1. Microbial reference strains and their pathological effects

Microbial Strains	Catalog number	Effects	Reference
Pseudomonas aeruginosa	*ATCC 15442*	Gastrointestinal diseases	[11]
Salmonella enteritidis	*ATCC 13076*	Gastrointestinal diseases	
Staphylococcus aureus	*ATCC 25923*	Foodborne, scalded skin syndrome	
Escherichia coli	*ATCC 8739*	Foodborne	
Escherichia coli	*ATCC 25922*	Foodborne	
Candida albicans	*ATCC 10231*	Candidiasis, opportunistic oral and genital infections	

2.6.2. Preparation of Bacterial Strains

Several colonies of each strain grown on Mueller Hinton agar were transferred to sterile saline (8.5 g/L) and adjusted to match the McFarland 0.5 turbidity (1.5 × 10^8 CFU/mL). Then, a 1.5 × 10^5CFU/mL bacterial suspension was prepared to be added to each microplate well for 1.5 × 10^6CFU/mL for antifungal activity, respectively.

2.6.3. Determination of the Minimum Inhibitory Concentration (MIC)

According to standard protocols [12], the determination of the minimum inhibitory concentration (MIC) values for each extract (OLEA, OLEN (non-encapsulated extracts), EOLEA and EOLEN (encapsulated extracts)) against both the tested bacterial and yeasts strains. The strain was cultured in either Mueller Hinton broth or Malt Extract broth (Sigma, Tunis, Tunisia) at the appropriate temperature (30°C or 37°C). Inocula were prepared by adjusting the turbidity of each culture. To achieve an optical density of 0.5 McFarland standards, which is equivalent to approximately 1–5 × 10^8 CFU/mL, the microbial culture must be allowed to reach a certain level of growth. This growth is necessary to obtain the desired concentration of cells concentrations. The broth was then diluted. The essential process was conducted in 96-well microtiter plates, utilizing both microbial reference strains.

The extracts were meticulously prepared in a sterile manner and subsequently transferred to sterile 96-well microtiter plates through two-fold serial dilutions utilizing the corresponding broth medium. The stock solution of the tested extracts was administered in a concentration of 500 mg/mL. A total of 80 microliters of the extract was used. In each well, the resazurin indicator solution (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) and the prepared oil suspension were introduced, with 10 µL of each oil dose added. Employing the latter reagent makes it possible to identify the presence of microorganisms in minuscule amounts of liquid within microtiter plates without needing a spectrophotometer. This method also includes the utilization of two control wells. Two types of plates were prepared for the experiment. The first plate contained well-containing microorganisms and resazurin, while the second plate contained only medium and resazurin to ensure sterile conditions. After preparation, the plates were placed in an incubator. The bacterial growth evaluation involved incubating the samples anaerobically at 37°C for a duration of 24 hours. Following the incubation period, the growth of bacteria was assessed by observing a color transition from blue to pink. The minimum dosage that indicated suppression of growth was determined.

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3. Results

3.1. Identification of Bioactive Compounds by HPLC-DAD- MS

3.1.1. Olive Leaf Extracts Characterization

The HPLC-DAD performed the metabolic profiling of olive leaf extracts- MS using electrospray in positive ionization mode. The identified metabolites are summarized in Table 2, which includes retention time (RT), experimental m/z, UV data (nm), and the proposed compound and subclass of those compounds. Furthermore, all of these results were complemented by the UV–Vis detection provided by the diode array detector (DAD) data, which were in accordance with several studies. When the reference compounds were available, phenols were compared with standards regarding Rt, UV, MS spectral characteristics. Briefly, sixteen phenolic compounds were identified by HPLC-DAD- MS and grouped in tyrosol, flavonoids, and hydroxycinnamic acid. As it is shown in Table 2, tyrosol was the major component, constituting 62% of the classes identified in the extract of olive leaf. The extract is also composed of flavones, constituting 31.25% of the classes identified in the extract of olive leaf, by flavanols and hydroxycinnamic acid. Ten phenolic compounds belonging to tyrosol were found in the olive leaf extract (Table 2), particularly the two major compounds oleuropein and hydroxytyrosol. Tyrosol content was determined using a five-point calibration curve of oleuropein (R^2 =0.9978) in the linearity range 10-50 µg/ml. Flavone content was determined using a five-point calibration curve of luteolin (R^2 =0.9972) in the linearity range 1-100 µg/ml. Hydroxycinnamic acid content was determined using a five-point calibration curve of chlorogenic acid (R^2 =0.9937) in the linearity range 10-50 µg/ml. Flavonol content was determined using a five-point calibration curve of rutin (R^2 =0.9981) in the linearity range 10-100 µg/ml. Peak 1 was identified as Hydroxytyrosol-glucoside by reference to standards. Peak 2 showed a molecular ion at 155 m/z. It was identified as hydroxytyrosol by comparison with RT, UV absorption, and MS spectra, which are commercial standards. Peak 13 showed a molecular ion at 702 m/z, which was attributed to oleuropein-diglucoside[13]. Peaks 14 and 15 showed the same molecular ion at 557 m/z, and both were assigned to two isomers, namely, Hydroxyoleuropein and Hydroxyoleuropein isomers. Finally, compound 16 with a molecular ion at 379 m/z was identified as an oleuropein-aglycone [13].

Flavonoids are another important group of phenolic compounds largely distributed in olive leaf extracts. Different flavonoid sub-classes were identified as flavonols and flavones (Table 2). Among them, four compounds were identified as flavones. Compounds 6, and 10 were identified as luteolin-diglucoside and Luteolin-glucoside according to their mass data and as previously reported by several authors [13,14]. Indeed, compound 8 was identified as Quercetin-rutinoside (rutin) [13]. Peak 9 showed a molecular ion at 625 m/z, indicating the presence of Verbascoside. The latter is a compound that belongs to the hydroxycinnamic acid subfamily, as previously reported by [14].

Table 2. DAD and MS data obtained after positive ionization of the samples.

Peak No.	Retention time Rt (min)	Molecular formula	UV λ_{max} (nm)	$[M+H]^+$ (m/z)	Phenolic Compound	Subclass
1	9.25	$C_{14}H_{19}O_8$	280	317	Hydroxytyrosol-glucoside	Tyrosol
2	9.63	$C_8H_{10}O_3$	280	155	Hydroxytyrosol	Tyrosol
3	11.31	$C_{25}H_{32}O_{12}$	330	525	Lingstroside	Tyrosol
4	13.03	$C_{18}H_{26}O_{11}$	320	419	Oleoside dimethylester	Tyrosol
5	13.55	$C_{17}H_{24}O_{11}$	320	405	Oleoside 11-methylester	Tyrosol
6	13.81	$C_{27}H_{29}O_{16}$	340	611	Luteolin-diglucoside	Flavone
7	15.09	$C_{21}H_{19}O_{10}$	341	433	Apigenin-glucoside	Flavone

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8	15.52	$C_{27}H_{29}O_{16}$	360	611	Quercetin-rutinoside (Rutin)	Flavonol
9	15.86	$C_{29}H_{35}O_{15}$	332	625	Verbascoside	Hydroxycinnamic acid
10	16.15	$C_{21}H_{19}O_{11}$	340	449	Luteolin-glucoside	Flavone
11	16.69	$C_{27}H_{29}O_{14}$	341	579	Apigenin-rutinoside	Flavone
12	17.04	$C_{19}H_{21}O_{7}$	330	363	Ligstroside-aglycone	Tyrosol
13	17.58	$C_{31}H_{41}O_{18}$	280	702	Oleuropein-diglucoside	Tyrosol
14	18.43	$C_{18}H_{35}O_{14}$	280	557	Hydroxyoleuropein	Tyrosol
15	19.07	$C_{18}H_{35}O_{14}$	280	557	Hydroxyoleuropein isomer	Tyrosol
16	19.71	$C_{19}H_{21}O_{8}$	280	379	Oleuropein-aglycon	Tyrosol

3.1.2. Antioxidant Activity of Olive Leaf Extract

It is widely recognized that phenolic substances extracted from plants have well-established antioxidant properties[15,16]. These compounds act in various ways, such as reducing agents, hydrogen donors, oxygen scavengers, or metal ion chelators[16,17]. The results of our study reveal a significant total concentration of phenolic compounds in the olive leaf extract obtained at neutral pH, reaching approximately 189.81 ± 0.24 mg EAG/g.In comparison, the extract obtained at an acid pH had a concentration of approximately 167.07 ± 0.84 mg EAG/g. These concentrations are lower than those reported by Xie et al [18], but higher than those obtained by Khelouf et al. [18] and Ghasemi et al. [20]. In this context, the extract obtained at neutral pH appears to have potentially stronger antioxidant activity than that obtained at acid pH.

The methodology used to assess the antioxidant activity of olive leaf extracts is based on the DPPH test. The anti-free radical activity of the extract is assessed by spectrophotometry at 517 nm, by observing the decrease in DPPH, accompanied by a change in color from violet to yellow[19]. The sensitivity of DPPH to the presence of active substances, even at low concentrations, means that it can quickly adjust its behavior according to the different samples. A comparison of IC50 values highlighted the significant effectiveness of oleuropein and hydroxytyrosol as antioxidants. The anti-free radical activity of the hydrolysate, composed mainly of hydroxytyrosol, was assessed using the DPPH test, and the results are summarized in Table 3. The hydrolysate obtained at acidic pH showed an IC50 of 0.68 µg/ml, while the extract obtained at neutral pH showed an IC50 of around 1.30 µg/ml, indicating significant antioxidant activity. These findings demonstrate that both extracts have similar or even greater antioxidant activity than pure extracts.Similar results were observed by Bouaziz et al.[10].

In summary, the antioxidant efficacy of olive leaf extract is mainly due to the phenolic compounds it contains. The antioxidant activity of these compounds is highly dependent on their chemical structure[18,20].It is generally recognised that increasing the number of hydroxyl groups in the structure of phenols improves their ability to act as hydrogen donors and inhibit oxidation. In addition, their solubility and partitioning behaviour also influence their reactive activity [10].

Table 3. IC50 of Chemlali olive leaf extract and its hydrolysate.

	IC50 [µg/mL]
BHT	0.63
Pure Hydroxytyrosol	0.60
Olive leaf extract obtained atacidic pH	0.68
Pure Oleuropein	1.10

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Olive leaf extract obtained at neutral pH	1.30

3.1.3. Encapsulation of Olive Leaf Extract Obtained in Two Different pH

Table 4 shows the concentrations of phenolic compounds in the extracts before and after spray drying. As the table shows, oleuropein is the main compound, closely followed by hydroxytyrosol, lingstroside aglycone, oleosidedimethylester, oleoside 11-methylester, rutin, as well as other compounds, in the case of the extract obtained at neutral pH (OLEN). In contrast, hydroxytyrosol is the predominant compound for the extract obtained at acidic pH (OLEA), closely followed by oleuropein. Interestingly, the 16 phenolic compounds identified in the liquid extract (before the spray drying) were still present in the powders (after encapsulation), indicating minimal degradation due to encapsulation. Hydroxyoleuropein remained the predominant phenolic compound before and after encapsulation for OLEN, while hydroxytyrosol was for OLEA extract, highlighting their thermal stability. This is why the food industry is increasingly interested in developing functional products containing hydroxyoleuropein and its hydrolysate, hydroxytyrosol, because of their superior nutritional properties and their ability to extend shelf life thanks to their antibacterial properties. It is also noteworthy that luteolin-diglucoside, luteolin-glucoside, and apigenin-rutinoside maintained similar values in both powders, which can be explained by the similar behavior of these phenolic compounds under different environmental conditions, such as high temperatures and pressures [21]. These observations highlight the importance of encapsulation processes in preserving phenolic compounds.

Finally, it should be noted that manufacturing the powdered phenolic microspheres and nanocapsules required high-speed homogenization, and various technological factors could have potentially damaged the phenolic compounds by disrupting the interfaces between the phenolic extract and the cover materials.

Table 4. The content of phenolic compounds in olive leafextracts and encaplsulatedextracts , expressed in µg/g.

Retention time Rt (min)	Phenolic Compound	OLEN before spray dryer (mg/g)	OLEA before spray dryer (mg/g)	OLEN After spray dryer (mg/g)	OLEA After spray dryer (mg/g)
9,25	Hydroxytyrosol-glucoside	2,23	1,67	1,33	1,17
9,63	Hydroxytyrosol	1,09	14,57	0,99	9,77
11,31	Lingstroside	0,34	0,40	0,56	0,52
13,03	Oleosidedimethylester	0,43	0,44	0,71	0,84
13,55	Oleoside 11-methylester	0,39	0,57	0,68	1
13,81	Luteolin-diglucoside	0,055	0,045	0,030	0,064
15,09	Apigenin-glucoside	0,041	0,031	0,033	0,024
15,52	Quercetin-rutinoside (Rutin)	0,37	0,23	0,23	0,10
15,86	Verbascoside	0,21	0,33	0,14	0,20
16,15	Luteolin-glucoside	0,14	0,19	0,038	0,043
16,69	Apigenin-rutinoside	0,091	0,082	0,056	0,048
17,04	Lingstroside-aglycone	0,49	0,65	0,49	0,82

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17,58	Oleuropein-diglucoside	1,87	1,24	1,58	0,87
18,43	Hydroxyoleuropein	12,84	3,03	8,86	1,78
19,07	Hydroxyoleuropein isomer	2,81	1,29	1,83	0,89
19,71	Oleuropein-aglycone	0,85	0,42	0,68	0,34
	Total Phenolics	24,29	25,24	18,27	18,53

3.2. Antibacterial Activity

Table 5 displays the MIC of olive leaf extract obtained at two different pH levels both before (OLEN and OLEA) and after encapsulation (EOLEN and EOLEA). The antimicrobial activity of the olive leaf extracts was evaluated against *Escherichia coli ATCC 25922 and ATCC 8739*, *Salmonella enteritidisATCC 13076*, *S. aureus ATCC 25923*, *Pseudomonas aeruginosa ATCC 15442* and *Candida albicans ATCC 10231*.

These results show that all four extracts have an undeniable antimicrobial effect. The MIC values are different due to their different compositions. This allows us to conclude that this antimicrobial activity comes mainly from the major compound present in each extract. In fact, the values of the MICs depend on the active ingredient content of the extracts and the sensitivity of the microbial genera to this active ingredient. The OLEN, and EOLEN**'**s active ingredient is a phenolic oleuropein polymer (Table2). Despite this, the active ingredient in OLEA and EOLEA is hydroxytyrosol, a phenolic monomer. The MIC values for these extracts are 2 to 10 times lower than those of the OLEN and EOLEN preparations (Table 5). This may be due to the low concentration of hydroxytyrosol, but its richness in other phenolic compounds gives its equally important antimicrobial activity.

The use of hydrolysis is therefore justified. The antimicrobial action of phenolic compounds has been well-known for a long time. Indeed,Takó et al. [22] shed that phenolic compounds possess strong antibacterial activities and act on various microorganisms.Lobiuc et al.[23] reported that the antimicrobial activity of phenolic compounds is due to their action on bacterial walls. The action of phenolic compounds is related to their ability to denature cell wall proteins and modify the cell wall**'**s structure. The destruction of the cell walls causes a leakage of cytoplasmic contents such as enzymes, potassium ions, or glutamate from the bacteria, leading to cell death. The action of phenolic compounds can be by modifying the wall**'**s structure by denaturing proteins and solubilizing its lipid constituents. They also reported that phenolic compounds enter the cell and act at the level of DNA replication.Ecevit et al.[24] explain the high bactericidal activity of phenolic compounds by their tendency to inhibit metabolic energy by blocking oxygen consumption.Antunes et al. [25] reported that Gram- bacteria are more resistant to phenolic compounds than Gram+ bacteria. The values obtained in this study do not fully support this property as we obtained lower MIC values for *P. aeruginosa*, *E. coli* and *S. enteritidis* Gram- than those for Gram+ *S. aureus* and *Bacillus cereus* (Table 5). The results also show that the MICs values of the genera *Pseudomonas* and *Bacillus* are somewhat higher than the rest of the strains studied. This resistance is not surprising because these two bacterial genera are known for their potent hydrolytic capacities, as these genera are soil bacteria and are involved in many plant polymer degradation reactions. The results obtained are significant for the type of bacteria such as *S. aureus*, *S. enteritidis*, *E. coli* and *P. aeruginosa*, among which species have become resistant to antibiotics and pose serious problems for hospitals and health in general.

Encapsulation results in a considerable reduction in the MIC of the extract against almost all microbial-tested strains (Table 5). The MICs value reductions were 2 to 4 fold. The reduction is particularly significant for *S. enteritidis,* dropping from 250 mg/mL to 62.5 mg/mL after encapsulation of the OLEN extract (Table 5). On the other hand, encapsulation does not seem to significantly impact efficacy against *P. aeruginosa* and *C. albicans* as the MICs of encapsulated and non-encapsulated OLEN extracts are comparable (Table 5). These results are discordant with those that Medfai et al. [26] showed. They demonstrated that the antimicrobial effectiveness was lost or preserved of olive leaf

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extracts encapsulated by spray-drying using maltodextrins, maltodextrins–pectin, and maltodextrins–gum Arabic as encapsulating agents. Although,Muzzalupo et al.[27] demonstrated variability of antimicrobial activity of olive leaf extracts as free or encapsulated in chitosan-tripolyphosphate nanoparticles. The observed reduction in MIC post-encapsulation suggests improved antimicrobial activity of the encapsulated olive leaf extract against tested microbial strains. The impact of encapsulation varies across microbial genera, underscoring the dependence of the encapsulation process's effectiveness on the specific properties of both the extract and microorganisms. Further investigation is warranted to elucidate the mechanisms underlying the observed changes in MIC after encapsulation and the specific factors influencing the effectiveness of the encapsulated extract.

In summary, the results propose that the encapsulation of olive leaf extract has the potential to enhance its antimicrobial activity. However, the extent of improvement is contingent on the bacterial strain and the pH of the extract, offering implications for the development of antimicrobial formulations utilizing olive leaf extract.

Table 5. The Minimum Inhibition Concentration (MIC) of the prepared olive leaf extracts

Strain	Minimum inhibitory concentration (mg/ml) of samples before encapsulation		Minimum inhibitory concentration (mg/ml) of encapsulated samples	
	olive leaf extract obtained at neutral pH (OLEN)	olive leaf extract obtained at acidic pH (OLEA)	olive leaf extract obtained at neutral pH (EOLEN)	olive leaf extract obtained at acidic pH (EOLEA)
Pseudomonas aeruginosa ATCC 15442	250	25	250	12.5
Escherichia coli ATCC 25922	125	25	62.5	12.5
Escherichia coli ATCC 8739	125	25	62.5	12.5
Salmonella enteritidis ATCC 13076	250	25	62.5	12.5
S. aureus ATCC 25923	500	50	250	25
Candida albicans ATCC 10231	250	125	250	125

4. Conclusions

In conclusion, the metabolic profiling of olive leaf extracts revealed a rich composition of phenolic compounds, with tyrosol, flavonoids, and hydroxycinnamic acid identified as major subclasses. The antioxidant activity assessment demonstrated the efficacy of the extracts, with notable differences between neutral and acidic pH conditions. Encapsulation was successfully employed as a preservation technique, maintaining the integrity of phenolic compounds and their distribution in both liquid and powder forms. The antibacterial activity against *E. coli ATCC 25922* and *S. aureus*

ATCC 25923 significantly improved after encapsulation, particularly for the acidic pH extract. The study suggests that encapsulation can potentially enhance the antimicrobial activity of olive leaf extract, opening avenues for developing functional products with extended shelf life and enhanced nutritional properties. However, further investigations are required to comprehend the underlying mechanisms and optimize encapsulation processes to maximize the benefits of olive leaf extract in various applications.

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