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Volatile and non-volatile profiles of olive pomace and its potential uses

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Abstract

Olive pomace is an environmentally harmful waste from the olive oil industry, containing large amounts of bioactive compounds that could be used in several areas. In the present study, the olive pomace, by-products, was fractionated successively using organic solvents of increasing polarity (cyclohexane (CYHA), ethanol (EtOH) and distilled water) to determine their biochemical composition (total phenolic content, total flavonoid content, HPLC, and GC-MS) and biological activities (antioxidant and antibacterial activities). In addition, the olive pomace was extract directly by the distilled water to determine its physicochemical characteristics (pH, humidity and quantity of oil). The study proved that olive pomace has a low content of total phenolic content in the three extracts (from 0.81 to 2.24 mg GAE/g dry residue). Likewise, for the content of total flavonoids, it presents in the CYHA and EtOH extracts of 0.5 and 0.4 mg QE/g dry residue, respectively. GC-MS data showed the detection of 6 volatile compounds in the cyclohexane extract which contains two major compounds were hexadecanoic acid (2.23%) and 9-octadecenoic acid (8.67%). Moreover, HPLC data showed that chrysin compound was the major one among the four detected ones in the organic extracts of olive pomace. The results showed that the CYHA extract exhibited the best antioxidant power exceeds 50% on the other hand, it is less than 25% for the ethanoic and aqueous extract. In the aqueous extract there is a stronger antibacterial activity against the strain *L. monocytogenes* (13 mm) than the reference (ampicillin) and the bacterial strain *B. cereus* (11 mm) associated with their phenolic activity.

1. INTRODUCTION

Olive pomace is an environmentally harmful waste from the olive oil industry, containing large amounts of bioactive compounds that could be used in several areas. In the present study, the olive pomace, by-products, was fractionated successively using organic solvents of increasing polarity (cyclohexane (CYHA), ethanol (EtOH) and distilled water) to determine their biochemical composition (total phenolic content, total flavonoid content, HPLC, and GC-MS) and biological activities (antioxidant and antibacterial activities). In addition, the olive pomace was extract directly by the distilled water to determine its physicochemical characteristics (pH, humidity and quantity of oil). The study proved that olive pomace has a low content of total phenolic content in the three extracts (from 0.81 to 2.24 mg GAE/g dry residue). Likewise, for the content of total flavonoids, it presents in the CYHA and EtOH extracts of 0.5 and 0.4 mg QE/g dry residue, respectively. GC-MS data showed the detection of 6 volatile compounds in the cyclohexane extract which contains two major compounds were hexadecanoic acid (2.23%) and 9-octadecenoic acid (8.67%). Moreover, HPLC data showed that chrysin compound was the major one among the four detected ones in the organic extracts of olive pomace. The results showed that the CYHA extract exhibited the best antioxidant power exceeds 50% on the other hand, it is less than 25% for the ethanoic and aqueous extract. In the aqueous extract there is a stronger antibacterial activity against the strain *L. monocytogenes* (13 mm) than the reference (ampicillin) and the bacterial strain *B. cereus* (11 mm) associated with their phenolic activity.

2. MATERIAL AND METHODS

2.1. Reagents

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma Aldrich (France): acetic acid, acetonitrile, cyclohexane, dichloromethane, methanol, dimethyl sulfoxide, Folin-Ciocalteu reagent (2 N), gallic acid, quercetin.

2.2. Pomace sample

The sample consists of olive pomace of the Zarrazi variety which comes from an industrial oil mill located in the Limawa region (South of Gabés-Tunisia) using a three-phase extraction process. A representative sample has been kindly provided for the completion of the work.

2.3. Preparation of the organic extracts

Ten grams of the olive pomace (10 g) were extracted successively with solvents of increasing polarity: CYHA, EtOH and distilled water. After each extraction, the extract was filtered using filter paper (Whatman N°. 2). Solvents were removed using a rotary evaporator (BUCHI, R-100) under pressure at 35°C. The dry residues obtained were placed in hemolysis tubes (5 mL) and kept at -20°C until subsequent analysis. The different residues obtained were evaluated for their chemical composition and biological activities.

2.4. Determination of the total phenolic content

The TPC of the olive pomace extracts was determined using the Folin-Ciocalteu method (Saoudi et al. 2021). Results were expressed as mg of gallic acid equivalents (GAE)/g dry residue (dr).

2.5. Determination of the total flavonoids content

The estimation of the TFC in the various extracts was determined according to the Dowd method as described by Saoudi et al. (2021). The results were expressed in milligrams of quercetin equivalents per g of dry residue (mg QE/g dr).

2.6. Chromatographic analysis

2.6.1. GC-MS analysis

For chemical identification of volatile compounds from the olive pomace extracts, the procedure of Kohoude et al. (2017), was used. The analysis was done on a gas chromatography system (7890A) coupled to a mass spectrometry. Chromatographic conditions were 50°C hold for 1 min, up to 250°C at the rate of 10°C/min and then 1 min isothermally at 250°C. Afterward, a second gradient was used at 300°C and 50°C/min and finally 300°C held at 3 min. The volatile compounds identification was done using mass spectra comparison with those obtained in NIST08 using AMDIS software.

2.6.2. HPLC analysis

The analysis of various olive pomace extracts to identify the phenolic compounds was performed using analytical HPLC-DAD as described by Ben Hassine et al. (2022). The phenolic compounds were identified by comparing the retention time of the unknown molecules with those of the standards.

2.7. Biological activities

The antioxidant activity of samples was determined using the DPPH. method as described by Saoudi et al. (2020). Furthermore, the antimicrobial activity of the extracts was assayed using the well diffusion method according to Sassi Aydi et al. (2020). The olive pomace extracts were tested individually against three human-pathogenic microbial strains: two Gram-positive bacteria (*Listeria monocytogenes*; *Bacillus cereus*) and one Gram-negative one (*Salmonella typhimurium*). The bacteria used were selected for their involvement in the oral and intestinal tract.

2.8. Statistical analysis

All measurements were carried out in triplicate. Two-way analysis of variance (ANOVA) was used for the significance calculation using the SPSS software 20.1 (Version IBM. 20.0. 2004). Statistical differences between the solvents used in the study were estimated using Tukey's test. The confidence limits were set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Extraction yield, total phenolic (TPC), total flavonoids content (TFC)

According to the literature, no previous studies have been reported on the effect of solvents on the yield extraction, TFC and the TPC of olive pomace extracts. The olive pomace was extracted using three organic solvents of increasing polarity (CYHA, EtOH and distilled water). Extracts showed lower yields of 3.4, 3.7, and 4.7% respectively (Table 1). From these esults it can be said that the gradual increase in solvent polarity influences the performance of the extracts. The extraction of polar and apolar compounds is largely dependent on the polarity of the solvents used. This explains why the three extraction solvents have different levels of phenolic compounds (Table 1). Statistically, there was a significant difference ($p \leq 0.05$) between the three extracts in terms of their TPC, as well as their TFC. The aqueous extract showed the highest TPC with 2.24 mg GAE/g of dr. The EtOH extract gained the second place, followed by the CYHA one, with respective TPC of 1.85 and 0.81 mg GAE/g of dr. (Table 1). The obtained values were higher than results reported by De Bruno et al. (2018), who used the same procedure. In addition, our results were in accordance with those found by Chanioti and Tzia, (2018) in their study when working on olive pomace by using natural deep eutectic solvents. The difference of the TPC between the used solvents could be influenced by the solubility of phenolic compounds, which depends on the type of solvent used (Ben Lataief et al, 2020). Interestingly, the aqueous extract showed no TFC, comparing to the other extracts. The total flavonoid content results showed that the pomace has a very low content even as the TFC in the range of 0 to 0.5 0.1 mg eq EQ/g of dry residue, for CYHA, EtOH and aqueous extracts (Table 1).

3.2. Chromatographic analysis

3.2.1. GC-MS analysis

GC-MS was used to identify volatile compounds in CYHA extracts of olive pomace. As far as we know, this work is an initial attempt to determine the volatile composition of olive pomace. The technique used was able to identify 6 volatile molecules which were styrene, 2-propenoic acid, hexadecanoic acid, 9-octadecenoic acid, oleic acid and 2-hexadecanol (Fig. 1; Table 2). The volatile profiles of pomace extracts showed the presence of 3 classes of organic compounds, which were, derivative of benzene, fatty acids, and fatty alcohol. The peak areas of these compounds were ranged from 0.07 (styrene) to 8.67% (9-octadecenoic acid). Most of the identified compounds have been reported to possess interesting biological activities. According to Calder, 2015, fatty acids, such as, oleic acid have human health benefits. In addition, 9-octadecenoic acid was known to have an antimicrobial activity (Muflihunna et al, 2021). In a recent study, styrene compound was identified in the volatile compounds composition of olive pomace (″alperujo″) (Cecchi et al, 2021).

Table 1. Yield, total phenolic (TPC) and total flavonoid (TFC) contents of various olive pomace extracts.

Samples	Yield (%)	TPC (mg GAE/ g dr)	TFC (mg QE/ g dr)
CYHA	3.4	0.81±0.2	0.50±0.1
EtOH	3.7	1.85±0.7	0.40±0.2
Aqueous	4.7	2.24±0.1	nd

CYHA: cyclohexane, EtOH: ethanol; nd: not detected.

Table 2. Volatile compounds tentatively identified in olive pomace

Sr. n°.	Compounds	RT (min)	Peak area percentage (%)	Class
1	Styrene	5.40	0.07	Derivative of benzene
2	2-propenoic acid	6.01	0.77	Fatty acid
3	Hexadecanoic acid	17.00	2.23	Fatty acid
4	9-octadecenoic acid	18.16	8.67	Fatty acid
5	Oleic acid	18.77	0.43	Fatty acid
6	2-Hexadecanol	19.02	1.18	Fatty alcohol

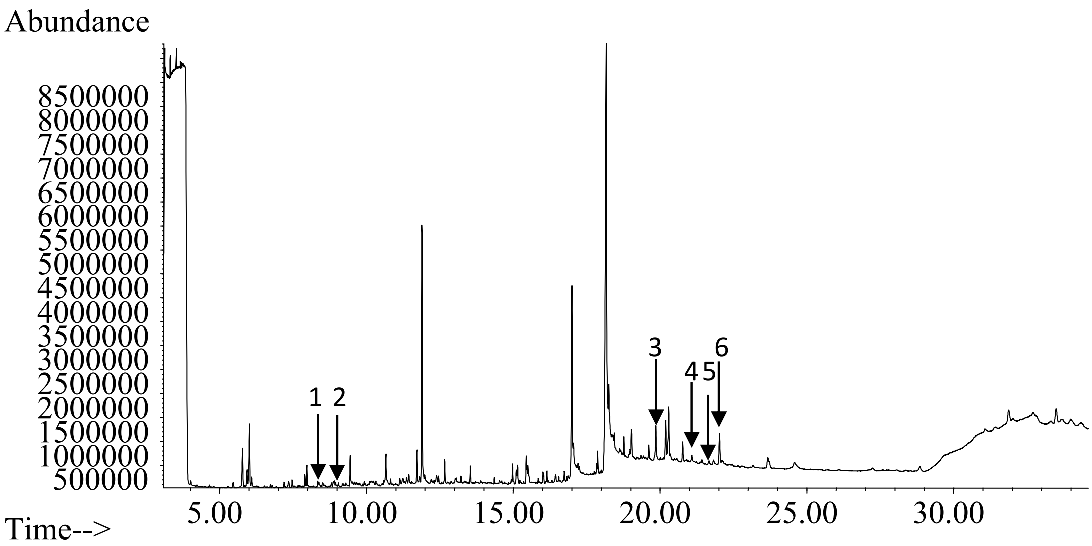

Fig. 1. GC-MS chromatograms of olive pomace extract. Peaks: (1) Styrene; (2) 2-propenoic acid; (3) Hexadecanoic acid; (4) 9-octadecenoic acid; (5) Oleic acid; (6) 2-Hexadecanol.

3.2.2. HPLC analysis

The phytochemical screening was established using HPLC-DAD to gain a deeper knowledge on the individual chemical compounds of the different olive pomace extracts. Phenolic compounds identified and quantified in the different extracts of pomace were presented in Table 3. A total of 4 phenolic compounds, which belong to phenolic acids and flavonoids classes, were detected in the pomace extracts. Among the identified compounds, salicylic acid was found in the three extracts (CYHA, EtOH, and aqueous), with respective concentrations of 0.22, 0.12, and 0.20 mg/g dr. In addition, chrysin compound was found in two extracts (CYHA and EtOH) with the highest concentrations of 0.38, and 0.37 mg/g dr respectively. The other two compounds (coumaric acid and rutin) were found only in CYHA extract with the same concentration of 0.32 mg/g dr. The previous work of Morsi et al. (2016), showed the presence of salicylic acid and rutin compounds in olive pomace. However, chrysin was usually detected in the olive oil (Gordon et al, 2001; Fernández-Calderón et al, 2020), and it is for the first time that can be identified in the olive pomace.

Table 3. Phenolic compounds identified (or tentatively identified) in the olive pomace

Sr. n°	RT (min)	Compounds	Extracts		
			CYHA	EtOH	Aqueous
Concentration (mg/g dr)					
1	24.62	Coumaric acid	0.32	nd	nd
2	38.48	Rutin	0.32	nd	nd
3	39.37	Chrysin	0.38	0.37	nd
4	44.49	Salicylic acid	0.22	0.12	0.20

RT: retention time; nd: not detected

3.3. Biological activities

3.3.1. Antioxidant activity

The antioxidant activity of olive pomace extracts was evaluated by the anti-radical DPPH test and compared to that of the synthetic antioxidant, Vit C (ascorbic acid). The inhibition percentages of the different extracts (CYHA, EtOH and aqueous) were illustrated in Fig.2. Statistically, there is a significant difference ($p \leq 0.05$) between the different extracts. The CYHA extract had the highest anti-free radical activity with 53.4% inhibition compared to the EtOH and aqueous extracts. The latter two showed low inhibition percentages of little more than 23% (Fig. 2). Some of the compounds found in the CYHA extract are known to have antioxidant activity. The latest study by Benbettaieb et al. (2018) showed the inhibitory effect of caffeine acid and coumaric acid on the free radical DPPH. These molecules have been considered as natural antioxidants. The statistical study showed that there is a high positive correlation between TFC and antioxidant activity (DPPH) with a *r value* = 0.73 (Table 5). The result gives an idea of the role played by phenolic compounds in the various extracts in neutralizing free radicals (Belaid et al, 2020).

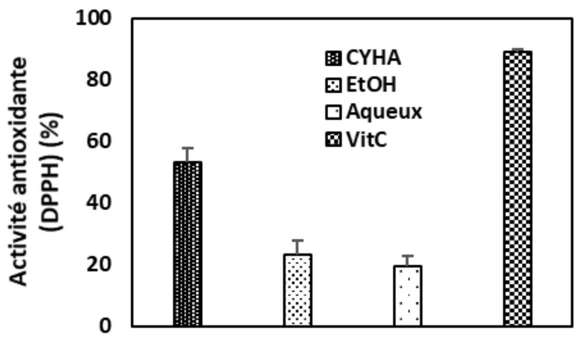

Fig. 2. Antioxidant activity of various extracts of olive pomace.

3.3.2. Antibacterial activity

The antimicrobial activity was performed against three pathogenic bacteria, including one Gram-negative (*Salmonella typhimuniu*) and two Gram-positive (*Listeria monocytogenes* and *Bacillus cereus*). In this study, the different extracts of olive pomace were evaluated for their antimicrobial activity using the disc diffusion method (Table 3). Only the aqueous extract showed good antibacterial activity against the two strains tested Gram-positive (13 mm inhibition for the *L. monocytogenes* strain and 10 mm inhibition for the *B. cereus* strain) (Table 4). However, the antimicrobial analysis carried out on the other 2 extracts of olive pomace (EtOH and CYHA) reveals a weak inhibitory effect against the development of these two strains tested, on the other hand they rendered without inhibitory effect on the Gram-negative strain (*S. typhimuniu*). Statistical analysis showed that there is a high positive correlation between the Gram-positive (*B. cereus*) strain and TPC, with a value equal to 0.97 (Table 5). The study of Jayalakshmi et al. (2014), confirms the idea that Gram-positive bacteria are more sensitive to Gram-negative, given the structural difference in their lipid layer.

Table 4. Antibacterial activity of different extracts of olive pomace.

	Inhibition diameter (mm)				
Bacterial strains	**CYHA**	**EtOH**	**Aqueous**	**Positif control (AB)**	**Negatif control (DMSO)**
Listeria monocytogenes	10	5	13	**11**	**-**
Salmonella typhimuniu	-	-	-	**12**	**-**
Bacillus cereus	-	6	10	**14.5**	**-**

CYHA: cyclohexane; EtOH: ethanol; AB: antibiotique; DMSO: dimethylsulfo oxyde.

Table 5 : Correlation matrix (Pearson (n))

Variables	TPC	TFC	DPPH	*B. cereus*	*L. monocytogenes*
TPC	1	-0.57	0.15	0.15	0.97
TFC	-0.57	1	0.73	-0.90	-0.33
DPPH	0.15	0.73	1	-0.96	0.40
B. cereus	0.15	-0.90	-0.96	1	-0.12
L. monocytogenes	0.97	-0.34	0.40	-0.12	1

3.4. Principal components analysis (PCA)

The PCA was carried out to better understand the relationship between the chemical composition (TPC and TFC) and the different biological activities (antioxidant and antibacterial) of olive pomace. The first two axes (F1 and F2) together accounted for 98.47% of the data variability. The major components (F1 and F2) accounted for 62.38 and 36.09% of the total variance of the data, respectively (Fig. 3). At the same time, the PCA figures express the extent to which the main components (F1 and F2) are correlated with the variables, as well as the correlations between different activities and chemical composition (TPC and TFC). The F1 axis showed a high positive correlation with antioxidant activity (DPPH) and TFC with (r = 0.85) and (r = 0.98), respectively (Table 6). Antibacterial activity (*B. cereus*) showed a negative correlation with TFC, with r-value equal to -0.90 (Table 5). In addition, the second axis (F2) was highly correlated with the bacterial strain (*L. monocytogenes*) and TPC by showing values equal to 0.99 and 0.92, respectively. If the two preceding figures (biplot) were to come together (Fig. 3), several observations and interpretations can be drawn; (i): extracts were located on the circle, based on their chemical composition and biological activities. (ii): The extracts of CYHA and EtOH, which contain the most TFC, were located close to the antioxidant activity (anti-DPPH). Thus, it is possible to suggest that polyphenolic compounds contained in aqueous extract contributes to bacterial strain inhibition (*L. monocytogenes*).

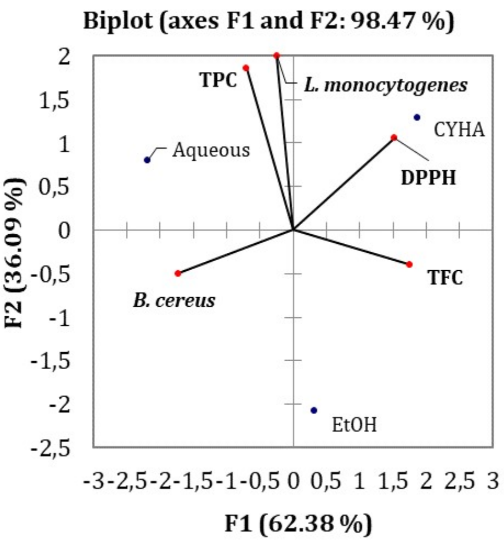

Fig. 3. Analysis of the chemical composition and antioxidant activity of various extracts of olive pomace in "biplot" components.

Table 6. Correlation between variables and axes

	F1	F2
TPC	-0.39	0.92
TFC	0.98	-0.20
DPPH	0.85	0.53
B. cereus	-0.97	-0.25
L. monocytogenes	-0.14	0.99

4. CONCLUSION

The agricultural and food industries generate appreciable quantities of by-products, which are for the most part little or no valued, and whose release into nature constitutes a great threat to the environment. As part of a development of these resources, olive pomace was the subject of a phytochemical and an evaluation of biological activities. The results found show that the olive have a moderate content of phenolic compounds. In addition, the various biological activities of the extracts have been linked in particular to its phenolic composition characterized by the presence of numerous phenolic acids and flavonoids which have been identified and quantified by quantified by HPLC-DAD. Moreover, several volatil compounds were identified in the cyclohexane extract by GC-MS. Olive pomace extracts contain molecules with antibacterial activity against Gram-positive bacteria. In addition, cyclohexane extract contains molecules with significant anti-radical activity.

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AUTHOR CONTRIBUTION STATEMENT

RR performed the sampling; manuscript writing and analyzed the data statistically. KEA helps in chromatographic analysis. AH and FBA performed the antibacterial analysis. SSA, MD, and SA conceived and validated the experiments, proofread the paper, and refined the manuscript to be ready for publication. All authors read and approved the final manuscript.

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