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Comprehensive Metabolite Fingerprinting of Australian Black and Green Olives and Their Antioxidant and Pharmacokinetics Properties

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Abstract: Polyphenols, especially flavonoids, are well-known for their bioactive antioxidant properties. Therefore, this study aimed to analyze Australian black (ripe) and green olives (unripe) for phenolic and non-phenolic metabolites, antioxidant activities, and pharmacokinetic properties. Liquid chromatography–mass spectrometry coupled with quadrupole–time of flight (LC–ESI–QTOF–MS/MS) was applied to elucidate the composition, identification, and characterization of bioactive metabolites from Australian olives. This study identified 110 metabolites, including phenolic acids, flavonoids, stilbenes, lignans, and other compounds (phenolic terpenes, tyrosols, fatty acids, and terpenoids). Luteolin (flavonoid) and verbascoside (hydroxycinnamic acid) are identified with higher concentrations in black olives. Black (ripe) olives were measured at a higher TPC (10.94 ± 0.42 mg GAE/g) and total antioxidant potential than green olives. The pharmacokinetic properties (absorption, distribution, metabolism, excretion, toxicity) of phenolic compounds for human health were evaluated to predict the potential of the most abundant metabolites in olives. Gastrointestinal absorption and Caco-2 cell permeability of metabolites in olives were also predicted. This study will develop into further research to identify the Australian olives' therapeutic, nutraceutical, and phytopharmaceutical potential.

Keywords: olives; antioxidants; melatonin; polyphenols; flavonoids; anthocyanins; bioavailability; LC–MS/MS

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1. Introduction

Olives (*Olea europaea*) are widely recognized as a rich source of phenolic compounds, which are known to possess a wide range of biological activities, including anti-oxidant, anti-inflammatory, anti-cancer, anti-bacterial, anti-diabetic, anti-aging, and cardioprotective effects [1]. Olives are a popular food worldwide, with a long consumption history dating back to ancient times [2]. Black and green olives are a type of fruit from the Oleaceae family, widely cultivated for culinary uses and nutritional benefits. Olives contain various nutrients, including healthy monounsaturated fats, fiber, vitamins, minerals, and antioxidants [3]. The antioxidants in olives, such as phenolic compounds and vitamin E, are believed to contribute to their anti-inflammatory and disease-fighting properties. In addition to their nutritional value, olives are a versatile ingredient in many Mediterranean and Middle Eastern dishes [4]. Black and green olives are different types of olives with distinct characteristics and uses. Black olives are ripe olives allowed to mature on the tree before being harvested. They are typically softer and milder in flavor compared to green olives. Black olives are often used in Mediterranean dishes such as pizza, salads, and sauces. They can also be used as a garnish or added to charcuterie boards. On the other hand, green olives are picked before they are fully ripe and are treated with lye or brine to remove their bitter taste. They have a firmer texture and a more pungent flavor than

black olives. Green olives are stuffed with pimentos, garlic, or cheese, and are often used in cocktails, such as martinis. Both black and green olives are rich in healthy fats, fiber, and anti-oxidants, making them a healthy and flavorful addition to any diet [5].

In recent years, there has been growing interest in the health benefits of olives and their phenolic compounds. Phenolic compounds are a diverse group of secondary plant metabolites. A significant category of secondary metabolites derived from plants are phenolic compounds, which typically exist in conjugated configuration with mono- and polysaccharides bound to more than one phenolic group [6]. Phenolic compounds may also be derivatives, such as esters and methyl esters. In this study, we used six in vitro antioxidant assays to assess the antioxidant capacity of black and green olives to evaluate the antioxidant potential of the olive extracts. We also identified the major phenolic compounds using high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). We measured Total Phenolic Content, Total Flavonoid Content, and Total Tannin Content. Using various in vitro techniques, the anti-oxidant activity associated with these polyphenols can be measured by scavenging free radicals or prolonging their generation, including the ferric reducing anti-oxidant power (FRAP) assay, the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation de-colorization assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) anti-oxidant assay, Ferrous ion chelating assay (FICA), and Hydroxyl Radical Scavenging Activity ($^{\bullet}$OH-RSA) assay. Phenolic extracts were further analysed using liquid chromatography coupled with electrospray-ionization triple quadruple time-of-flight mass spectrometry LC–ESI–QTOF–MS/MS to identify and characterize 96 compounds from black and green olives. They comprised phenolic acids (27), lignans (8), flavonoids (45), stilbene (3), and other compounds (27). This supports the theory that phenolic compounds in black and green olives can contribute to anti-oxidant activities [7].

The findings of this study shed light on the phenolic composition and antioxidant capacity of black and green olives, highlighting their potential as a valuable source of food-based ingredients for industrial applications. These results can aid in developing new functional foods, nutraceuticals, and pharmaceuticals that harness the beneficial compounds present in olives. The study emphasizes the importance of understanding the unique properties of black and green olives and their potential contribution to the food and health industries.

2. Materials and Methods

2.1. Reagents and Chemicals

Bioactives were extracted and characterized using analytical and LC-MS grade chemicals. Sigma-Aldrich (Castle Hill, NSW, Australia) provided most of the chemicals utilized for extraction and characterization. Gallic acid, Folin–Ciocalteu's phenol reagent, L-ascorbic acid, sodium phosphate, vanillin, hexahydrate aluminium chloride, iron (III) chloride hexahydrate (Fe[III]$Cl_3 \cdot 6H_2O$), sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, trichloroacetic acid, hydrochloric acid, ethylenediaminetetraacetic acid (EDTA), chloride, iron (III), ferrozine, iron (II), ammonium molybdate, sulphate heptahydrate, chloride, 3-hydroxybenzoic acid, catechin, iron (II) sulphate heptahydrate, potassium ferrocyanide (III), DPPH, 2,4,6 tripyridyl-s-triazine (TPTZ), and are all components of the Folin–Ciocalteu's phenol reagent and those used to measure the amount of polyphenols and antioxidant potential, ABTS, were acquired from Sigma Aldrich (Castle Hill, NSW, Australia). From Chem-Supply Pty Ltd. (Adelaide, SA, Australia), we bought sodium carbonate anhydrous and hydrogen peroxide (30%), and we purchased 98% H_2SO_4 from RCI Labscan (Rongmuang, Thailand). Thermo Fisher Scientific Inc. supplied methanol, ethanol, acetonitrile, formic acid, glacial acetic acid, and iron (III) chloride anhydrous, as HPLC and LC-MS grade chemicals (Scoresby, VIC, Australia). Thermo Fisher Scientific provided 96-well plates for various in vitro bioactivity and antioxidant tests (Scoresby, VIC, Australia). HPLC vials (1 mL) were also acquired from Agilent equipment (Melbourne, VIC, Australia).

2.2. Preparation and Extraction of Phenolic Compounds

Australian-grown olives (ripe and unripe) were purchased from a local market in Melbourne, Australia. Then, olives were washed, pitted to remove the seeds, and cut into small pieces with a knife on the same day and kept at room temperature for two days to dry. Then, they were dried in an oven at 45 ± 5 °C for four days and ground/crushed with a grinder. Three-gram ground/crushed samples were taken in triplicate in 50 mL falcon tubes and 30 mL 80% acidified methanol with 1% formic acid added and mixed with ultra-turrax for 2 min at 10,000 rpm to homogenize the samples. After that, these samples were placed in an orbital shaker (Labwit, ZWYR-240 incubator shaker, Ashwood, VIC, Australia) for 16 h at 10 °C and 150 rpm. After the period, ultrasound was used to facilitate the extraction of bioactives as described in our previous study [8]. The samples were centrifuged (Hettich Chilled Centrifuge, ROTINA380R, Tuttlingen, Baden-Württemberg, Germany) at 8000 rpm for 20 min. The supernatant was filtered with a 0.45 µm syringe filter and stored at 20 °C for LC-MS/MS and in vitro antioxidant assays.

2.3. Measurement of Phenolic Contents and Antioxidant Activities of Olives

The measurements of phenolic contents and antioxidant experiments were performed in triplicate according to the techniques of Ali et al. [9,10]. The potential of each plant extract was determined using 96-well plates. The absorbance was calculated with a Multiskan Go microplate photometer (Thermo Scientific, Waltham, MA, USA) to create standard curves against various concentrations of standards.

The DPPH free radical scavenging activity of all trials was calculated following the Sharifi-Rad et al. [11] method. The ferric-reducing activity of all trials were determined by following the process developed by Chou et al. [12]. The ABTS radical scavenging capability was assessed using the method of Zahid et al. [13]. The •OH-RSA was determined using the Fenton-type reaction technique of Bashmil et al. [14] with some modifications. The methods of Ali et al. [9] were used to measure the Fe^{2+} chelating activity of all samples. A detailed description of each method is given in the Supplementary Material.

2.4. LC–ESI–QTOF–MS/MS Characterization of Metabolites in Olives

The approaches of Ali et al. [10,15] were used to identify and characterize polyphenols from sample extracts. The phenolic compounds were characterized using an Agilent 6520 Accurate Mass Q-TOF LC-MS and an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA). The parting was carried out using a Synergi Hydro-RP 80 Å reverse phase column (250 mm 4.6 mm, 4 m particle size) and a guard column of endangered C18 ODS (4.0 2.0 mm) (Phenomenex, Lane Cove, NSW, Australia). The mobile phase comprised water/formic acid (99.9:0.1, *v*/*v*; eluent A) and acetonitrile/formic acid (99.9:0.1 *v*/*v*; eluent B). The gradient summary was as follows: 10–20% B (0–10 min), 20–25% B (10–20 min), 25–30% B (20–25 min), 30–45% B (25–35 min), 45–80% B (35–50 min), 90–100% B (50–55 min), 100% B (55–57 min), 100–10% B (57–58 min), and 10% B (58–60 min). A 6 µL aliquot was injected for each extract, and the normal flow rate was 0.6 mL/min. Peaks were observed when the capillary and nozzle voltages were set to 3.5 kV in both the negative and positive ion modes, at 500 V correspondingly. Furthermore, the following parameters were sustained: (i) 9 L/min sheath gas flow rate at 325 °C, (ii) 325 °C temperature of nitrogen gas, and (iii) 45 psi nitrogen gas nebulization. MS/MS studies were accomplished in automated mode with collision energy (10, 20, and 40 eV) for disintegration using a whole mass scan fluctuating from *m*/*z* 50 to 1500. Peak identification was made positively and negatively, with the LC–ESI–QTOF–MS/MS MassHunter workstation software utilized for instrument control, data acquisition, and processing (Qualitative Analysis, 152 versions B.06.01, Agilent Technologies, Santa Clara, CA, USA).

2.5. Pharmacokinetic Properties of Metabolites in Olives

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were calculated by following the method described by Ali et al. [16].

2.6. Data Analysis

Data from the phenolic content assays and antioxidant assays were expressed as means and standard deviations. MetaboAnalyst (5.0) was used for heatmap analysis while RStudio was used for Venn diagram analysis.

3. Results and Discussion

3.1. Measurement of Phenolic Content and Antioxidant Activities

The secondary bioactive metabolites, phenolic acids, and flavonoids are significant and have various positive health effects [17]. These substances are considered multifunctional as metal chelators, free radical scavengers, hydrogen atom donors, and reducing agents [16]. Six in vitro antioxidant assays were used in the current study to assess the antioxidant capacity of black and green olives (Table 1).

Table 1. Measurement of antioxidant activities and Phenolic Content.

Variables	Black Olives	Green Olives
TPC (mg GAE/g)	10.94 ± 0.42	7.58 ± 0.35
TFC (mg QE/g)	4.94 ± 0.34	3.13 ± 0.57
TCT (mg CE/g)	1.11 ± 0.09	0.61 ± 0.03
DPPH (mg AAE/g)	19.35 ± 2.83	10.71 ± 0.36
FRAP (mg AAE/g)	2.63 ± 0.26	3.97 ± 0.61
ABTS (mg AAE/g)	10.35 ± 1.54	9.55 ± 0.45
FICA (µg EDTA/g)	1240 ± 31.09	750 ± 17.45
$^{\bullet}$OH-RSA (mg AAE/g)	17.46 ± 0.67	9.62 ± 0.46

According to the above table, given values are mean ± S.D per gram dried weight where the number of samples per sample is three (n = 3). Total phenolic content (TPC); total flavonoid content (TFC); total condensed tannins (TCT); AAE (ascorbic acid equivalents); CE (catechin equivalents); EDTA (ethylenediaminetetraacetic acid); GAE (gallic acid equivalents); QE (quercetin equivalents); 2,2-azio-bis-3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS); (2,2-diphenyl-1-picrylhydrazyl assay (DPPH); ferrous ion chelating activity (FICA); ferric reducing anti-oxidant power (FRAP); hydroxyl-radical scavenging activity ($^{\bullet}$OH-RSA).

Black and green olives are known for their high content of polyphenols, which can be determined through various measures such as TPC, TFC, and TTC. In the present study, the TPC and TFC values of black olives were significantly higher than those of green olives, at 10.94 ± 0.42 mg GAE/g and 4.94 ± 0.34 mg CE/g, respectively. On the other hand, green olives had a lower TCT (0.61 ± 0.03 mg GAE/g) than black olives (1.11 ± 0.09 mg GAE/g). It is worth noting that variations in TPC may be influenced by several factors such as time, temperature, solvent-to-sample ratio, cultivar, solvent concentration, and geographical location [14]. Additionally, the procedure used to calculate the TPC may also impact on how phenolics are estimated. These TPC results align with earlier studies' descriptions, which claimed that phenolic compounds can be found in significant amounts in olive and olive leaves [18–20]. The TFC compared favourably to the Data formally provided by Ons Rekik and his co-authors [21].

The DPPH and the ABTS, the two most common in vitro antioxidant tests, assess the plant extracts' capacity for total antioxidant by determining whether they can scavenge the body's free radicals or give hydrogen to them. Black olives had the higher ABTS (10.35 ± 1.54 mg AAE/g) and DPPH (19.35 ± 0.83 mg AAE/g) values, whereas green olives had the lower ABTS (9.55 ± 0.45 mg AAE/g) and DPPH (10.71 ± 0.36 mg AAE/g) values. This suggests that black olives have more antioxidant potential than green olives. The findings of Bouaziz et al. [22] are similar to our results. The samples that were taken when the olives had turned black (2.69–10.96 g/mL) had smaller IC_{50} values, which suggested stronger antioxidant capacity. Green fruit extracts (olives), on the other hand, showed greater IC_{50} values and lesser antioxidant potential (31.87–180.95 g/mL). The ABTS and DPPH activities were also reported to be higher compared to Australian finger lime, mountain pepper berries, and tamarind [23]. This is most likely because of the strong radical inhibition brought through an elevated level of total phenols and flavonoids, par-

ticularly ortho-hydroxylated phenolics such as hydroxytyrosol, quercetin, and luteolin at the mature stage. The data in the literature on the DPPH and the ABTS assays demonstrate that the two are occasionally not closely associated. Due to the involvement of two distinct action processes and two distinct radicals, they frequently do not yield the same consequences [24,25].

The ferrous ion chelating assay (FICA) was used to assess the ability of native Australian fruits and herbs to chelate metals. Black olives showed the highest FICA value (1240 ± 80.0 mg EDTA/g) compared to green olives, with a value of 750 ± 70.0 mg EDTA/g. Additionally the greatest value of OH-RSA (17.46± 0.67 mg AAE/g) was found in black olives as compared to green olives (9.62 ± 0.46 mg AAE/g), which is crucial for inhibiting lipid peroxidation for stopping oxidized metal ions from transitioning [26]. The $^{\bullet}$OH-RSA assay is a crucial method for understanding the mechanism of lipid peroxidation, as it helps to scavenge free $^{\bullet}$OH radicals that cause DNA damage. A surplus of various reactive oxygen species, for instance, hydrogen peroxide (H_2O_2), superoxide radicals ($^{\bullet}O_2^-$), and hydroxy radicals ($^{\bullet}$OH) can lead to multiple pathologies. Therefore, it is essential to include anti-oxidant bioactives as a regular part of the diet to counteract many pathological disorders [27].

Furthermore, green olives had higher FRAP values (3.97 ± 0.61 mg AAE/g) compared to black olives, with FRAP values of 2.63 ± 0.26 mg AAE/g. The FRAP assay measures a sample's total antioxidant capacity by evaluating its ability to reduce Fe^{3+} to Fe^{2+}. These results suggest that black olives have higher metal chelating and hydroxyl radical scavenging activities, while green olives have higher reduced power.

3.2. LC-MS/MS Screening and Characterization of Phenolic Compounds

Human health has long benefited from the usage of natural foods. Because fruits contain a variety of phenolics as well as non-phenolic bioactive metabolites, their use as preventive and therapeutic supplements in nutraceuticals has expanded. The extracts were further analysed using LC–ESI–QTOF–MS/MS to support the theory that phytochemicals in black and green olives potentially contribute to antioxidant activities (Figures S1 and S2). A total of 110 metabolites were identified in Black and green olives (Table 2).

Table 2. LC–ESI–QTOF–MS/MS screening of Phytochemicals in Australian-grown Black and Green Olives.

No.	Proposed Compounds	Molecular Formula	RT (min)	Mode of Ionization	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Mass Error (ppm)	MS/MS Product Ions	Samples
	Phenolic acids								
	Hydroxybenzoic acids								
1	3-*O*-Galloylquinic acid	$C_{14}H_{16}O_{10}$	4.339	$[M + H]^+$	345.0817	345.0826	2.6	327, 283, 153, 125	BO, GO
2	3-*O*-Methylgallic acid	$C_8H_8O_5$	6.738	$[M + H]^+$	185.0445	185.0456	5.9	167, 141, 123	GO, BO
3	Protocatechuic acid	$C_7H_6O_4$	12.067	$[M - H]^-$	153.0188	153.0170	−1.8	109	BO
4	Benzoic acid	$C_7H_6O_2$	16.298	$[M + H]^+$	123.0446	123.0449	2.4	105	BO, GO
5	*p*-Hydroxybenzoic acid	$C_7H_6O_3$	17.529	$[M - H]^-$	137.0239	137.0238	−0.7	93	GO, BO
6	Syringic acid	$C_9H_{10}O_5$	22.488	$[M - H]^-$	197.0450	197.0455	1.6	182, 163, 153	BO, GO
7	Ellagic acid	$C_{14}H_6O_8$	28.754	$[M - H]^-$	300.9985	300.9951	−5.3	284	BO
8	Paeoniflorin	$C_{23}H_{28}O_{11}$	33.967	$[M - H]^-$	479.1554	479.1565	2.3	461, 317	GO, BO
	Hydroxycinnamic acids								
9	Feruloyl tartaric acid	$C_{14}H_{14}O_9$	4.339	$[M + H]^+$	327.0711	327.0690	−6.4	309, 177, 163, 151	GO
10	Feruloyl glucose	$C_{16}H_{20}O_9$	4.488	$[M + H]^+$	357.1180	357.1175	−1.4	195, 177, 149	GO, BO
11	3-*p*-Coumaroylquinic acid	$C_{16}H_{18}O_8$	4.676	$[M + H]^+$	339.1080	339.1087	2.1	191, 119	BO
12	3-Sinapoylquinic acid	$C_{18}H_{22}O_{10}$	4.676	$[M + H]^+$	399.1291	399.1293	0.5	223, 191	BO
13	Sinapic acid	$C_{11}H_{12}O_5$	10.234	$[M - H]^-$	223.0612	223.0618	2.7	193, 179, 149, 134	BO, GO
14	Dihydroferulic acid	$C_{10}H_{12}O_4$	13.816	$[M - H]^-$	195.0658	195.0656	−1.0	177, 151, 135	BO, GO
15	Caffeic acid 4-*O*-glucoside	$C_{15}H_{18}O_9$	14.236	$[M - H]^-$	341.0873	341.0859	−4.1	179, 135	GO
16	3-Caffeoylquinic acid	$C_{16}H_{18}O_9$	15.157	$[M - H]^-$	353.0873	353.0875	0.6	191, 179, 161, 135	BO, GO
17	*p*-Coumaric acid 4-*O*-glucoside	$C_{15}H_{18}O_8$	16.453	$[M - H]^-$	325.0924	325.0925	0.3	163	GO, BO

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Mode of Ionization	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Mass Error (ppm)	MS/MS Product Ions	Samples
18	Caffeic acid	$C_9H_8O_4$	18.326	$[M-H]^-$	179.0345	179.0343	−1.1	135	GO, BO
19	Cinnamic acid	$C_9H_8O_2$	19.374	$[M+H]^+$	149.0602	149.0600	−1.3	105	BO, GO
20	1,2-Disinapoylgentiobiose	$C_{34}H_{42}O_{19}$	20.274	$[M-H]^-$	753.2242	753.2244	0.3	529, 223, 205	GO
21	1-Sinapoyl-2-feruloylgentiobiose	$C_{33}H_{40}O_{18}$	20.527	$[M-H]^-$	723.2137	723.2103	−4.7	529, 499	GO, BO
22	Verbascoside	$C_{29}H_{36}O_{15}$	20.632	$[M-H]^-$	623.1976	623.1974	−0.3	461, 161, 133	BO, GO
23	3,5-Diferuloylquinic acid	$C_{27}H_{28}O_{12}$	21.675	$[M-H]^-$	543.1503	543.1496	−1.3	193, 191, 134	GO
24	Hydroxycaffeic acid	$C_9H_8O_5$	23.442	$[M-H]^-$	195.0294	195.0305	5.6	177, 151	BO
25	Ferulic acid	$C_{10}H_{10}O_4$	26.363	$[M-H]^-$	193.0501	193.0510	4.7	178, 149, 134	GO, BO
26	Caffeic acid 3-*O*-glucuronide	$C_{15}H_{16}O_{10}$	29.084	$[M-H]^-$	355.0666	355.0687	5.9	179	BO, GO
27	*p*-Coumaric acid	$C_9H_8O_3$	29.725	$[M-H]^-$	163.0395	163.0395	0.0	119	BO, GO
	Flavonoids								
	Anthocyanins								
28	Petunidin 3-rhamnoside	$C_{22}H_{23}O_{10}$	9.674	$[M]^+$	447.1291	447.1287	−0.9	317	BO
29	Delphinidin	$C_{15}H_{11}O_7$	20.848	$[M]^+$	303.0505	303.0516	3.6	303	BO
30	Cyanidin	$C_{15}H_{11}O_6$	21.076	$[M]^+$	287.0556	287.0576	7.0	287	BO
31	Cyanidin 3-glucoside	$C_{21}H_{21}O_{11}$	21.662	$[M]^+$	449.1084	449.1121	8.2	287	BO
32	Cyanidin 3-rutinoside	$C_{27}H_{31}O_{15}$	22.092	$[M]^+$	595.1663	595.1657	−0.9	287	BO
	Flavanols								
33	(+)-Gallocatechin	$C_{15}H_{14}O_7$	14.021	$[M-H]^-$	305.0662	305.0671	3.0	139	BO, GO
34	(+)-Catechin 3-*O*-gallate	$C_{22}H_{18}O_{10}$	16.159	$[M-H]^-$	441.0822	441.0844	5.0	289, 245	GO
35	Prodelphinidin dimer B3	$C_{30}H_{26}O_{14}$	24.791	$[M-H]^-$	609.1245	609.1273	4.6	591, 539	BO
36	(−)-Epicatechin	$C_{15}H_{14}O_6$	25.878	$[M-H]^-$	289.0712	289.0727	5.2	245, 205	BO, GO
	Flavonols and dihydroflavonols								
37	3,7-Dimethylquercetin	$C_{17}H_{14}O_7$	16.655	$[M-H]^-$	329.0662	329.0673	3.3	301, 283, 165, 121	GO
38	Quercetin 3-rutinoside (Rutin)	$C_{27}H_{30}O_{16}$	20.779	$[M-H]^-$	609.1456	609.1459	0.5	301	BO
39	Myricetin 3-*O*-rhamnoside	$C_{21}H_{20}O_{12}$	21.652	$[M-H]^-$	463.0877	463.0900	5.0	317	BO
40	Isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside	$C_{28}H_{32}O_{16}$	21.675	$[M-H]^-$	623.1612	623.1593	−3.0	315	GO, BO
41	Quercetin 3-*O*-arabinoside	$C_{20}H_{18}O_{11}$	22.876	$[M-H]^-$	433.0771	433.0773	0.5	301	BO, GO
42	Dihydroquercetin (Taxifolin)	$C_{15}H_{12}O_7$	23.076	$[M-H]^-$	303.0505	303.0517	4.0	217, 125	BO
43	Dihydromyricetin 3-*O*-rhamnoside	$C_{21}H_{22}O_{12}$	24.626	$[M-H]^-$	465.1033	465.1035	0.4	319, 301, 151	BO
44	Myricetin 3-*O*-rutinoside	$C_{27}H_{30}O_{17}$	26.545	$[M-H]^-$	625.1405	625.1434	4.6	317	GO
45	Quercetin	$C_{15}H_{10}O_7$	32.243	$[M-H]^-$	301.0348	301.0355	2.3	271, 179, 151, 121	BO
46	Isorhamnetin	$C_{16}H_{12}O_7$	32.321	$[M-H]^-$	315.0505	315.0500	−1.6	300, 151, 107	BO
47	Kaempferol 7-*O*-glucoside	$C_{21}H_{19}O_{11}$	53.800	$[M-H]^-$	446.0849	446.0854	1.1	285	GO
	Flavones								
48	Apigenin 6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	20.999	$[M-H]^-$	593.1507	593.1509	0.3	269	BO, GO
49	Quercetin-3-rhamnoside (Quercitrin)	$C_{21}H_{20}O_{11}$	21.604	$[M-H]^-$	447.0928	447.0932	0.9	301	BO, GO
50	6-Hydroxyluteolin	$C_{15}H_{10}O_7$	23.726	* $[M-H]^-$	301.0348	301.0350	0.7	283, 257, 175	BO
51	Apigenin 6-C-glucoside	$C_{21}H_{20}O_{10}$	23.340	$[M-H]^-$	431.0978	431.0981	0.7	269	GO, BO
52	Luteolin	$C_{15}H_{10}O_6$	24.651	$[M-H]^-$	285.0399	285.0397	−0.7	267, 151, 109	GO, BO
53	Nobiletin	$C_{21}H_{22}O_8$	27.296	$[M+H]^+$	403.1393	403.1404	2.7	373, 343, 211	BO
54	Apigenin 7-*O*-glucoside	$C_{21}H_{24}O_9$	28.452	$[M-H]^-$	419.1342	419.1327	−3.6	269	GO
	Flavanones								
55	8-Prenylnaringenin	$C_{20}H_{20}O_5$	18.368	$[M+H]^+$	341.1384	341.1392	2.3	323, 221, 147	GO
56	Neoeriocitrin	$C_{27}H_{32}O_{15}$	24.067	$[M-H]^-$	595.1663	595.1643	−3.4	459, 287, 151	BO
57	Naringin	$C_{27}H_{32}O_{14}$	25.739	$[M-H]^-$	579.1714	579.1730	2.8	271	GO, BO
58	Eriodictyol	$C_{15}H_{12}O_6$	28.080	$[M-H]-$	287.0556	287.0563	2.4	269, 151	BO
59	Naringenin	$C_{15}H_{12}O_5$	30.167	$[M-H]^-$	271.0607	271.0601	−2.2	253, 229, 151	BO, GO
60	Hesperidin	$C_{28}H_{34}O_{15}$	33.307	$[M-H]^-$	609.1820	609.1828	1.3	301	GO
61	Liquiritigenin	$C_{15}H_{12}O_4$	37.582	$[M-H]^-$	255.0658	255.0648	−3.9	237, 135, 93	BO
62	6-Geranylnaringenin	$C_{25}H_{28}O_5$	45.281	$[M-H]^-$	407.1859	407.1851	−2.0	389, 287, 271	BO
	Isoflavonoids								
63	Irilone	$C_{16}H_{10}O_6$	4.887	$[M+H]^+$	299.0550	299.0544	−2.0	299	GO
64	Glycitin	$C_{22}H_{22}O_{10}$	9.674	$[M+H]^+$	447.1291	447.1286	−1.1	447	BO, GO
65	2′-Hydroxyformononetin	$C_{16}H_{12}O_5$	11.048	$[M+H]^+$	285.0763	285.0762	−0.4	277, 229	BO
66	Daidzin 4′-*O*-glucuronide	$C_{27}H_{28}O_{15}$	20.192	$[M-H]^-$	591.1350	591.1375	4.2	429, 253	BO
67	Dihydroformononetin	$C_{16}H_{14}O_4$	23.855	$[M+H]^+$	271.0970	271.0958	−4.4	271	BO
68	Puerarin 4′-*O*-glucoside	$C_{27}H_{30}O_{14}$	25.313	$[M-H]^-$	577.1558	577.1560	0.3	415	GO
69	Phloretin	$C_{15}H_{14}O_5$	29.324	$[M-H]^-$	273.0763	273.0763	0.0	255, 167, 147, 125, 119	BO
70	Sativanone	$C_{17}H_{16}O_5$	31.200	$[M-H]^-$	299.0920	299.0924	1.3	283, 255, 163	BO, GO
71	3′-Hydroxymelanettin	$C_{16}H_{12}O_6$	32.039	$[M-H]^-$	299.0556	299.0554	−0.7	284	BO, GO
72	Violanone	$C_{17}H_{16}O_6$	34.200	$[M-H]^-$	315.0869	315.0864	−1.6	297, 163	BO, GO
	Stilbenes								
73	3′-Hydroxy-3,4,5,4′-tetramethoxystilbene	$C_{17}H_{18}O_5$	30.043	$[M-H]^-$	301.1076	301.1070	−2.0	283, 255	BO, GO
74	Resveratrol 3-*O*-glucoside (Polydatin)	$C_{20}H_{22}O_8$	32.392	$[M-H]^-$	389.1237	389.1237	0.0	227	BO, GO
75	4′-Hydroxy-3,4,5-trimethoxystilbene	$C_{17}H_{18}O_4$	33.113	$[M-H]^-$	285.1127	285.1118	−3.2	269, 253, 241, 211	BO, GO
	Lignans								
76	Secoisolariciresinol	$C_{20}H_{26}O_6$	24.726	$[M-H]^-$	361.1651	361.1653	0.6	343, 331, 315, 165	BO, GO
77	7-Hydroxymatairesinol	$C_{20}H_{22}O_7$	24.825	$[M-H]^-$	373.1288	373.1281	−1.9	343, 313, 298, 285	GO
78	Lariciresinol	$C_{20}H_{24}O_6$	26.023	$[M+H]^+$	361.1651	361.1658	1.9	343, 237, 137	BO, GO
79	Matairesinol	$C_{20}H_{22}O_6$	30.607	$[M+H]^+$	359.1494	359.1498	1.1	341, 299, 235, 137	BO, GO

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Mode of Ionization	Theoretical (*m*/*z*)	Observed (*m*/*z*)	Mass Error (ppm)	MS/MS Product Ions	Samples
80	Syringaresinol	$C_{22}H_{26}O_8$	30.607	$[M + H]^+$	419.1706	419.1708	0.5	419	BO, GO
81	Schisantherin A	$C_{30}H_{32}O_9$	30.665	$[M - H]^-$	535.1968	535.1969	0.2	535	GO, BO
82	Medioresinol	$C_{21}H_{24}O_7$	31.405	$[M + H]^+$	389.1600	389.1601	0.3	389	BO, GO
83	Schisandrol B	$C_{23}H_{28}O_7$	35.575	$[M + H]^+$	417.1913	417.1916	0.7	399, 383, 369	BO
	Other compounds								
84	Quinic Acid	$C_7H_{12}O_6$	4.218	$[M - H]^-$	191.0556	191.0566	5.2	127, 93	GO, BO
85	Melatonin	$C_{13}H_{16}N_2O_2$	4.281	$[M + H]^+$	233.1284	233.1284	0.0	185, 152, 93	BO, GO
86	Hydroxytyrosol 4-*O*-glucoside	$C_{14}H_{20}O_8$	4.898	$[M - H]^-$	315.1080	315.1081	0.3	153	GO, BO
87	Pyrogallol	$C_6H_6O_3$	8.893	$[M + H]^+$	127.0395	127.0399	3.1	107, 97	BO, GO
88	Hydroxytyrosol	$C_8H_{10}O_3$	10.964	$[M - H]^-$	153.0552	153.0551	0.3	123	BO, GO
89	4-Ethylguaiacol	$C_9H_{12}O_2$	13.816	* $[M - H]^-$	151.0759	151.076	0.7	135, 119, 107	BO, GO
90	Oleoside 11-methylester	$C_{17}H_{24}O_{11}$	14.554	$[M - H]^-$	403.1241	403.1244	0.7	223, 165	BO, GO
91	Tyrosol	$C_8H_{10}O_2$	16.262	$[M - H]^-$	137.0603	137.0605	1.5	119, 107, 93	BO, GO
92	1,3,5-Trimethoxybenzene	$C_9H_{12}O_3$	17.593	$[M - H]^-$	167.0708	167.0711	1.8	137, 121, 111	BO, GO
93	Esculetin	$C_9H_6O_4$	17.873	$[M - H]^-$	177.0188	177.0197	5.1	133	GO, BO
94	*p*-Anisaldehyde	$C_8H_8O_2$	18.326	$[M - H]^-$	135.0446	135.0447	0.7	119	GO, BO
95	Umbelliferone	$C_9H_6O_3$	20.622	* $[M - H]^-$	161.0239	161.0242	1.9	133, 117	GO, BO
96	Ligstroside	$C_{25}H_{32}O_{12}$	21.295	$[M - H]^-$	523.1816	523.1816	0	505, 361, 319, 277	BO
97	Urolithin B	$C_{13}H_8O_3$	21.798	$[M - H]^-$	211.0395	211.0398	1.4	183, 167	BO, GO
98	*p*-HPEA-AC	$C_{10}H_{12}O_3$	22.831	$[M + H]^+$	181.0864	181.0859	−2.8	163, 139, 121	BO, GO
99	Oleuropein	$C_{25}H_{32}O_{13}$	23.922	$[M - H]^-$	539.1765	539.1750	−2.8	521, 377	BO, GO
100	Coumarin	$C_9H_6O_2$	24.189	$[M + H]^+$	147.0446	147.0450	2.7	119, 103	BO, GO
101	3,4-DHPEA-EDA	$C_{17}H_{20}O_6$	24.626	$[M - H]^-$	319.1182	319.1185	0.9	275, 195	BO
102	Salvianolic acid G	$C_{20}H_{18}O_{10}$	24.651	$[M - H]^-$	417.0822	417.0820	−0.5	399, 373, 237, 219	GO
103	Mellein	$C_{10}H_{10}O_3$	30.515	$[M - H]^-$	177.0552	177.0557	2.8	133, 105	BO, GO
104	Acetyl eugenol	$C_{12}H_{14}O_3$	35.444	$[M + H]^+$	207.1021	207.1021	0.0	189, 174, 109	BO, GO
105	Ricinoleic acid	$C_{18}H_{34}O_3$	48.542	$[M - H]^-$	297.2430	297.2439	4.9	279, 185	BO, GO
106	Corosolic acid	$C_{30}H_{48}O_4$	49.217	$[M - H]^-$	471.3475	471.3475	0.0	453, 427	BO, GO
107	Carvacrol	$C_{10}H_{14}O$	49.840	$[M - H]^-$	149.0967	149.0967	0.0	133, 105	GO, BO
108	Kaurenoic acid	$C_{20}H_{30}O_2$	50.365	$[M - H]^-$	301.2168	301.2175	2.3	283, 255	BO
109	Oleic acid	$C_{18}H_{34}O_2$	56.431	$[M - H]^-$	281.2481	281.2491	3.3	263, 237, 59	BO, GO
110	Linoleic acid	$C_{18}H_{32}O_2$	58.572	$[M - H]^-$	279.2324	279.2329	1.6	221, 204, 131, 127	BO, GO

Green olives (GO), Black olives (BO), retention time (RT). * = compounds were identified in both modes (positive and negative).

Black and green olives were analysed qualitatively using untargeted LC–ESI–QTOF–MS/MS in the ($[M - H]^-$/$[M + H]^+$) positive and negative ionization modes. Depending on the respective *m*/*z* value and MS/MS spectra in both ionization modes, the phenolic compounds from both samples were identified using Agilent's LC-MS Qualitative Software and Personal Compound Database & Library (PCDL). The mass error of just under ten ppm was used as a benchmark to select additional MS/MS characterization, identification, and compound verification. As shown in Table 2, 110 compounds from black and green olives were discovered and described in this study using LC–ESI–QTOF–MS/MS. They comprise phenolic acids (27), lignans (8), flavonoids (45), stilbene (3), and other compounds (27).

3.2.1. Phenolic Acids

With a carboxyl group, phenolic compounds fall under the category of phenolic acids. Phenolic acid's antioxidant, antibacterial, anticancer, anti-inflammatory, and cardiovascular properties have been thoroughly investigated. Olives, both black and green, contain phenolic acid species. The two phenolic acid subclasses, hydroxybenzoic acids (8) and hydroxycinnamic acids, were found in this investigation (19). Hydroxybenzoic and hydroxycinnamic acids are subclasses of phenolic acids found in olives and have been extensively studied for their health benefits. These compounds act as antioxidants, reducing oxidative stress and cellular damage caused by free radicals. They also possess anti-inflammatory properties that can help prevent chronic inflammation associated with numerous diseases, including cancer, diabetes, and heart disease. Additionally, hydroxybenzoic acids and hydroxycinnamic acids have been found to have cardiovascular benefits by lowering blood pressure and may have anticancer and anti-diabetic properties. Moreover, their antimicrobial properties suggest potential use in preventing and treating infections.

Hydroxybenzoic Acids

In this present study, compounds 1 to 8 were identified in both black and green olives, and are considered to fall under hydroxybenzoic acid derivatives in negative and positive modes of ionization ($[M-H]^-$/$[M+H]^+$). Compound 1, with $[M+H]^+$ at m/z 345.0826, was putatively characterized as 3-*O*-galloylquinic acid. Alkhalidy et al. [28] found that it can boost the synthesis of adiponectin, a hormone involved in controlling blood sugar levels and fatty acid breakdown, which suggests a potential role in managing obesity and diabetes [28]. Another study found that galloyl quinic acid can inhibit the growth of human colon cancer cells and may have potential as an anti-cancer agent [29].

Compound 2, with $[M+H]^+$ at m/z 185.0456, was putatively identified in black and green olives as 3-*O*-methylgallic acid. Compound 3 was designated as protocatechuic acid in black olives, with the molecular formula $C_7H_6O_4$ and a precursor ion at m/z 153.0170 in the negative ionization mode [30]. Protocatechuic acid has been shown to have neuroprotective effects and can protect against oxidative-stress-induced neuronal damage. It has been suggested that this compound may have the potential as a therapeutic agent for neurodegenerative diseases such as Alzheimer's and Parkinson's disease [31]. Compound 6, with $[M-H]^-$ at m/z 137.0238, was characterized as *p*-hydroxybenzoic acid in black and green olives. Previously, protocatechuic acid 4-*O*-glucoside and p-hydroxybenzoic acid have already been identified in different species of olives [27].

Compound 4 was detected in black and green olives, with $[M+H]^+$ at m/z 123.0449, and was tentatively characterized as benzoic acid. Benzoic acid has been shown to have anti-tumour effects and can inhibit the growth of certain cancer cells, including colon and breast cancer cells [32]. According to a study by Panzella [33], benzoic acid can improve skin hydration and reduce the appearance of wrinkles [33]. Two compounds (compounds 6 and 7) were detected in black and green olives in negative ionization modes and tentatively assigned to ellagic acid and Paeoniflorin, with $[M-H]^-$ at m/z 300.9951 and 479.1565, respectively. A study by Aishwarya et al. [34] found that ellagic acid reduces fat accumulation in the liver, a key factor in developing fatty liver disease. Another study found that ellagic acid protects skin cells from damage caused by UV radiation, which can contribute to skin aging and skin cancer [34]. Paeoniflorin has been found to have anxiolytic and antidepressant effects in animal studies. It may work by modulating the activity of neurotransmitters in the brain [35].

Hydroxycinnamic Acids

In the present work, we characterized 19 hydroxycinnamic acids, among which, Compounds 10, 13, 14, 16, 17, 18, 19, 21, 22, 25, 26, and 27 were found in both black and green olives. Among these 19 compounds, five were identified in positive ionization modes, and the remaining 14 were characterized in negative ionization modes. Compound 9, with $[M+H]^+$ at m/z 327.0690, was tentatively characterized as feruloyl tartaric acid and found only in green olives. At the same time, two more (compound 11 and 12) were detected in black olives and identified to be 3-*p*-coumaroylquinic acid and 3-sinapoylquinic acid with $[M+H]^+$ at m/z 339.1087 and 399.1293, respectively. However, two compounds (compounds 10 and 13) were identified in black and green olives in positive and negative modes of ionization and designated as feruloyl glucose and sinapic acid, respectively. This sinapic acid possesses anti-oxidant, anti-inflammatory, and anti-cancer properties and provides skin and cardiovascular health [36].

Compounds 15 and 17 produced fragments at m/z 179 and 163 after the loss of [M-H-162] from the parent ions. m/z 179 and m/z 163 are the characteristic mass of caffeic acid and *p*-coumaric acid, while m/z 162 represents the glycosal moiety. Therefore, compounds 15 and 17 could be identified as caffeic acid 4-*O*-glucoside and *p*-coumaric 4-*O*-glucosde. Previously, these compounds were also identified in lemon aspen, strawberry gum, and lemongrass [16,37]. Compound 18 at m/z 179.0343 generated a product ion at m/z 135 after the loss of carbon dioxide unit (44 Da). Compound 18 was identified as caffeic acid. Caffeic acid, like sinapic acid, has been found to possess antioxidant,

anti-inflammatory, anti-cancer, anti-viral, and anti-diabetic properties. Additionally, caffeic acid also demonstrates neuroprotective properties, which can help to prevent or slow the progression of neurological diseases and may also benefit digestive health by promoting the growth of beneficial gut bacteria [38]. Compound 19, showing its $[M + H]^+$ at m/z 149.0600, was identified as Cinnamic acid in black and green olives. Cinnamic acid offers a range of beneficial properties, including anti-cancer, anti-microbial, anti-inflammatory, and antioxidant properties. Additionally, it possesses unique properties, such as anti-hypertensive and anti-thrombotic properties, which can help reduce blood pressure and prevent blood clotting, respectively. Furthermore, it has anti-allergic properties that can alleviate allergy symptoms and anti-ulcer properties that can protect against stomach ulcers by promoting healing and reducing inflammation [39]. Verbascoside (compound 22) is one of the most abundant phenolic compounds in black olives. Compound 25 was identified as ferulic acid. Ferulic acid possesses various properties, including anti-inflammatory, anti-diabetic, anti-cancer, anti-aging, cardiovascular health, and liver health benefits, as well as immune system support by increasing the production and function of white blood cells, which play an important role in fighting off infections and diseases [40].

3.2.2. Flavonoids

Several dietary plant items contain a category of phenolic chemicals known as flavonoids. Due to their anti-oxidant and anti-inflammatory properties, as well as their capacity to modify some enzyme processes, flavonoids have gained attention [41]. Based on the constitution and form of both B and C rings, flavonoids are further split into subgroups [42]. Seven flavonoid subclasses of chemicals were found in this assay, which include anthocyanins (5), flavanols (4), flavonols and dihydro flavonols (11), flavones (7), flavanones (8), and isoflavonoids (11).

Flavanols

Based on MS/MS data, we identified seven flavanols. Compounds 33 and 36 in black and green olives had $[M - H]^-$ at m/z 305.0671 and 289.0727 and were tentatively characterized as gallocatechin and epicatechin, respectively. Both gallocatechin and epicatechin offer a variety of health benefits. These compounds have been shown to provide cardiovascular health benefits, improve blood sugar regulation, support cognitive function, promote skin health, exhibit anti-cancer properties, and even provide immune system support [43]. In addition, compound 34, with the chemical formula $C_{22}H_{18}O_{10}$, showed $[M - H]^-$ at m/z 441.0822 and was putatively identified as (+)-catechin 3-*O*-gallate. Compound 35 was only detected in black olives, with a precursor ion at a negative ionization mode at m/z 609.1273, tentatively representing the prodelphinidin dimer B3. The prodelphinidin dimer B3 has several unique health benefits, such as anti-inflammatory, anti-diabetic, anti-cancer, and neuroprotective properties, and is beneficial for skin and cardiovascular health [44].

Flavonols and Dihydroflavonols

In this work, eleven flavonols and dihydroflavonols were detected in negative ionization mode only, including compounds 37, 44, and 47, which were only detected in green olives showing at m/z 329.0673, 625.1434, 446.0854, respectively. They were tentatively characterized as 3,7-dimethylquercetin, myricetin 3-*O*-rutinoside, and kaempferol 7-*O*-glucoside, respectively. Compounds 40 and 41 were putatively identified in black and green olives with negative ionization mode at m/z 623.1593 and 433.0773. They were designated as isorhamnetin 3-*O*-glucoside 7-*O*-rhamnoside and quercetin 3-*O*-arabinoside, respectively. In black olives, compounds 38, 39, 42, 43, 44, and 46 were observed with $[M - H]^-$ at m/z 609.1459, 463.0900, 303.0517, 465.1035, 301.0355, and 315.0500, respectively. They were tentatively identified to be kaempferol 3,7-*O*-glucoside, myricetin 3-*O*-rhamnoside, dihydroquercetin, dihydromyricetin 3-*O*-rhamnoside, quercetin, and isorhamnetin, respectively.

Flavones

Seven flavones were identified in olives. Among these, compound 53 was detected in black olives with $[M + H]^+$ at m/z 403.1404 and was tentatively identified as nobiletin. Nobiletin has various health benefits, including anti-allergic, anti-viral, anti-diabetic, and anti-microbial properties, as well as anti-angiogenic properties, which can inhibit the formation of new blood vessels [45]. This is particularly relevant to cancer, as tumours require new blood vessels to grow and spread. In addition, in negative ionization modes, Compounds 48, 49, 51, and 52 were found in both black and green olives with $[M - H]^-$ at m/z 593.1509, 447.0932, 431.0981, and 285.0397, respectively. They were tentatively assigned as apigenin 6,8-di-C-glucoside, quercitrin, apigenin 6-C-glucoside, and luteolin, respectively. Luteolin is the most abundant flavonoid in olives. Compound 54 was tentatively identified in green olives with $[M - H]^-$ at m/z 419.1327, and was designated as Apigenin 7-*O*-glucoside. In various studies, apigenin 7-*O*-glucoside has been found to exhibit unique properties such as anti-obesity, anti-atherosclerotic, anti-neuroinflammatory, anti-oxidant, anti-inflammatory, and anti-diabetic effects [46]. Compound 50, with the chemical formula $C_{15}H_{10}O_7$, was found in black olives showing $[M - H]^-$ at m/z 301.0350 and was tentatively identified as 6-hydroxyluteolin. Research suggests that 6-hydroxyluteolin may have several health benefits, including anti-inflammatory, anti-cancer, anti-diabetic, anti-viral, and anti-bacterial properties. Additionally, it has been studied for its potential to protect against neurodegenerative diseases such as Alzheimer's and Parkinson's disease [47].

Flavanones

Seven flavanones (compound 55 to compound 62) were putatively identified in black and green olives (Table 2). Compound 55 was detected at ESI^+ m/z 341.1392 in green olives and was designated as 8-Prenylnaringenin. 8-Prenylnaringenin has some unique properties, such as estrogenic activity, which means it can bind to estrogen receptors in the body and potentially benefit menopausal symptoms and bone health. It also has been shown to have neuroprotective, anti-inflammatory, and anti-oxidant effects, as well as potential benefits for cardiovascular health and cancer prevention [48]. Compounds 49 and 52 were tentatively identified in negative ionization mode in black olives at ESI^- m/z 300.0654 and characterized as Neoeriocitrin and 6-Geranylnaringenin, respectively. Compounds 56, 58, 61, and 62 were only identified in black olives and were named as neoeriocitrin, eriodictyol, liquiritigenin, and 6-geranylnaringenin, respectively. Compound 57, with the molecular formula $C_{27}H_{32}O_{14}$ and $[M - H]^-$ at m/z 579.1714, was tentatively identified as naringin in black and green olives. Naringin provides several potential health benefits. These benefits include its antioxidant and anti-inflammatory properties and ability to regulate cholesterol and blood sugar levels. Additionally, naringin may offer immune system support [49]. Previously, naringin was also identified in lemongrass and wattle seeds [37]. Compound 61 was detected at ESI^- m/z 407.1851 in black olives and tentatively characterized as hesperidin. In addition to health benefits such as anti-cancer, anti-inflammatory, cardiovascular, and skin health effects, Hesperidin has also been found to have neuroprotective effects. Studies have shown that it may improve cognitive function, memory, and learning ability and protect against neurodegenerative diseases such as Alzheimer's and Parkinson's. Hesperidin may also have anti-anxiety and anti-depressant effects by regulating neurotransmitters in the brain [50].

3.2.3. Isoflavonoids

Thirteen isoflavonoids (compound 63 to compound 72) were also detected in olives. Compound 63 was putatively characterized at ESI^+ m/z 299.0544 in green olives and tentatively characterized as Irilone. Irilone is a natural compound found in plants with promising potential for various health benefits. It has potent anti-oxidant and anti-inflammatory activities and can protect the brain and nervous system, potentially reduce the growth of cancer cells, and has anti-microbial effects [51]. Compound 68 was characterized at ESI^- m/z 477.1286 in green olives in negative ionization mode and was designated puerarin

4′-*O*-glucoside. Compounds 65, 66, 67, and 69 were only detected in the black olives at m/z 285.0762, 591.1375, 271.0985, and 273.0763, respectively, and were putatively characterized as 2′-hydroxyformononetin, daidzin 4′-*O*-glucuronide, dihydroformononetin, and phloretin, respectively. 2′-Hydroxyformononetin is well-known for reducing oxidative stress and inflammation, improving bone health and density, and potentially reducing the risk of certain cancers. It may also benefit cardiovascular health, including lowering blood pressure and improving cholesterol levels [52]. Dihydroformononetin has been found to have anti-diabetic effects, including improving insulin sensitivity, and reducing blood sugar levels. It may also benefit weight loss and reduce inflammation in the body. Additionally, it may have anti-cancer properties, particularly in reducing the growth of cancer cells in the breast and colon. The compounds 64, 70, 71, and 72, with the molecular formulae $C_{22}H_{22}O_{10}$, $C_{17}H_{16}O_5$, $C_{16}H_{12}O_6$, and $C_{17}H_{16}O_6$, respectively, were identified in both black and green olives and putatively characterized as glycitin, sativanone, 3′-hydroxymelanettin, and violanone, respectively. Compound 64, with $[M + H]^+$ at m/z 447.1291, was designated as glycitin. Glycitin has been associated with various health benefits. It has potent antioxidant and anti-inflammatory properties, can improve bone density, reduce the risk of heart disease, and potentially have anti-cancer effects. Its unique health benefits include neuroprotective effects, anti-allergic properties, skin health benefits, anti-obesity effects, and anti-aging properties [41].

3.2.4. Stilbenes and Lignans

Three stilbenes (compounds 73, 74, and 75) and eight lignans (compounds 76 to 83) were detected in this work. Compounds 73, 75, and 74) were detected in negative ionization mode at ESI^- m/z 301.1070, 285.1118, and 389.1237, respectively, and were designated as 3′-hydroxy-3,4,5,4′-tetramethoxystilbene, 4′-hydroxy-3,4,5-trimethoxystilbene, and polydatin, respectively. Compound 77 was putatively characterized at ESI^- m/z 373.1281 as 7-hydroxymatairesinol, and was found only in green olives. On the other hand, compound 83 was only detected in black olives with $[M + H]^+$ at m/z 417.1916, and was tentatively characterized as schisandrol B. Schisandrol B has promising potential for various health benefits. It has potent anti-oxidant and anti-inflammatory properties, can protect the brain and nervous system, potentially reduce the growth of cancer cells, and has protective effects on the liver [53]. Compounds 76 and 81 were putatively identified in black and green olives at ESI^- m/z 361.1653 and 535.1969, and were characterized as secoisolariciresinol and schisantherin A, respectively. Four more compounds (78, 79, 80, and 82) with $[M + H]^+$ at m/z 361.1658, 359.1498, 419.1708, and 389.1601 were detected in both black and green olives and designated as lariciresinol, matairesinol, syringaresinol, and medioresinol, respectively. Medioresinol is a natural compound found in certain plant foods that has potential health benefits, including anti-oxidant and anti-inflammatory properties, cardiovascular health improvement, and digestive health benefits [54].

3.2.5. Other Compounds

A total of 27 other compounds were found in the samples. Melatonin (Compound 85) was identified in black and green olives. Melatonin is well-known due to its health properties. Compound 88 (hydroxytyrosol) was identified in black and green olives at ESI^- m/z 153.0551, and was further confirmed through MS/MS spectra which generated a characteristic product ion at m/z 123 (Figure 1). Tyrosols and oleuropein are natural compounds reported in olive oil and olive leaves, and have potential health benefits. Tyrosols have been shown to have antioxidant and anti-inflammatory properties, which may help to reduce the risk of chronic diseases such as cancer and cardiovascular disease. They may also have a beneficial effect on cognitive function and mood. Oleuropein has been found to have antimicrobial properties, which may help to boost the immune system and protect against infections. It may also have anti-inflammatory and anti-oxidant effects, and a potential role in reducing blood pressure and improving cardiovascular health [55,56]. When it comes to preventing various oxidative-stress-related disorders, including cardiovascular, cancer, and

neurological disorders, polyphenols and chemical substances obtained from plant-based meals play a crucial role [57].

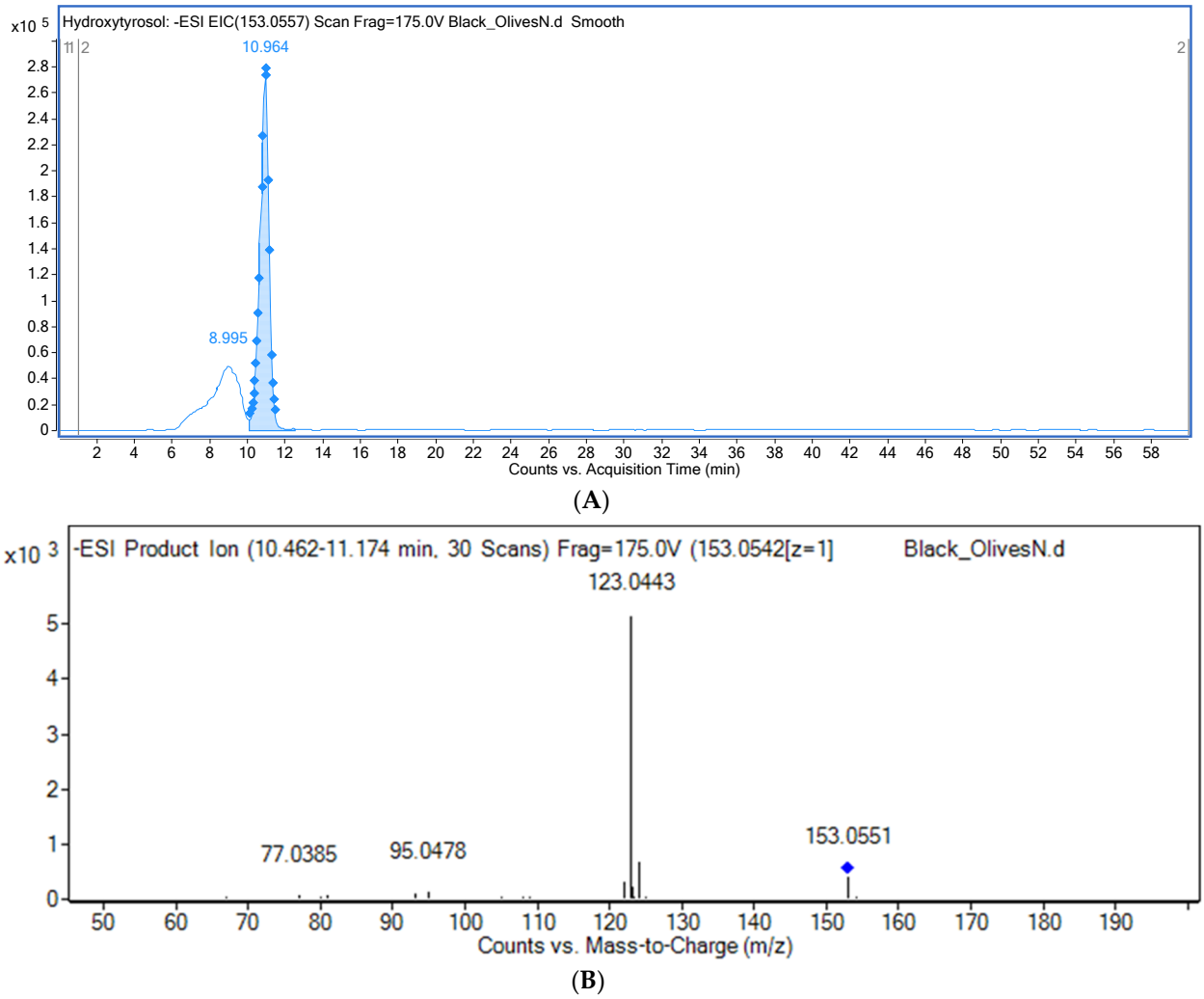

Figure 1. LC-MS/MS identification of hydroxytyrosol (compound 88). Chromatogram (**A**) and MS/MS spectra (**B**) of hydroxytyrosol at m/z 153.0551 are given in negative ionization mode in black olives.

3.3. Pharmacokinetics (ADMET) Properties

3.3.1. Oral Bioavailability

The oral bioavailability of the compounds was predicted using the bioavailability radar. It was obtained using the method of Ali et al. [15]. The results of radar bioavailability are given in Table S1 and Figure 2.

Figure 2 predicts that only melatonin (a) and hydroxytyrosol (b) in olives have predicted oral bioavailability. No other compound predicted oral bioavailability. It is interesting to discuss that most phytochemicals do not have oral bioavailability. They could be bioavailable in the gastrointestinal part of the body.

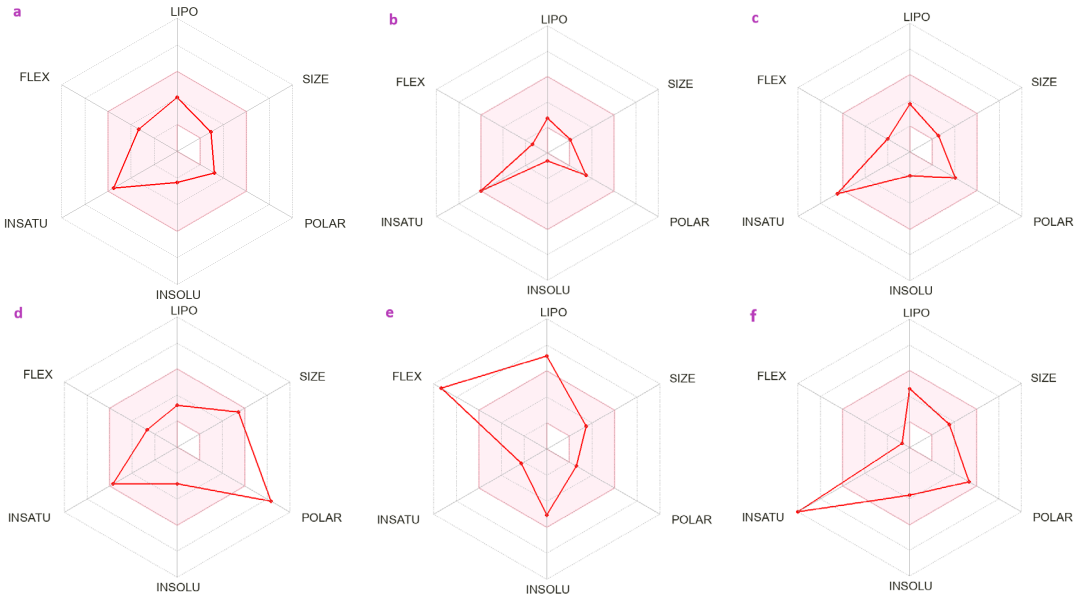

Figure 2. Radar bioavailability of melatonin (**a**), hydroxytyrosol (**b**), syringic acid (**c**), cyanidin 3-*O*-glucoside (**d**), oleic acid (**e**), and luteolin (**f**). The pink area of the bioavailability radar represents the optimal range for each property.

3.3.2. Absorption and Distribution

The absorption and distribution of phytochemical metabolites were evaluated using the BOILED-Egg method and the pkCSM platform. The results are given in Tables S2 and S3 and Figure 3.

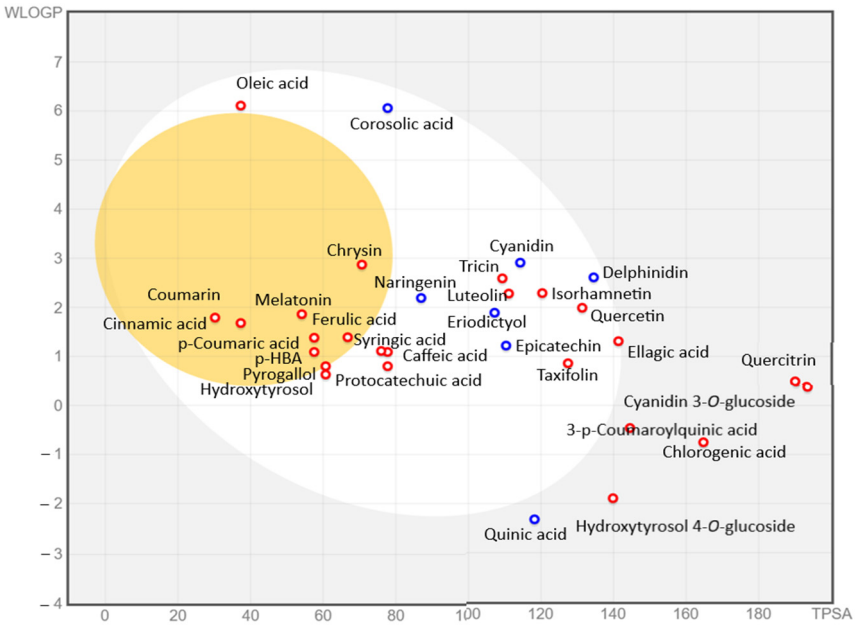

Figure 3. The BOILED-egg method was used to evaluate the absorption of metabolites. Blue dots indicate molecules predicted to be expelled from the CNS by P-glycoprotein, and the red dots indicate molecules predicted not to be expelled from the CNS by P-glycoprotein. The egg yolk area predicts the phenolic metabolites that passively penetrate the blood–brain barrier. The egg white area predicts which phenolic compounds will be absorbed through the gastrointestinal tract.

It is shown in Figure 3 that taxifolin, gallic acid, hydroxytyrosol, pyrogallol, protocatechuic acid, caffeic acid, syringic acid, tricin, eriodictyol, cyanidin, delphinidin, epicatechin, luteolin, naringenin, corosolic acid, ellagic acid, and quercetin pass into the egg white, which predicts the absorption of these metabolites into the gastrointestinal tract. Furthermore, melatonin, cinnamic acid, coumarin, p-hydroxybenzoic acid (*p*-HBA), cinnamic acid, *p*-coumaric acid, chrysin, and linoleic acid pass the blood–brain barrier (Tables S2 and S3). Melatonin found in olives has significant importance for human health. It is efficient at modulating oxidative stress, inflammatory markers, control of hypertension, neurodegenerative diseases, and metabolic syndrome. Previously, we reported melatonin in coffee arabica from different origins [8]. Cinnamic acid (94.83%), ellagic acid (86.68%), ferulic acid (93.69%), p-coumaric acid (93.49%), p-hydroxybenzoic acid (83.96), cyanidin (87%), delphinidin (77%), chrysin (93.76%), luteolin (81%), coumarin (97%), pyrogallol (83%), corosolic acid (100%), tricin (90%), naringenin (91%), eriodictyol (75%), linoleic acid (92%), melatonin (94%), and oleic acid (92%) are predicted to have the highest human gastrointestinal absorption. At the same time, cinnamic acid (1.72), *p*-coumaric acid (1.21), *p*-hydroxybenzoic acid (1.15), Chrysin (0.95), taxifolin (0.92), coumarin (1.65), pyrogallol (1.12), naringenin (1.03), linoleic acid (1.57), melatonin (1.22), hydroxytyrosol (1.10), and oleic acid (1.56) are predicted with the highest Caco-2 permeability (Table S3). If the predicted value of any metabolite is more than 0.90, then that compound is considered to have a higher Caco-2 permeability. It is worth noting that the metabolites with smaller molecular weight have higher absorption, either in the human intestinal system or in Caco-2 cells. On the other hand, the big molecules could be beneficial to the gastrointestinal tract and to modulate the gut microbiota in the colon [16].

3.3.3. Metabolism, Excretion, and Toxicity

Cytochrome P450 is a vital protein that plays a role in the metabolism of drug molecules. The calculated metabolism and excretion of metabolites from olives are presented in Table S4. The CYP model is used to predict the metabolism of metabolites as a substrate or inhibitor of the proteins CYP1A2, CYP3A4, CYP2D6, CYP2C9, and CYP2C19. The inhibition of these proteins by some compounds could elevate the concentration of other drug molecules, which results in the higher toxicity of elevated compounds, vice versa. The metabolites with higher total clearance (Table S4) have a higher bioavailability and metabolism in the liver. The predicted toxicity results are presented in Table S5, which indicates that most of the metabolites in olives do not have toxicity. For example, hydroxytyrosol 4-*O*-glucoside and hydroxytyrosol have predicted AMES toxicity, while corosolic acid and linoleic acid have predicted hepatotoxicity, while oleic acid did not predict any toxicity. Confirming the safety of plant extracts is the first step in drug discovery [58]. Chlorogenic acid, 3-*p*-coumaroylquinic acid, Pyrogallol, Naringenin, Linoleic acid, Eriodictyol, and Chrysin are predicted to have the lowest maximum tolerated doses in humans, which potentially indicates that these metabolites are required in minute quantities. Very minute quantities of the predicted metabolites with toxicity in olives do not adversely affect human health, or the quantities of these metabolites could be reduced during processing. Oleic acid is the main essential fatty acid in olives and has the highest gastrointestinal absorption. Overall, the metabolites in olives have great importance for human health.

4. Conclusions

The results of this study indicate that black olives (ripe) have considerably more potent antioxidant activity than green olives (unripe), possibly because of their comparatively great diversity of phenolic chemicals. This could also be due to the presence of anthocyanins and condensed tannins. A total of 110 metabolites were putatively identified in Australian-grown olives; many of them have not previously been reported in olives. Moreover, the pharmacokinetic properties (absorption, metabolism, distribution, excretion, toxicity), oral and gastrointestinal bioavailability, and blood–brain barrier permeability could be of great

interest to understand their potential on human health. This study will be helpful for further in vivo research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10060354/s1, Figure S1: Base peak chromatograms (BPC) of black and green olives in positive (black) and negative (blue) modes; Figure S2: Chromatograms and MS/MS spectra of some selected compounds; Table S1: Predicted absorption and distribution of selected compounds.; Table S2: Pharmacokinetic properties of selected compounds; Table S3: Radar bioavailability properties of selected compounds; Table S4: Predicted metabolism and excretion of selected compounds; Table S5: Predicted toxicity of abundant phenolic compounds.

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