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Biological quality and phytochemical profiling of olive fruits using gas chromatography–mass spectrometry (GCMS) analysis

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Abstract

The quality of olive fruit (OF) is widely affected due to geographical variation, affecting OF's chemical composition and biological properties. It is a novel and first-time study to evaluate the quality variation of 42 olive samples from different geographical origins based on phytochemical profile and their biological activities. The study reports the presence of unique chemical markers responsible for the difference in quality and biological activity of the olive samples. Biological activity (cytotoxic and antimicrobial) with GCMS phytochemical profile was evaluated. GCMS analysis confirmed the presence of 111 volatile compounds from various chemical classes with range (%) and average (%): esters (21.61–60.49) and 44.62, alcohols (20.73–49.2) and 38.06, hydrocarbons (3–38.88) and 15.39, ketones (0.16–3.87) and 0.75, acids (0.07–2.62) and 0.27, and aldehydes (0.12–1.47) and 0.45. The predominant ester was 13-methyl-pentadecanoic acid methyl ester, a differentiation marker between these samples. Cytotoxicity assay showed a significant inhibitory effect against MCF7 (8–64%) and HCT116 (0.11–44%) cell lines, whereas the extracts with the highest cytotoxicity observed were O17 (52.00 ± 2.00) and O25 (64.00 ± 4.88). The antimicrobial activity exhibited a range of zones of inhibition (mm) against *P. aeruginosa* (0.00–17.00), *E. coli* (0.00–15.00), *S. aureus* (0.00–13), and resistant *S. aureus,* i.e., MRSA (0.00–12.00). The extracts with the highest antimicrobial activity, i.e., O8 and O39 had identical MIC and MBC of 12.5 and 25 µg/ml against *P. aeruginosa*. In contrast, an MIC (50 and 25) and MBC (100 and 50) against *E. coli* were determined for O39 and O8. The statistical PCA and K-mean cluster analysis ($P < 0.05$) confirmed the presence of a high number of esters, alcohols, and hydrocarbons in GCMS data. Moreover, O8, O23, O25, and O39 were suggested as comparatively better varieties than those OF samples ($P = 0.001$). The presence of distinct volatile markers in these 42 OF samples may be further studied as a potential source of antimicrobials, food preservatives and therapeutic purposes.

Keywords Olive fruit, GCMS, Cytotoxicity, Antimicrobial, Geographical origin

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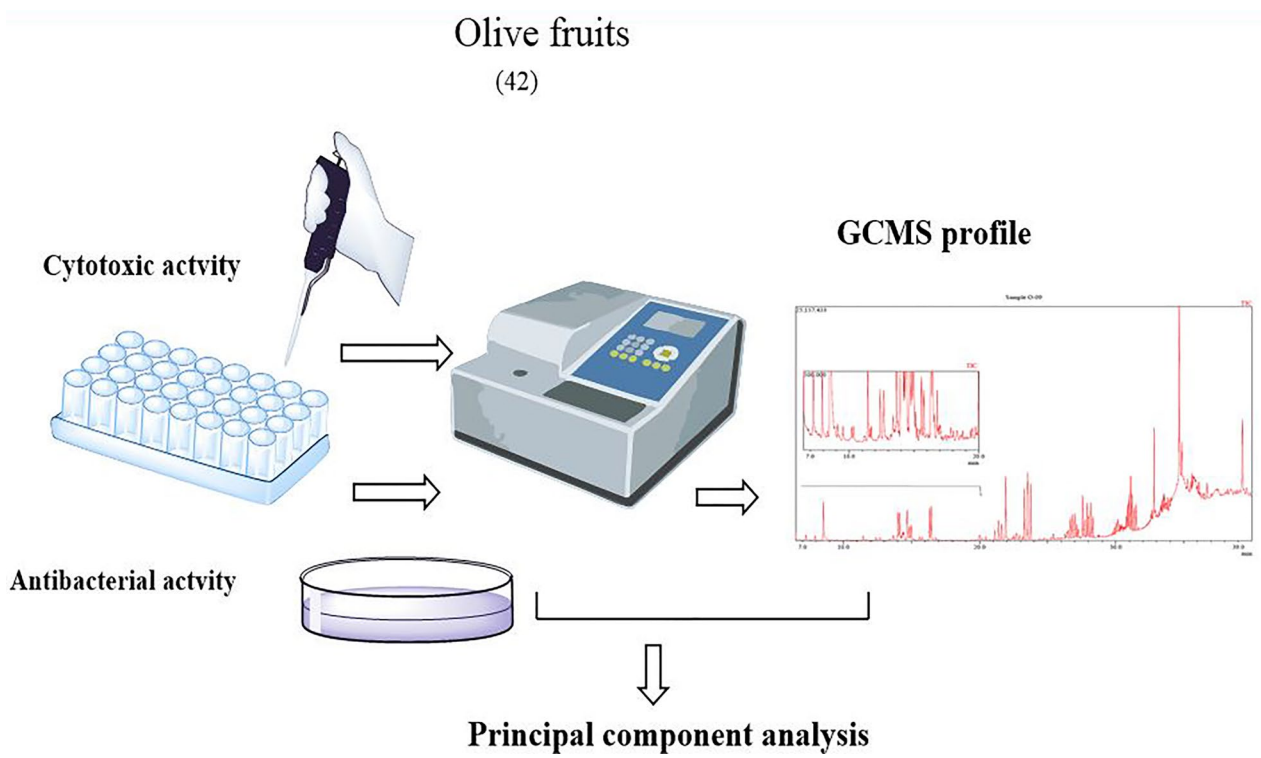
Graphical abstract



Background

Olea europaea L. is a small tree belonging to the family Oleaceae, commonly known as olive in English and Zaitoon in Arabic. *O. europaea* produces edible fruits, the main source of olive oil; olive fruit and its oil have a long history of nutritional and medicinal values. Olive oil is the major dietary fat of the traditional healthy "Mediterranean diet", which has been strongly associated with reduced prevalence of cardiovascular diseases and certain types of cancer [38]. The tree has been cultivated for centuries in tropical and warm temperate regions, particularly in the Mediterranean. Spain, Italy, and Tunisia produce over 50% of the global production of olives, with a production volume of approximately 12.5 million metric tons in 2020 [22]. The quality and chemical composition of olive fruit and oil are affected by several factors, including agricultural practices, cultivars, genetics, and seasonal and environmental factors [19]. Moreover, the storage and transporting conditions could significantly impact olive products' quality, taste, and health benefits. The health uses of olive fruit and leaves have been reported to include respiratory tract, urinary tract infections, and GIT diseases, while the oil is applied to the scalp to prevent hair loss and to fracture limbs [11, 28, 36]. Apart from folk use, the fruit and leaves have recently been reported for anti-oxidant effects with potential skin benefits [15].

The health benefits of olive oil, as well as its unique flavor and taste, are principally attributed to the occurrence of high amounts of monounsaturated fatty acids (MUFAs). These MUFAs are oleic acid (18:1) and palmitoleic acid (16:1) and functional bio-actives, including phenolics, tocopherols, phospholipids, and carotenoids with multiple biological properties [23]. The biological properties of olive oil and its components have been suggested due to its anti-oxidant, anti-cancerous, and antimicrobial activities and the modulation of gene functions [40]. Olive leaf and seed extracts have shown considerable activity against bacteria, fungi, and viruses in several in vitro and in vivo studies [12]. Virgin olive oil has been reported to reduce the count of inoculated *Salmonella enteritidis* and *Listeria monocytogenes* by approximately 1000 CFU/g in salad and mayonnaises, suggesting a strong protective effect against foodborne pathogens [37].

Similarly, olive oil has shown strong bactericidal activity against several *Helicobacter pylori* strains, including three antibiotic-resistant strains [42]. Olive oil affects cancer development through biological roles [40]. The constituents of olive oil, e.g., oleic acid and another

hydrocarbon known as squalene, are essential in inhibiting cancerous cell growth [49]. The phenolic constituents, including oleuropein and hydroxytyrosol, have exhibited cancer cell inhibition effects in several studies and strong anti-oxidant activity [49]. The cytotoxic effect of olive oil constituents has been demonstrated against different cell lines, including breast, prostate, and colorectal cancerous cell lines [40]. The most popular tool to determine the amount of phytochemical profile of samples is gas chromatography–mass spectrometry (GC–MS). This analytical tool has been widely employed to study the fatty acid composition of olive oil for quality assessment and authentication purposes. Variations between olive oil from different origins have been reported based on the fatty acid content. In one study, the amount of oleic acid (18:1), a monounsaturated fatty acid, was found in different ratios in Syria, Greek, and commercial oils, with the highest amount present in Syrian oil [3].

Moreover, Syrian oil contained the highest amount of polyunsaturated fatty acid linolenic acid (18:3), followed by Greek oil. Overall, Syrian oil contained the highest content of unsaturated fatty acids compared to Greek and commercial oils. This shows an enormous variation of the phytochemical profile and its composition in *O. europaea* samples based on geographical origin.

The objective of the study is to evaluate the quality of an enormous number of olive samples (42) from different origins in terms of phytochemical profile using GCMS, as well as a comparative biological evaluation of the cytotoxicity and antimicrobial activity in correlation to the volatile chemical markers observed.

Material and methods

Olive (Olea europaea) fruit samples

The 42 seedless fresh-olive fruit (OF) samples from different geographical origins, as reported in our previous study, were collected during the year 2021 at local markets and properly coded; Egyptian (O1–O3), Greek (O–O9), Jordan (O10–O19), Moroccan (O20–O27), Palestinian (O28), Spanish (O29–O32), Syrian (O33–O39), and Turkish (O40–O42) samples [6].

Preparation of OF samples

Liquid–liquid extraction was performed where the samples (1 g of the methanolic extracts) were dissolved and partitioned in n-hexane solvent (20 mL). The LLE process was repeated 3 times, and the n-hexane solvent was collected and evaporated. The final extract was dissolved in 10 mL GCMS grade solvent for a final concentration of 1 mg/mL of the extract. The n-hexane extracted samples were filtered (0.2 μm), diluted (5 ppm), and subjected to GCMS. The GC–MS chemical study for qualitative and quantitative analysis of the phytochemical profile is performed and reported herein.

GC–MS profiling for volatile compounds

The GC separation was performed using a Shimadzu-2010 plus gas chromatograph equipped with a split/splitless injector and coupled with QP2010 Ultra MS detector. The column used for the application was a nonpolar Rxi-5MS capillary column (30 m × 0.25 mm, 1.00 μm; Restek Corporation); whereas, helium was used as a carrier gas (1.5 mL/min). For GCMS analysis, the oven was initially maintained at 50 °C for 1 min, ramped to 150 °C at a rate of 5 °C/min (held for 1 min), and finally ramped to 280 °C at a rate of 10 °C/min (held for 5 min). The temperatures for the ion source and MS transfer lines were maintained at 250 and 280 °C, respectively. Mass spectra (33–450 m/z) were acquired following a 6.5-min solvent delay. Shimadzu GCMS Solution® (V. 4.52) was used for data acquisition, processing, and GC–MS control. The area normalization method (%content) was used for semi-quantification [48]; whereas, compound identification was achieved by mass spectral searching within the NIST11 Library database.

Cell lines and culture conditions

Cancer cell lines, MCF7 (human breast adenocarcinoma), and HCT116 (human colorectal carcinoma) were obtained and sub-cultured in RPMI-1640 media (10% FBS) at 37 °C, 5% CO_2, and 100% relative humidity.

Cytotoxicity assay

The MTT assay was performed using MCF7 and HCT116 cells to evaluate OF-samples' cytotoxicity. One concentration for each of the 42-OF-samples, i.e., 100 μg/mL (DMSO 0.1%; n = 3), was tested for comparative evaluation of the samples. The positive control used in the study was doxorubicin (5 μM) [1, 2].

Microorganisms and culture media

Bacterial strains: *Staphylococcus aureus* (ATCC-25923), methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC-43300), *Pseudomonas aeruginosa* (ATCC-15442), and *Escherichia coli* (ATCC-35218); whereas, the culture media: Muller Hinton agar (Oxoid, CM-0337) and Muller Hinton broth (Oxoid, CM-0405) were used for agar diffusion (MIC) and broth dilution method (MBC), respectively.

Preparation of the standard inoculum

The tested organisms were grown on Muller Hinton agar medium (37 °C, 24 h.), and selected colonies were inoculated with the help of a sterile loop in Muller Hinton broth

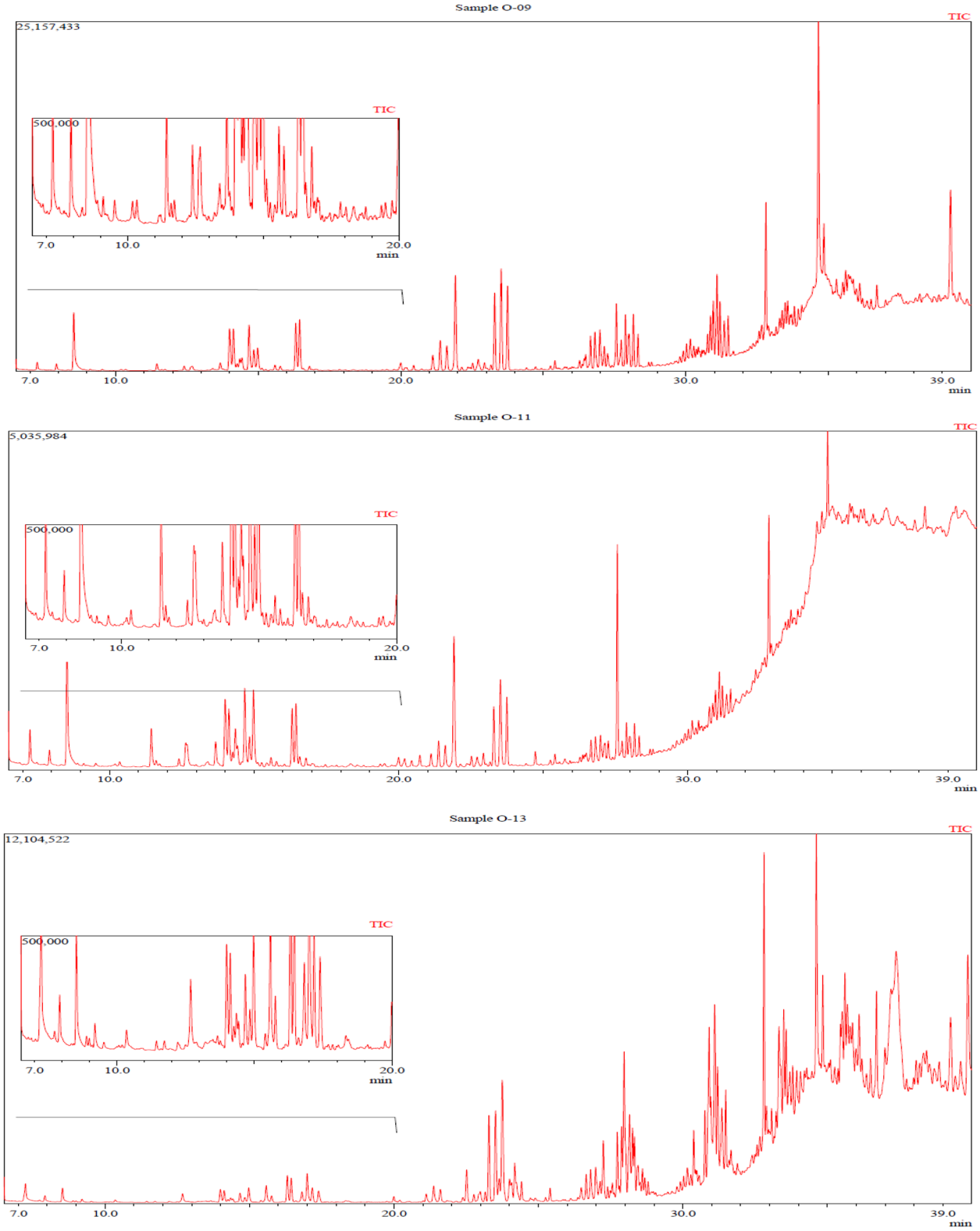


Fig. 1 Representative GC–MS chromatograms for olive samples

to form a homogenous suspension of bacterial strains. The suspension was standardized (0.5 McFarland turbidity) using calibrated Vitek Densichek Biomerieux Analyzer.

Agar diffusion assay

The assay was performed for individual bacterial strains suspended in Mueller Hinton broth in three directions

Table 1 Cytotoxic inhibition activity of the 42 extracts against two cell lines (MTT 72 h, % ± SD)

Sample origin	Sample code	MCF7	HCT116
Egyptian green olive plain	O1	8.00 ± 0.34	22.00 ± 2.77
Egyptian natural olive red	O2	24.00 ± 3.99	10.00 ± 1.00
Egyptian olive Kalamata	O3	22.00 ± 4.56	10.00 ± 0.99
Greek olive nafplion green	O4	14.00 ± 2.77	12.00 ± 2.00
Greek olive Cretan green	O5	13.00 ± 1.10	2.00 ± 0.05
Greek olive jumbo green	O6	22.00 ± 3.00	17.00 ± 1.11
Greek olive Kalamata jumbo black	O7	14.00 ± 2.39	1.20 ± 0.09
Greek olive Kalamata medium black	O8	60.00 ± 7.30	13.00 ± 2.34
Greek stuffed olive with pepper	O9	45.00 ± 5.56	29.00 ± 3.01
Spanish olive whole black	O10	33.00 ± 1.10	17.00 ± 2.03
Spanish olive whole green	O12	23.00 ± 2.33	19.00 ± 1.44
Spanish stuffed olive pine green	O11	33.00 ± 0.92	2.00 ± 0.09
Spain black olive	O13	23.00 ± 2.34	1.00 ± 0.03
Turkish green large olive	O14	35.00 ± 3.00	14.00 ± 2.00
Turkish grilled olive	O15	27.00 ± 3.01	14.00 ± 1.00
Turkish cracked green olive	O16	14.00 ± 1.50	8.00 ± 0.44
Jordan black olive in oil	O17	52.00 ± 2.00	34.00 ± 3.22
Jordan green olive in oil	O18	16.00 ± 1.40	19.00 ± 1.67
Jordan green olive with zaatar	O19	26.00 ± 3.60	44.00 ± 5.03
Jordan apple green olive	O20	27.00 ± 2.99	14.00 ± 1.98
Jordan green olive with lemon	O21	32.00 ± 2.56	16.00 ± 2.87
Jordan black olive in brine	O22	21.00 ± 2.10	9.00 ± 0.95
Jordan green olive in brine	O23	45.00 ± 1.98	2.00 ± 0.06
Jordan green olive	O24	25.00 ± 3.45	15.00 ± 1.56
Jordan green balad olive	O25	64.00 ± 4.88	25.00 ± 1.00
Jordan green nusi olive	O26	22.00 ± 2.11	15.00 ± 2.33
Moroccan green olive andalas	O27	12.00 ± 2.00	14.00 ± 1.03
Moroccan green black olive	O28	22.00 ± 2.87	5.00 ± 0.05
Moroccan green olive with lemon	O29	30.00 ± 2.65	0.11 ± 0.01
Moroccan dried olive black	O30	21.00 ± 1.10	19.00 ± 1.01
Moroccan green olive with herbs	O31	21.00 ± 2.54	18.00 ± 3.00
Moroccan green olive with red peppers	O32	17.00 ± 2.71	16.00 ± 4.32
Moroccan cocktail olive	O33	30.00 ± 2.33	17.00 ± 3.22
Moroccan purple olive	O34	24.00 ± 2.00	9.00 ± 0.93
Syrian black olive salqin	O35	19.00 ± 2.34	5.50 ± 0.56
Syrian green olive nybaly	O36	25.00 ± 2.00	2.00 ± 0.05
Syrian black olive with garlic	O37	26.00 ± 1.00	21.00 ± 1.98
Syrian black large olive	O38	30.00 ± 3.00	14.00 ± 2.45
Syrian black selptin olive	O39	41.00 ± 6.30	24.00 ± 5.22
Syrian black Kalamata olive	O40	8.00 ± 0.67	7.00 ± 0.99
Syrian green olive	O41	25.00 ± 2.30	7.00 ± 0.19
Palestine green olive	O42	41.00 ± 3.32	5.00 ± 0.92
Standard drug	***Doxorubicin***	***85.11 ± 5.25***	***37.0 ± 2.01***

Table 2 Evaluation of the antimicrobial activity of the 42 olive variety extracts against four reference bacterial strains using agar diffusion assay

Sample origin	Sample code	Bacterial strains			
		P. aeruginosa **ATCC – 15,442**	***E. coli*** **ATCC – 35,218**	***S. aureus (MRSA)*** **ATCC –43,300**	***S. aureus*** **ATCC -25,923**
		Diameter of inhibition zone (mm ± SD)			
Egyptian green olive plain	O1	R	R	R	N. D
Egyptian natural olive red	O2	12 ± 1.0	R	R	R
Egyptian olive Kalamata	O3	14 ± 1.0	15 ± 1.0	R	R
Greek olive nafplion green	O4	R	N. D	N. D	N. D
Greek olive Cretan green	O5	R	12 ± 1.0	R	R
Greek olive jumbo green	O6	R	R	R	13 ± 1.0
Greek olive Kalamata jumbo black	O7	R	R	R	R
Greek olive Kalamata medium black	O8	17 ± 1.1	12 ± 1.0	R	11 ± 1.0
Greek stuffed olive with pepper	O9	R	N.D	R	N. D
Spanish olive whole black	O10	R	R	R	R
Spanish olive whole green	O11	R	R	R	R
Spanish stuffed olive pine green	O12	R	R	R	R
Spain black olive	O13	11 ± 1.0	R	R	R
Turkish green large olive	O14	R	R	R	R
Turkish grilled olive	O15	R	N. D	R	N. D
Turkish cracked green olive	O16	11 ± 1.0	N. D	R	R
Jordan black olive in oil	O17	R	N. D	R	N. D
Jordan green olive in oil	O18	R	R	R	R
Jordan green olive with zaatar	O19	R	R	R	N.D
Jordan apple green olive	O20	R	11 ± 1.0	R	R
Jordan green olive with lemon	O21	R	R	R	R
Jordan black olive in brine	O22	12 ± 1.0	R	R	R
Jordan green olive in brine	O23	12 ± 1.0	R	R	11 ± 1.0
Jordan green olive	O24	13 ± 1.0	R	R	N. D
Jordan green balad olive	O25	11 ± 1.0	R	R	R
Jordan green nusi olive	O26	R	N.D	R	N.D
Moroccan green olive andalas	O27	R	N. D	R	N. D
Moroccan green black olive	O28	12 ± 1.0	R	R	N. D
Moroccan green olive with lemon	O29	12 ± 1.0	N. D	R	N. D
Moroccan dried olive black	O30	R	N.D	R	R
Moroccan green olive with herbs	O31	12 ± 1.0	12 ± 1.0	R	N.D
Moroccan green olive with red peppers	O32	13 ± 1.0	R	R	N.D
Moroccan cocktail olive	O33	R	R	R	11 ± 1.0
Moroccan purple olive	O34	R	N. D	R	N. D
Syrian black olive salqin	O35	R	12 ± 1.0	R	R
Syrian green olive nybaly	O36	R	N. D	R	N. D
Syrian black olive with garlic	O37	13 ± 1.0	R	R	N. D
Syrian black large olive	O38	R	R	R	R
Syrian black selptin olive	O39	13 ± 1.0	15 ± 1.1	R	12 ± 1.1
Syrian black Kalamata olive	O40	11 ± 1.0	R	R	13 ± 1.0
Syrian green olive	O41	R	12 ± 1.0	12 ± 1.0	R
Palestine green olive	O42	14 ± 1.0	R	12 ± 1.1	13 ± 1.0
Standards	Amikacin	21 ± 0.00	23 ± 0.00	–	–
	Vancomycin	–	–	18 ± 0.00	16 ± 0.00
Vehicle	DMSO	R	R	R	R

R resistant, *mm* millimeter, *N.D* not determined, *DMSO* dimethyl sulfoxide, *SD* standard deviation

Table 3 Assessment of MIC (µg/ml) and MBC (µg/ml) of the selected extracts against two bacterial strains

Sample origin	Sample code	Bacterial strains			
		P. aeruginosa (ATCC – 15,442)		*E. coli* (ATCC – 35,218)	
		MIC	MBC	MIC	MBC
Syrian black selptin olive	O39	12.5	25	50	100
Greek olive Kalamata medium black	O8	12.5	25	25	50

MIC minimum inhibitory concentration, *MBC* minimum bactericidal concentration, *ATCC* American Type Culture Collection

of the agar plate using sterile cotton swabs (100 µL), as reported [21].

MIC and MBC determination

For MIC and MBC, 200 µL of the selected optimum extracts (O17 and O25) (100 µg/ml) were added to the first column of the 96-well microtiter plates, followed by the addition of 100 µL Mueller Hinton broth to the second column. Individual extract (100 µL) was transferred from the first well into the subsequent wells to produce twofold dilutions (50, 25, 12.5, 6.2, and 3.1 µg/ml). The adjusted McFarland bacterial suspension (10 µL of 0.5) in the Muller Hinton broth of either *P. aeruginosa* or *E. coli* was added to each well, and the experiment was repeated three times. The plates were incubated overnight (37 °C) according to the clinical and laboratory standard institute, and the MIC and MBC were determined (CLSI M26– A, 1998).

Statistical analysis

The results were analyzed using SPSS (statistical package for social science students) software V 22.0 using principal component analysis and k-mean cluster analysis.

Results

GCMS profiling

The GCMS revealed the presence of 111 compounds from various chemical classes with a descending order (largest to smallest) of percentages/chemical class: esters (21.61–60.49%), alcohols (20.73–49.2%), hydrocarbons (3–38.88%), ketones (0.16–3.87%), acids (0.07–2.62%), and aldehydes (0.12–1.47%). Regarding the average (%) of 111 volatile compounds/chemical classes, the 42-OF samples showed a descending pattern of esters (44.62%), alcohols (38.06%), hydrocarbons (15.39%), ketones (0.75%), aldehydes (0.45%), others (0.41), acids (0.27%), and ethers (0.06%) as shown in Additional file 1: Table S1.

Among the 42-OF samples, O34 exhibited the highest (60.49%), whereas O23 revealed the lowest (21.61%) concentration for the ester compounds present in these samples. The major among these esters compounds was 13-methyl-pentadecanoic acid methyl ester, with an average occurrence of 11.9%. The representative chromatograms for OF-GCMS analysis are shown in Fig. 1 for sample Greek stuffed olive with pepper (O-09), Spanish stuffed olive pine green (O-11), Spain black olive (O-13), which shows the (tR) maximum up to 39 min for each sample. At the same time, the peak height is proportional to the concentration of a compound in the sample, which is given in more detail in Additional file 1: Table S1.

Cytotoxic assay

The 42-OF-samples exhibited cytotoxic activity within the range of 8–64% and 0.11–44% against MCF7 and HCT116 cell lines, respectively. Among the 42-OF-samples, the most significant inhibition was observed for O17 and O25 on MCF7 (64% and 52%) and HCT116 (25% and 34%), respectively (Table 1).

Antimicrobial activity

The antimicrobial activity for 42-OF-samples revealed significant zones of inhibition (mm) against the tested strains: *P. aeruginosa* (0.00–17.00), *E. coli* (0.00–15.00), *S. aureus* (0.00–13), and resistant *S. aureus,* i.e., MRSA (0.00–12.00). The maximum zones of inhibition (mm ± SD) were observed for O8 and O39 as *E. coli* (12 ± 1.0) for O8 and (15 ± 1.1) for O39; *P. aeruginosa* (17 ± 1.1) for O8 and (13 ± 1.0) for O39. These two extracts did not exhibit any activity against the resistant *S. aureus* (MRSA), while significant activity was observed against the sensitive *S. aureus* (25,923) as: (11 ± 1.0) for O8 and (12 ± 1.1) for O39. Likewise, O42 showed a zone of inhibition against *P. aeruginosa* (14 ± 1.0), S. *aureus,* i.e., MRSA (12 ± 1.1), and *S. aureus* (13 ± 1.0). Except for O41 and 42, none of the OF samples revealed any activity against MRSA strain, as shown in Table 2. Further evaluation of the OF-samples with optimum activity, i.e., O39 and O8 revealed an identical MIC and MBC of 12.5 and 25 mg/ml against *P. aeruginosa.* In contrast, a MIC (50 and 25) and MBC (100 and 50) against *E. coli* were determined for O39 and O8, as shown in Table 3.

Statistical analysis

SPSS V 22.0 was used to classify and analyze the data.

Principal component analysis (PCA)

To evaluate the variability and correlation of the components in the extracted data, PCA was applied where the

Table 4 PCA analysis for GCMS, cytotoxicity and antimicrobial dataset

Components	PC1	PC2	PC3	PC4	PC5
PCA for GCMS data set					
O1	0.165	0.677	− 0.069	0.516	− 0.237
O2	0.752	0.577	0.297	0.051	− 0.067
O3	0.291	0.732	0.374	0.353	0.202
O4	0.582	0.786	0.140	0.091	− 0.080
O5	0.626	0.624	0.409	0.187	0.087
O6	0.454	0.698	0.398	0.291	0.171
O7	0.829	0.504	0.196	0.092	− 0.075
O8	0.719	0.595	0.337	0.098	− 0.007
O9	0.561	0.713	0.374	0.170	0.012
O10	0.780	0.556	0.242	0.127	− 0.019
O11	0.294	0.559	0.728	− 0.038	− 0.028
O12	0.289	0.399	0.851	0.025	− 0.042
O13	0.793	0.118	− 0.016	0.526	− 0.125
O14	0.216	0.887	0.363	0.151	0.074
O15	0.823	0.504	0.229	0.111	− 0.002
O16	0.486	0.803	0.263	0.186	0.082
O17	0.842	0.334	0.309	0.201	0.163
O18	0.437	0.168	0.725	− 0.061	− 0.240
O19	0.080	0.133	0.966	0.106	0.015
O20	0.912	0.174	0.251	0.170	0.155
O21	0.146	0.593	0.651	0.234	0.305
O22	0.213	0.792	0.465	0.213	0.096
O23	0.026	0.483	0.151	0.691	0.016
O24	0.660	0.639	0.329	0.177	0.067
O25	0.505	0.830	0.202	0.104	− 0.005
O26	0.693	0.659	0.238	0.063	− 0.108
O27	0.309	0.112	0.008	0.697	− 0.209
O28	0.479	0.838	0.227	0.062	− 0.081
O29	0.779	0.589	0.163	0.046	− 0.097
O30	0.729	0.120	− 0.014	0.593	− 0.121
O31	0.619	0.561	0.388	0.287	0.206
O32	0.745	0.474	0.334	0.254	0.157
O33	0.165	0.259	0.937	0.021	− 0.037
O34	0.923	0.226	0.178	0.057	0.003
O35	0.474	0.845	0.210	0.113	− 0.039
O36	0.686	0.661	0.264	0.046	− 0.114
O37	0.703	0.499	0.372	0.237	0.176
O38	0.677	0.616	0.324	0.188	0.082
O39	0.590	0.156	0.782	0.006	− 0.060
O40	0.820	0.513	0.219	0.042	− 0.082
O41	0.647	0.624	0.236	0.030	− 0.132
O42	0.866	0.246	0.271	0.118	0.182
Individual %variance	36.27	31.61	17.03	6.50	2.72
Cumulative %variance	36.27	67.89	84.92	91.43	94.16

Components	PC1	PC2
PCA for cytotoxicity and antimicrobial assay		
MCF7_activity	0.328	0.797
HCT116_activity	− 0.412	0.765

Table 4 (continued)

Components	PC1	PC2
P. aeruginosa	0.676	0.097
E. coli	0.487	− 0.071
S. aureus (MRSA)	0.551	− 0.136
S. aureus (25,923)	0.672	0.283
Individual %variance	28.75	22.21
Cumulative %variance	28.75	50.96

Eigenvalue was applied to classify the data. The PCA for 42-OF samples suggested five components (PC1−PC5) with a cumulative %variance of 94.16 and an individual %variance for each component: PC1 (36.27), PC2 (31.61), PC3 (17.03), PC4 (6.50), and PC5 (2.72). The major variability of 36% was exhibited by PC1 loaded with OF-samples: O2, O5, O7, O8, O10, O13, O15, O17, O20, O24, O26, O29–32, O34, O36–39, and O40–42. This implicates the presence of major amounts of volatile compounds in these groups. The next major variability of 31.61% as represented by PC2 consisted of the OF-samples: O1, O3–4, O6, O9, O14, O16, O22, O25, O28, and O35. The samples with more potent cytotoxicity and antimicrobial activities, i.e., O25, O39 and O42 (PC1), O17 and O8 (PC2) are loaded in these two components where a distribution of an enormous number of volatile components has been observed. The remaining components with %variability and OF samples are briefly presented in Table 4.

The PCA for cytotoxicity and antimicrobial activity produced two components with a cumulative variability of 50.96% and individual variability of 28.75% (PC1) and 22.21% (PC2). The components loaded in PC1 consisted of antimicrobial activity, which showed more component variation of 28.75% compared to cytotoxicity activities loaded in PC2. The data suggest more variation in OF samples regarding antimicrobial activity due to lack of activity at most instances, particularly against the resistant strain of MRSA. On the contrary, the OF samples revealed a comparatively high activity for cytotoxicity assay, representing a low variation in components (Table 4 and Fig. 2).

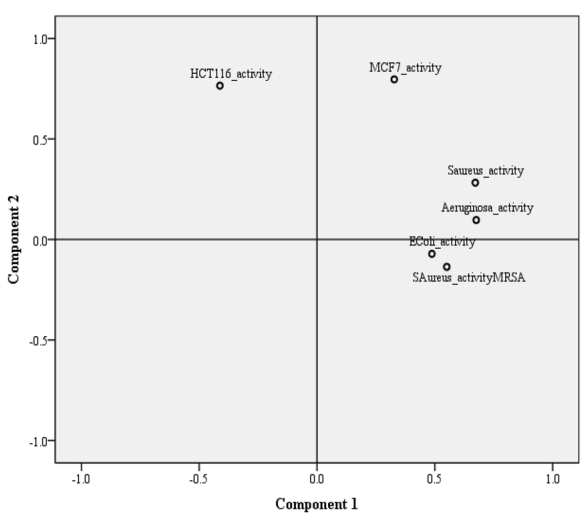


Fig. 2 Three-dimensional representation for cytotoxicity and antimicrobial assay components

K-mean cluster analysis

The K-mean cluster analysis classifies the data into clusters based on the nearest mean among the data set. For GCMS, six clusters with high F-value and significance ($P = 0.001$) were observed as cluster 1 (2 samples), cluster 2 (1 sample), cluster 3 (1 sample), cluster 4 (15 samples), cluster 5 (1 sample), and cluster 6 (91 samples). The two samples in cluster 1 and one sample in cluster 5 represent the ester chemical class of volatile oils; one sample in cluster 2 represents alcohols, and one in cluster 3 represents hydrocarbons. These samples denote the presence of more amounts (%) of such chemical classes in the 42-OF samples. This may be appropriately explained by the high quantity of esters (44.62%) followed by alcohols (38.06%), and hydrocarbons (15.39%) among the chemical classes reported by GCMS in these 42-OF samples. The cluster with chemical class distribution is shown in Table 5 and Fig. 3.

Regarding K-mean cluster analysis for cytotoxicity and antimicrobial activity, four clusters were proposed: cluster 1 (2 samples), cluster 2 (4 samples), cluster 3 (21 samples), and cluster 4 (15 samples). As denoted in Fig. 4, cluster 1 represents two extracts (OF-41 and 42) which are the only samples with activity against *S. aureus* (MRSA). Cluster 2 represents samples with activity against all cell lines and microbial strains except MRSA. These extracts are O8, O23, O25, and O39. The K-mean effectively distinguished the GCMS samples with more amounts of chemical compounds and the

Table 5 K-mean cluster analysis for GCMS, cytotoxicity and antimicrobial dataset

Factors	*F*-value	Significance	Clusters	Samples
K-mean cluster analysis for GCMS data				
Zscore: O1	28.643	0.00	1	2
Zscore: O2	242.133	0.00	2	1
Zscore: O3	129.049	0.00	3	1
Zscore: O4	162.693	0.00	4	15
Zscore: O5	300.179	0.00	5	1
Zscore: O6	202.767	0.00	6	91
Zscore: O7	297.313	0.00	Total	111
Zscore: O8	285.570	0.00		
Zscore: O9	225.880	0.00		
Zscore: O10	290.002	0.00		
Zscore: O11	107.871	0.00		
Zscore: O12	194.302	0.00		
Zscore: O13	58.976	0.00		
Zscore: O14	228.918	0.00		
Zscore: O15	371.892	0.00		
Zscore: O16	227.807	0.00		
Zscore: O17	531.912	0.00		
Zscore: O18	48.366	0.00		
Zscore: O19	381.985	0.00		
Zscore: O20	937.118	0.00		
Zscore: O21	227.174	0.00		
Zscore: O22	137.451	0.00		
Zscore: O23	14.340	0.00		
Zscore: O24	298.218	0.00		
Zscore: O25	188.534	0.00		
Zscore: O26	169.493	0.00		
Zscore: O27	5.561	0.00		
Zscore: O28	148.621	0.00		
Zscore: O29	218.282	0.00		
Zscore: O30	36.668	0.00		
Zscore: O31	265.768	0.00		
Zscore: O32	305.279	0.00		
Zscore: O33	528.296	0.00		
Zscore: O34	200.560	0.00		
Zscore: O35	174.198	0.00		
Zscore: O36	178.966	0.00		
Zscore: O37	256.120	0.00		
Zscore: O38	320.560	0.00		
Zscore: O39	280.236	0.00		
Zscore: O40	259.254	0.00		
Zscore: O41	75.926	0.00		
Zscore: O42	1374.378	0.00		
Cluster analysis for cytotoxicity and antimicrobial assay				
Zscore: MCF7_activity	13.189	0.000	1	2
Zscore: HCT116_activity	7.854	0.000	2	4
Zscore: *P. aeruginosa*	10.465	0.000	3	21
Zscore: *E. coli*	2.885	0.048	4	15
Zscore: *S. aureus* (MRSA)	0.00	–	Total	42
Zscore: *S. aureus* (25,923)	5.372	0.003		

samples with more potency against the cell lines and microbial strain studied. The K-mean for cytotoxicity and antimicrobial activity is shown in Table 5 and Fig. 4.

The data produced with the current methodology were analyzed with the help of descriptive, PCA, and K-mean cluster analysis, which revealed a significant difference in terms of phytochemical profile as well as a correlation for the biological activities linked with a specific class and volatile chemicals of the phytochemical class in these olive samples. The statistical models fully support the significance and correlation of the data extracted based on the current methodology.

Discussion

Olive oil and fruits are considered healthy because of their nutritional and medicinal properties. Bioactive compounds like triterpenoids and phenolics contribute to their high quality. Olive is known to be produced by over a hundred cultivars with different colors, shapes, and sizes where the quality and oil content of olive may be compromised, prone to various environmental or geographical conditions [5, 33]. For instance, many studies have previously reported the variation in natural products such as black seeds and black pepper varieties [7, 8]. Current research collected 42 available olive fruit (OF) samples of different sizes, colors, and shapes. The selection for these samples was non-specific and generalized, i.e., all the samples available at all the stores, hypermarkets, suppliers, shops, etc., were searched and collected irrespective of any targeted numbers or geographical origin.

GCMS analysis was performed for the extracts where several volatile chemical classes were reported, consisting of acids, alcohols, esters, ethers, hydrocarbons, aldehydes, and ketones. The predominant among these chemical classes was the ester chemical class (44.62%), followed by alcohols (38.06%) and hydrocarbons (15.39%). The predominant volatile compounds in these chemical classes were: 13-methyl-pentadecanoic acid methyl ester (esters), 2, 4-bis (1, 1-dimethyl ethyl) -phenol (alcohols), o-cymene (hydrocarbons), 2-pentadecanone, 6, 10, 14-trimethyl- (ketones), 3-hydroxy-dodecanoic acid (acids), and 4-methyl- benzaldehyde

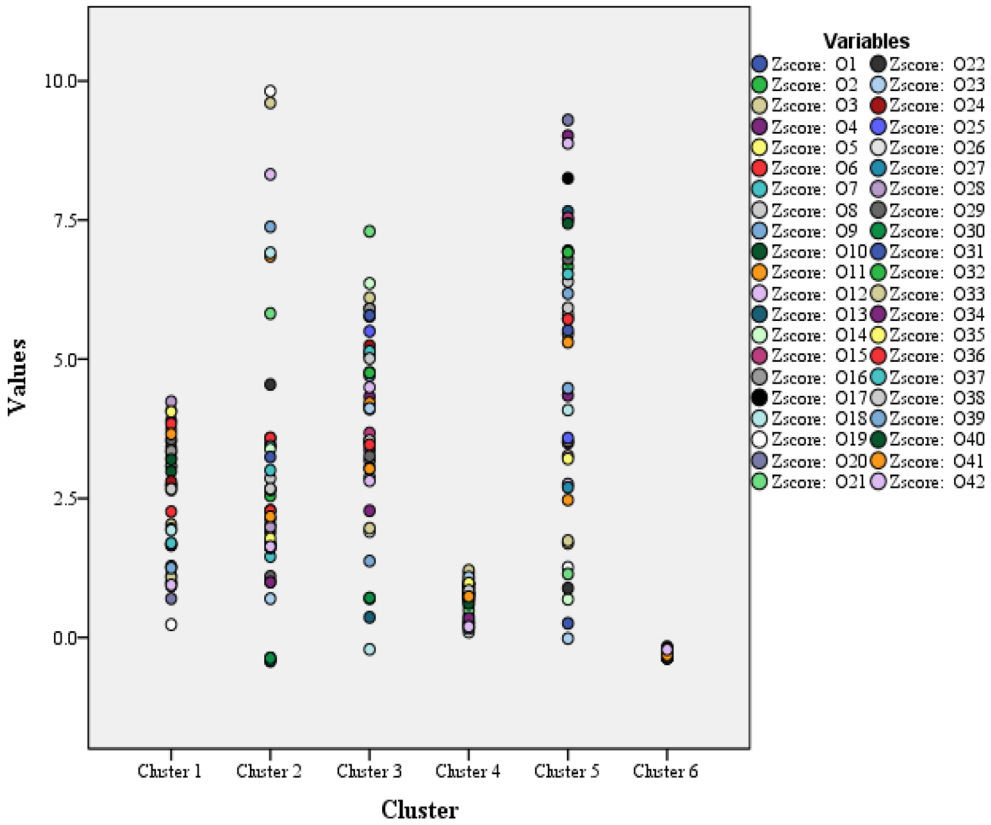


Fig. 3 Cluster distribution for the GCMS data of 42-OF-samples

(aldehydes). Although the study of quality variation was reported previously for olive fruit, based on the comparative evaluation of fatty acids [3], this is the first report to evaluate 42-OF samples from different geographical origins based on the phytochemical profile and biological properties of the samples.

As olive oil consumption has been linked to the prevention of cancer [26, 31], these 42-OF-samples were tested for cytotoxic potential against various cell lines. An intra-comparative evaluation was performed for the 42-extracts, using MCF7 and HCT116 cell lines, where the two samples, O17 (Jordan black olive) and O25 (Jordan green balad olive) exhibited the maximum activity with an inhibition of 52% for O17 (Jordan black olive) and 64% for O25 (Jordan green balad olive). The findings in our study corroborate previous reports [9, 25]. The OF extracts in this study exhibited feeble antiproliferative effects against one cell line, showing strong inhibition against the other type. This may be due to the varied genetic context of each cell line, the uncertain response of cell lines against the tested compounds, as well as the use of non-standardized crude extracts against these cell lines by different researchers, which may affect the outcomes of the study [10, 32]. The antiproliferative effects, though, have been attributed to many compounds in most OF samples; 3, 4- (dihydroxyphenyl) -ethanol elenolic acid di-aldehyde and 3, 4- (dihydroxyphenyl) -ethanol elenolic acid ester secoiridoid aglycone has been reported as the most potent cytotoxic compounds at many instances [10]. This again supports the same phytochemical marker of olive to be used for a comparative quality evaluation and standardization of the olive samples. The GCMS analysis herein for the 42 OF samples showed the presence of 17-pentatriacontene in high concentration in O17 (Jordan black olive), whereas 10-octadecenal, trichloroacetic acid, hexadecyl ester, and citronellol epoxide were found in maximum amounts in O25 (Jordan green balad olive) compared to other cultivars. This may be a valuable biomarker for further mechanistic studies on OF.

It is of utmost importance to mention here that a consistent trend of halogenated and sulphonated compounds (chlorinated/fluorinated/sulphurous) have been observed in the phytochemical profile of most of the samples, as reported previously in an enormous amount of literature [4, 14, 20, 27, 34, 35, 39, 41, 43–47] [52]. Though most of these chemical compounds have been reported in these

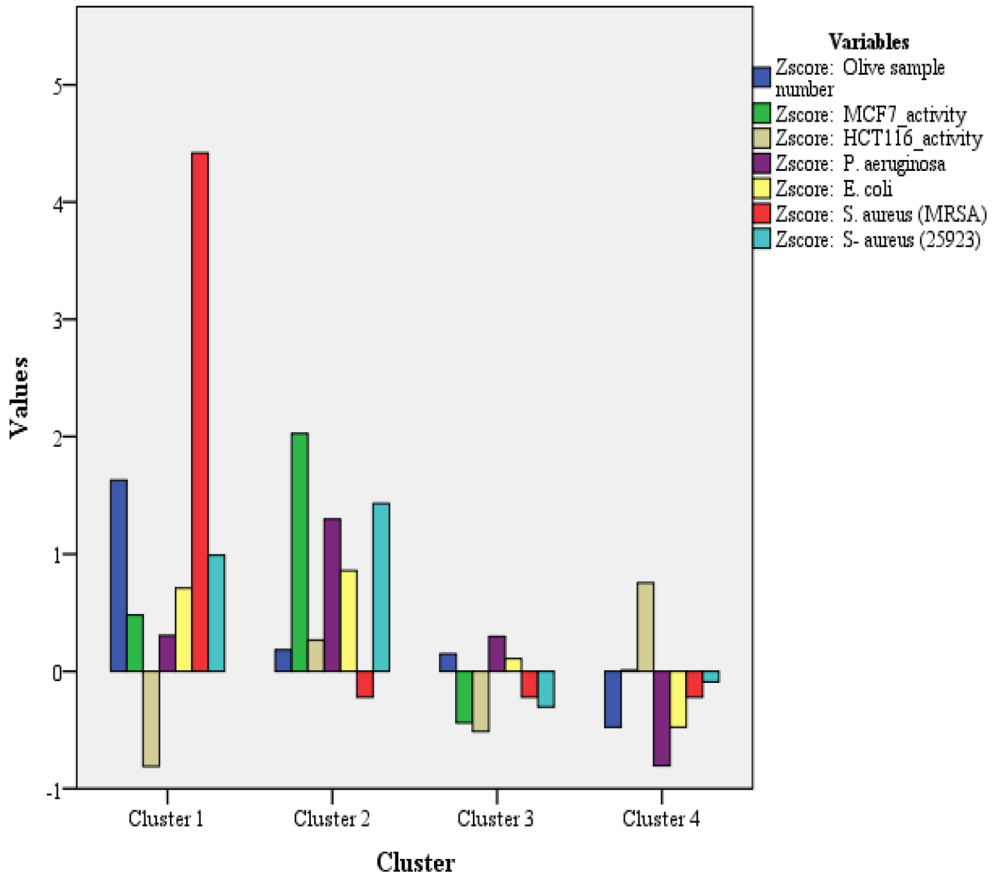


Fig. 4 Cluster distribution for cytotoxicity and antimicrobial data of 42-OF-samples

studies, they are not related to the chemistry of natural products. The authors suggest that such volatile components originate from environmental contamination during cultivation or instrumental and solvent contamination during extraction and GCMS analysis. Therefore, such compounds should be carefully evaluated when interpreting the results and selecting chemical markers for the quality control of medicinal plants.

The 42-OF-samples were studied for their antimicrobial potential against various bacterial strains. Similar to cytotoxicity, the general antimicrobial screening showed three OF samples: O8 (Greek olive, Kalamata medium black), O39 (Syrian black, selptin olive), and O42 (Palestine green olive) with significant inhibition of the tested microorganisms. The MICs and MBCs were calculated for the most potent extracts of O8 (Greek olive, Kalamata medium black) and O39 (Syrian black selptin olive), which showed a similar MIC of 12.5 µg/ml against *P. aeruginosa.* In contrast, MICs of 25 (O8) and 50 (O39) µg/ml with MBCs of 50 (O8) and 100 (O39) were observed against *E. coli*. A previous study reported a MIC of 0.625–1.25 mg/mL for olive oil against *Salmonella typhimurium* and *Staphylococcus aureus.* The other samples presented a lack of inhibitory effect, possibly due to dilute concentrations compared to Guo et al. work [18, 29]. The GCMS profile for these samples (O8 and O39) suggested the presence of high amounts of 2-hexyl-1-decanol, 2-butyl-1-octanol, 3, 7, 11-trimethyl-1-dodecanol, n-nonadecanol, (Z) -7-hexadecenal, (Z) -9-octadecenoic acid, hexyl ester, octacosyl trifluoroacetate, nonadecyl pentafluoropropionate, octatriacontyl trifluoroacetate, and octacosyl heptafluorobutyrate, compared to other cultivars.

Aldehydes might be potential chemicals responsible for antimicrobial effects [16, 30]. The organic acids might also be responsible for antimicrobial effects, though the amount might not be high, while the esters might not be the active ingredients responsible for antimicrobial effects [17, 50, 51]. Phenolic compounds might be another category responsible for antimicrobial effects. However, our GC–MS results did not report these compounds [13, 24].

The statistical analysis using GCMS-PCA proved the presence of high amounts of esters, alcohols, and hydrocarbons with significant quantities of ethers, aldehydes,

acids, ketones, and aldehydes. PCA revealed more variation in antimicrobial data for biological activities than cytotoxicity, where the OF samples presented significant antiproliferative effects. The antimicrobial activity was sparse, and only three extracts showed reasonable activity. For the activity against MRSA, only O41 (Syrian green olive) and O42 (Palestine green olive) showed considerable activity, whereas the rest of the samples were ineffective to retard the growth of MRSA. The K-mean cluster analysis of GCMS data and biological activities scrutinized the most effective four extracts of O8 (Greek olive, Kalamata medium black), O23 (Jordan green olive), O25 (Jordan green balad olive), and O39 (Syrian black selptin olive). The MIC and MBC evaluation for the two most effective extracts of O8 (Greek olive, Kalamata medium black) and O39 (Syrian black selptin olive) revealed a low IC_{50} value against the tested organisms.

The outcomes of this research study with statistical calculations were the variation in the quality of food and herbs, as prone to various factors of environmental and geographical variations, including watering, salinity, humidity, temperature, altitude, processing, packaging, and storage, etc.[7, 8]. Hence, a proper evaluation using instrumental metabolomics analysis is of utmost importance to determine the quality of food and herbal products.

Conclusion

The GCMS analysis for 42-OF samples showed the presence of one hundred and eleven compounds which belong to the chemical classes of acids, alcohols, esters, ethers, aldehydes, hydrocarbons, and ketones. Among the 42-OF samples, O17 (Jordan black olive) and O25 (Jordan green balad olive) exhibited the most potent activity against the cancer human cell lines, whereas O8 (Greek olive Kalamata medium black) and O39 (Syrian black selptin olive) had the highest levels of inhibition against the tested microorganisms. These samples showed the presence of a unique pattern of volatile chemical compounds with a predominant amount of esters which may be considered markers and valuable sources for further mechanistic studies of anticancer and antimicrobial natural products. Olive fruit may be a potential source to prevent microbial growth in food products using Olea-based food preservatives.

Novelty and potential impact of the study

This is the first study comparing the quality variation of 42 olive samples from different geographical locations worldwide. The study aimed to evaluate the food quality of olive used in the Saudi Arabian market, as an enormous proportion of the local inhabitants are using olive and their oil for various food, nutraceutical, health, and therapeutic purposes. It is critical to know the quality of the olive in terms of its phytochemical and phytochemical profile as most of the health and therapeutic properties of olive are imparted due to the presence of these novel and active phytochemicals. This study evaluated the quality variation of olive samples not merely in terms of their phytochemical profile using GCMS; the samples were also subjected to various comparative biological evaluations (cytotoxic and antimicrobial). The purpose was to standardize the quality of olive samples as per WHO guidelines, where the chemical, physical, and biological evaluations of the samples are of utmost necessity. This unique quality variation study, with the help of GCMS and biological evaluation, may be useful for the food and nutraceutical manufacturers for the quality control of marketed products, as well as for the end-user to be aware of the olive product with good quality in terms of active components and health effects. This study is equally important for the researchers in further studies that evaluate the impact of geographical origins and other factors on the quality of olive oil.

Additionally, the study investigated the correlation between phytochemical profile and biological activities wherein specific classes with unique volatile chemicals were responsible for most of the olive activities. Notably, such volatile substances may act as potential markers for researchers to select the most potent olive products and explore their mechanistic properties for food and nutraceutical manufacturers for quality control and standardization purposes of olive products.

Abbreviations

OF	*Olea europaea* Fruit
GCMS	Gas chromatography mass spectrometry
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00413-8.

Additional file 1. Supplementary file for the GCMS profile of 42-olive samples.

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Author contributions

RA (conceived idea and designed the study); RA, MR (literature review); MA (wrote introduction); AA, NHA, AMA, SSA (cytotoxicity and antimicrobial activity); (GCMS analysis); MR, NHA, FSA, AFA, SSA (material and methods,

and results write up); MMA, RA (statistical analysis and write up); RA, MR (discussion); RA, MR, MA, AA (final revision and approval). All authors read and approved the final manuscript.

Availability of data and materials
The data used in this study are completely provided during submission. For GCMS, a supplementary file has been uploaded.

Declarations

Ethics approval and consent to participate
The study did not use any animal or human subjects; hence, ethical approval or volunteer consent was not required.

Consent for publication
The authors give consent for the publication and its relevant data.

Competing interests
No conflict of interest exists among the authors.

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References

1. Abdalla AN, Abdallah ME, Aslam A, Bader A, Vassallo A, Tommasi ND, Malki WH, Gouda AM, Mukhtar MH, El− Readi MZ, Alkahtani HM. Synergistic Anti leukemia effect of a novel Hsp90 and a pan cyclin dependent kinase inhibitors. Molecules. 2020;25(9):2220.
2. Abdalla AN, Malki WH, Qattan A, Shahid I, Hossain MA, Ahmed M. Chemosensitization of HT29 and HT29− 5FU cell lines by a combination of a Multi− Tyrosine Kinase Inhibitor and 5FU downregulates ABCC1 and Inhibits PIK3CA in light of their importance in Saudi Colorectal Cancer. Molecules. 2021;26(2):334.
3. Abdelrahman MH, Hussain RO, Shaheed DS, AbuKhader M, Khan SA. Gas chromatography− mass spectrometry analysis and in vitro biological studies on fixed oil isolated from the waste pits of two varieties of Olea europaea L. OCL. 2019;26:28.
4. Abdul Rahim MH, Zakaria ZA, Mohd Sani MH, Omar MH, Yakob Y, Cheema MS, Ching SM, Ahmad Z, Abdul KA. Methanolic extract of Clinacanthus nutans exerts antinociceptive activity via the opioid/nitric oxide− mediated, but cGMP− independent, pathways. Evidence− Based Complement Altern Med. 2016;13:2016.
5. Ahmad R, Ahmad N, Al− Anaki WS, Ismail FA, Al− Jishi F. Solvent and temperature effect of accelerated solvent extraction (ASE) coupled with ultra− high− pressure liquid chromatography (UHPLC− PDA) for the determination of methyl xanthines in commercial tea and coffee. Food Chem. 2020;1(311): 126021.
6. Ahmad R, Ahmad N, Aljamea A, Abuthayn S, Aqeel M. Evaluation of solvent and temperature effect on green accelerated solvent extraction (ASE) and UHPLC quantification of phenolics in fresh olive fruit (Olea europaea). Food Chem. 2021;16(342): 128248.
7. Ahmad R, Ahmad N, Amir M, Aljishi F, Alamer MH, Al− Shaban HR, Alsadah ZA, Alsultan BM, Aldawood NA, Chathoth S, Almofty SA. Quality variation and standardization of black pepper (Piper nigrum): a comparative geographical evaluation based on instrumental and metabolomics analysis. Biomed Chromatogr. 2020;34(3): e4772.
8. Ahmad R, Ahmad N, Mohd AM, AIJHISI F, Alamer MH, Al− Shaban HR, Alsultan BM, Alsadah ZA, Aldawood NA, Chathoth S, Aslam KH. Variation in Nigella sativa quality and its standardization via instrumental analysis: a study based on geographical origin. Not Bot Horti Agrobo Cluj− Napoca. 2020;48(3):1141–54.
9. Allouche Y, Warleta F, Campos M, Sanchez− Quesada C, Uceda M, Beltran G, Gaforio JJ. Anti− oxidant, antiproliferative, and pro− apoptotic capacities of pentacyclic triterpenes found in the skin of olives on MCF− 7 human breast cancer cells and their effects on DNA damage. J Agric Food Chem. 2011;59(1):121–30.
10. Bačkorová M, Bačkor M, Mikeš J, Jendželovský R, Fedoročko P. Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. Toxicol In Vitro. 2011;25(1):37–44.
11. Belkhadar J, Claisse R, Fleurentin J, Yaunos C. Repertory of standard herbal drugs in the Moroccan pharmacopoeia. J Ethnopharmacol. 1991;35:123–43.
12. Bensehaila S, Ilias F, Saadi F, Zaouadi N. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L.) leaves. Asian J Dairy Food Res. 2022;41(2):237–41.
13. Bianco A, Melchioni C, Ramunno A, Romeo G, Uccella N. Phenolic components of Olea europaea–isolation of tyrosol derivatives. Nat Prod Res. 2004;18(1):29–32.
14. Byeon YS, Lim ST, Kim HJ, Kwak HS, Kim SS. Quality characteristics of wheat malts with different country of origin and their effect on beer brewing. J Food Qual. 2021;26(2021):1–2.
15. Cadiz− Gurrea MD, Pinto D, Delerue− Matos C, Rodrigues F. Olive fruit and leaf wastes as bioactive ingredients for cosmetics—A preliminary study. Anti− oxidants. 2021;10(2):245.
16. Chen L, Liu Q, Zhao X, Zhang H, Pang X, Yang H. Inactivation efficacies of lactic acid and mild heat treatments against *Escherichia coli* strains in organic broccoli sprouts. Food Control. 2022;1(133): 108577.
17. Chen L, Zhao X, Li R, Yang H. Integrated metabolomics and transcriptomics reveal the adaptive responses of Salmonella enterica serovar Typhimurium to thyme and cinnamon oils. Food Res Int. 2022;1(157): 111241.
18. de Carvalho AB, Caselli F, Rodrigues V, Paiva− Martins F, Marques MP. Antiproliferative activity of olive oil phenolics against human melanoma cells. Lett Drug Des Discovery. 2017;14(9):1053–9.
19. El Qarnifa S, El Antari A, Hafidi A. Effect of maturity and environmental conditions on chemical composition of olive oils of introduced cultivars in Morocco. J Food Qual. 2019;14(2019):1–4.
20. El Shafay SM, Ali SS, El− Sheekh MM. Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. Egypt J Aquatic Res. 2016;42(1):65–74.
21. El− Said H, Ashgar SS, Bader A, AlQathama A, Halwani M, Ascrizzi R, Flamini G. Essential oil analysis and antimicrobial evaluation of three aromatic plant species growing in Saudi Arabia. Molecules. 2021;26(4):959.
22. FAO. 2021. World Food and Agriculture - Statistical Yearbook 2021. Rome. https://doi.org/10.4060/cb4477en
23. Farhangi H, Ajilian M, Saeidi M, Khodaei GH. Medicinal fruits in holy Quran. Int J Pediatr. 2014;2(3.2):89–102.
24. Feng X, Ng VK, Mikš− Krajnik M, Yang H. Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. Food Bioprocess Technol. 2017;10:89–102.
25. Fogli S, Arena C, Carpi S, Polini B, Bertini S, Digiacomo M, Gado F, Saba A, Saccomanni G, Breschi MC, Nieri P. Cytotoxic activity of oleocanthal isolated from virgin olive oil on human melanoma cells. Nutr Cancer. 2016;68(5):873–7.
26. Franceschi S, Favero A, Conti E, Talamini R, Volpe R, Negri E, Barzan L, La Vecchia C. Food groups, oils and butter, and cancer of the oral cavity and pharynx. Br J Cancer. 1999;80(3):614–20.
27. Gautam V, Kohli SK, Arora S, Bhardwaj R, Kazi M, Ahmad A, Raish M, Ganaie MA, Ahmad P. Anti− oxidant and antimutagenic activities of different fractions from the leaves of Rhododendron arboreum Sm. and their GC− MS profiling. Molecules. 2018;23(9):2239.
28. Ghazanfar SA, Al− Al− Sabahi AM. Medicinal plants of northern and central Oman (Arabia). Econ Bot. 1993;47:89–98.

29. Guo L, Gong S, Wang Y, Sun Q, Duo K, Fei P. Antibacterial activity of olive oil polyphenol extract against *Salmonella Typhimurium* and *Staphylococcus aureus*: possible Mechanisms. Foodborne Pathog Dis. 2020;17(6):396–403.
30. Hai Y, Zhou D, Lam YL, Li X, Chen G, Bi J, Lou X, Chen L, Yang H. Nanoemulsified clove essential oils– based edible coating controls *Pseudomonas* spp– causing spoilage of tilapia (*Oreochromis niloticus*) fillets: working mechanism and bacteria metabolic responses. Food Res Int. 2022;159:111594.
31. Hodge AM, English DR, McCredie MR, Severi G, Boyle P, Hopper JL, Giles GG. Foods, nutrients and prostate cancer. Cancer Causes Control. 2004;15:11–20.
32. Jaeger S, Duran– Frigola M, Aloy P. Drug sensitivity in cancer cell lines is not tissue– specific. Mol Cancer. 2015;14:1–4.
33. Lavee S. World olive encyclopedia. Int Olive Oil Council. 1996;1:61–106.
34. Liu H, Zhang W, Chen H. Low polar volatile constituents from Isodon excisa by GC– MS. J Med Coll PLA. 2012;27(6):334–42.
35. Liu MH, Zhang Q, Zhang YH, Lu XY, Fu WM, He JY. Chemical analysis of dietary constituents in *Rosa roxburghii* and *Rosa sterilis* fruits. Molecules. 2016;21(9):1204.
36. Markhali FS, Teixeira JA, Rocha CM. Olive tree leaves—A source of valuable active compounds. Processes. 2020;8(9):1177.
37. Medina E, Romero C, Brenes M, De Castro A. Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J Food Prot. 2007;70(5):1194–9.
38. Mentella MC, Scaldaferri F, Ricci C, Gasbarrini A, Miggiano GA. Cancer and Mediterranean diet: a review. Nutrients. 2019;11(9):2059.
39. Priya V, Abinaya G. GCMS analysis of bioactive components of Ficus mollis, vahl leaves. J Med Plants Stud. 2018;6(2):98–100.
40. Rahmani AH, Albutti AS, Aly SM. Therapeutics role of olive fruits/oil in the prevention of diseases via modulation of anti– oxidant, anti– tumour and genetic activity. Int J Clin Exp Med. 2014;7(4):799.
41. Ravi R, Husna Zulkrnin NS, Rozhan NN, Nik Yusoff NR, Mat Rasat MS, Ahmad MI, Hamzah Z, Ishak IH, Mohd Amin MF. Evaluation of two different solvents for Azolla pinnata extracts on chemical compositions and larvicidal activity against *Aedes albopictus* (Diptera: Culicidae). J Chem. 2018;13(2018):1–8.
42. Romero C, Medina E, Vargas J, Brenes M, De Castro A. In vitro activity of olive oil polyphenols against Helicobacter pylori. J Agric Food Chem. 2007;55(3):680–6.
43. Sen S, Dehingia M, Talukdar NC, Khan M. Chemometric analysis reveals links in the formation of fragrant bio– molecules during agarwood (Aquilaria malaccensis) and fungal interactions. Sci Rep. 2017;7(1):1–4.
44. Sheoran N, Nadakkakath AV, Munjal V, Kundu A, Subaharan K, Venugopal V, Rajamma S, Eapen SJ, Kumar A. Genetic analysis of plant endophytic Pseudomonas putida BP25 and chemo– profiling of its antimicrobial volatile organic compounds. Microbiol Res. 2015;1(173):66–78.
45. Sophia A, Faiyazuddin M, Alam P, Hussain MT, Shakeel F. GC–MS characterization and evaluation of antimicrobial, anticancer and wound healing efficiency of combined ethanolic extract of Tridax procumbens and *Acalypha indica*. J Mol Struct. 2022;15(1250): 131678.
46. Sujatha J, Asokan S, Rajeshkumar S. Phytochemical analysis and anti– oxidant activity of chloroform extract of *Cassis alata*. Res J Pharm Technol. 2018;11(2):439–44.
47. Sulaiman FA, Nafiu MO, Yusuf BO, Muritala HF, Adeyemi SB, Omar SA, Dosumu KA, Adeoti ZJ, Adegbesan OA, Busari BO, Otohinoyi DA. The GC– MS fingerprints of *Nicotiana tabacum* L extract and propensity for renal impairment and modulation of serum triglycerides in Wistar rats. J Pharm Pharmacogn Res. 2020;8(3):191–200.
48. Topi D. Volatile and chemical compositions of freshly squeezed sweet lime (Citrus limetta) juices. J Raw Mater Process Foods. 2020;1(1):22–7.
49. Waterman E, Lockwood B. Active components and clinical applications of olive oil. Altern Med Rev. 2007;12(4):331–42.
50. Zhao X, Chen L, Wu JE, He Y, Yang H. Elucidating antimicrobial mechanism of nisin and grape seed extract against Listeria monocytogenes in broth and on shrimp through NMR– based metabolomics approach. Int J Food Microbiol. 2020;16(319): 108494.
51. Zhao L, Zhao MY, Phey CP, Yang H. Efficacy of low concentration acidic electrolyzed water and levulinic acid combination on fresh organic lettuce (Lactuca sativa Var. Crispa L.) and its antimicrobial mechanism. Food Control. 2019;101:241–50.
52. Semwal P, Painuli S, Badoni H, Bacheti RK. Screening of phytoconstituents and antibacterial activity of leaves and bark of Quercus leucotrichophora A. Camus from Uttarakhand Himalaya. Clinical Phytoscience. 2018;4:1–6.

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