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Chemical Composition of Lipoidal and Flavonoidal Extracts from Egyptian Olive Leaves with *In vitro* Biological Activities

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Abstract

The olive leaves (*Olea europaea* L.) have long been utilized for their various beneficial effects in the folk medication due to their various active phyto-constituents such as sterols, fatty acids, phenolics and flavonoids. Therefore, the study aimed to investigate the chemical composition of lipoidal and flavonoidal extracts from Egyptian olive leaves, as well as assaying the *in vitro* biological activities (antioxidant, scavenging, anti-diabetic, anti-Alzheimer and anti-arthritic activities).

It was found that 43 compounds (85.36%) were identified by GC/MS in petroleum ether *O. europaea* extract. Among these compounds, 10 unsaturated hydrocarbons, 9 saturated hydrocarbons, 9 fatty alcohols, 9 fatty acid methyl esters and 6 phytosterols were noticed. *β*-Sitosterol (1.53%), stigmasterol (1.02%), Δ^5-avenasterol (0.96%) and campesterol (0.84%) have the greatest values. Furthermore, UPLC- MS/MS negative ion mode technique clarified the presence of 3 phenolic acids, 4 flavonoid aglycones, 8 flavonoid monoglycosides, 3 flavonoid diglycosides and two seco-iridoids in the methanolic *O. europaea* extract. It is worth to mention that both petroleum ether and methanol extracts of *O. europaea* leaves proved remarkable biological activities (antioxidant, scavenging, anti-diabetic, anti-Alzheimer and anti-arthritic activities) with significant effect of methanol extract more than that of petroleum ether one. Our findings proved that the biological activities could be attributed to the combination of lipoidal and flavonoidal constituents. The study concluded that the methanolic *O. europaea* extract contains more effective phyto-constituents and exhibited higher biological efficiency than petroleum ether one.

Keywords: Olive Leaves, Lipids, Flavonoids, Antioxidant, Anti-diabetic, Anti-Alzheimer, Anti-Arthritic.

Introduction

Olea europaea L. tree is categorized as a member of the *Oleaceae* family which is considered as one of the oldest known agricultural plants in the world, especially in the Mediterranean region. In the Arabic language, it is acknowledged as Zaitoon and in the English language as Olive [1].

Olive leaves are considered as agricultural waste by-products which resulted through olive oil production, accounting for 10% of the weight of all harvested olive trees [2]**.** Olive leaves are a good source of valuable constituents with a variety of health-promoting effects because they contain large amounts of lipoidal and phenolic components [3]**.** The leaves phytoconstituents varied qualitatively as well as quantitatively as a result of numerous conditions, such as genotypes, collection times, surroundings circumstances, geographical locations, and exposition to sunlight [4]**.**

Historically, *O. europaea* leaves have been used to treat neurological and rheumatic conditions in Lebanon, as well as to relieve joint and cramps in certain areas of Iran. Therefore, they were utilized in the folk medicine as a traditional herbal tea with

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numerous curative benefits, such as gout, arteriosclerosis, and diabetes mellitus [5].

Actually, the previous study carried out by Dekanski [6] verified that *O. europaea* leaves extracts have strong antioxidant and free radical scavenging properties, making them suitable for usage in a variety of treatments. Therefore, the researchers recently have become more interested in detailed study on advantages of *O. europaea* leaves as antioxidant, anti-atherosclerotic, antihypertensive, antibacterial, and anti-mutagenic agents [7].

The present study was designed to investigate the chemical composition of lipoidal and flavonoidal extracts from Egyptian *O. europaea* leaves, as well as assaying *in vitro* biological activities.

Material and Methods

1. Phyto-chemical Investigation

1.1. Plant material

Olive leaves were collected in January 2023 from private olive farm in El-Slaheya Elgdeda, Sharkia Governorate, Egypt. The specimen of the leaves was identified by Therese Labib Youssef, a taxonomist of Botanical Orman Garden, Giza, Egypt.

1.2. Plant extraction

The dried powdered olive leaves (400g) were defatted with petroleum ether and then extracted with methyl alcohol several times till complete extraction.The obtained two extracts were separately concentrated under reduced pressure at 45^{o}C using the rotary evaporator.The concentrated extracts were kept separately in tightly closed containers in refrigerator for chemical and biological investigation.

1.3. Quantification of total polyphenols and tannins

Concentration of the total polyphenols was quantified in both petroleum ether and methanol extracts of Egyptian olive leaves as mg gallic acid/100 gm using Folin Ciocalteu reagent according to the method suggested by Singleton and Rossi [8]. In addition, total tannin content was assessed using tannic acid as a reference based on the method described by Broadhurst and Jones [9].

1.4. Identification of the lipoidal constituents in the petroleum ether extract

Using GC/MS technique, a Shimadzu GC/MS-QP5050A, the petroleum ether extract of olive leaves was investigated. By comparing the spectral fragmentation patterns of the compounds with those of available database libraries (Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA)) as well as published research papers, the lipoidal compounds have been identified. Based on peak area integration, quantitative determination was carried out.

1.5. Identification of the major phenolics and flavonoids

UPLC-QTOF-MS/MS negative ion mode was carried out on a Vendor/ Specstriple quadruple instrument for characterization of the major phenolics and flavonoids in the methanol extract of olive leaves, where HPLC-MS system was composed of an autosampler injector (Switzerland), waters corporation (Milford, MA01757, U.S.A) and mass spectrometer. ColumnACQUITY UPLC-BEH Waters (X select HSS T3)C18 1.7 µm- 2.1 × 50 mm. Mobile phase elution was made with the flow rate of 0.3 mL/min using gradient mobile phase comprising two eluents: eluent A is 5 mM ammonium formate buffer pH 3 containing 1% methanol and eluent B is 100 % acetonitrile. The peaks and spectra were processed using the Analyst TF 1.7.1software and tentatively identified by comparing its retention time (Rt) and mass spectrum with reported data.

1.6. Isolation and structure elucidation of the principal flavonoids

One gram of the methanol extract of olive leaves were subjected to Ready-made chromatographic plates (20x20cm) coated with silica gel F254 for the detection of flavonoids using a developing system chloroform- ethyl acetate -acetone (5:1:4) [10]**.** The bands giving yellow color after spraying with $ALCL_3$ spray reagent were marked and scratched separately [11]. The isolated flavonoids were identified by a number of spectroscopic investigations and compared with previous literature.

2. *In vitro* Biological Activities

All the biological activities were assayed in both petroleum ether and methanolic *O. europaea* leaves extracts and the analyses were carried out in three replicates.

2.1. Antioxidant Activity

Total antioxidant capacity (TAC) was assessed as mg gallic acid/gm by evaluating the green phosphate/Mo^{5+} complex at wavelength (λ) 695 nm based on the method suggested by Prieto [12]. Also, the iron reducing power (IRP) was tested as µg/mL using ascorbic acid as standard according to the method demonstrated by Oyaizu [13].

2.2. Free Radical Scavenging Activities

The scavenging activity was assayed by determining the ability of each plant extract to scavenge the free radicals. The median inhibitory concentration (IC_{50}) that required from the tested extract to inhibit 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radicals was calculated according to the method suggested by Rahman[14] . Furthermore, the scavenging activity against 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals was evaluated by calculating inhibition percent (%) of the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical

using ascorbic acid as standard based on the method described by Arnao [15].

2.3. Anti-Diabetic Activity

The anti-diabetic activity was assessed in both petroleum ether and methanol extracts of Egyptian olive leaves by calculating inhibition percents (%) of α-amylase and α-glucosidase enzymes according to the methods established by Wickramaratne [16] and Pistia-Brueggeman and Hollingsworth [17], respectively using acarbose as standard drug.

2.4. Anti-Alzheimer's Activity

It was determined by calculating the inhibition percent (%) of acetyl cholinesterase (AChE) enzyme using donepezil as standard drug according to Ellman's method [18].

2.5. Anti-Arthritic Activity

This assay was carried out by calculating percent (%) of protein denaturation [19] and proteinase inhibition [20] using the diclofenac sodium that was prepared using the method suggested by Meera [21] as standard drug.

3. Statistical Analysis

One-way analysis of variance (one-way ANOVA) was carried out using Statistical Package for Social Sciences (SPSS for windows, version 11.0) for evaluating the statistical correlations (positive and negative) among the different biological measurements. The correlation was considered significant at $P < 0.05$ and considered highly significant at $P < 0.01$.

Results and Discussion

1. Phyto-chemical Investigation

1.1. Quantification of total polyphenols and tannins

The concentration of total polyphenols and total condensed tannins was quantified in both petroleum ether and methanol extracts of Egyptian olive leaves.The results were explained in table 1, where the methanol extract showed greater values of polyphenols (106.67 mg/100g) and tannins (42.67 µg/ml) than that exist in the petroleum ether extract (35.82 mg/100g &14.33µg/ml, respectively).

The quantification assay was in agreement with the study reported by Ibrahim [22] and supported consequently by Mohamed [23] who stated that olive leaves are considered as a rich source of phenolic constituents**.** Moreover, Salah, [24] studied the extraction yield and total polyphenols content in eight olive cultivars leaves and established that the methanol was the best solvent for olive leaves extraction, as it produced high polyphenols content.

Table 1. Content of total polyphenols and total condensed tannins in petroleum ether and methanol extracts of Egyptian olive leaves.

Plant extract	Total Polyphenols (mg gallic acid/100 gm)	Total Condensed Tannins (µg/ml)
Petroleum ether	35.82 ± 0.06	14.33 ± 0.02
Methanol	**106.67 ± 0.25**	**42.67 ± 0.10**

Values were calculated from three replicates and expressed as mean ± SE.

1.2. GC/MS identification for the lipoidal constituents

The petroleum ether extract of Egyptian olive leaves was subjected to GC/MS investigation, and the constituents were identified by comparing their spectral fragments to those of the available database archives Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA)]. The contents and composition of the lipoidal constituents are presented in table 2. Fourty three compounds (85.36%) were identified in the lipoidal matter of olive leaves, among which 10 unsaturated hydrocarbons, 9 saturated hydrocarbons, 9 fatty alcohols, 9 fatty acid methyl esters and 6 phytosterols were characterized.

5-Octadecene (5.02%) and 10-heneicosene (4.08%) were the principal unsaturated hydrocarbons, while docosane (4.05%) and pentadecane (3.39%) were the chief identified saturated hydrocarbons. Furthermore, heptadecanol and eicosanol were the major identified fatty alcohols in the lipoidal matter with the values of 4.08% and 4.79%, respectively.

Additionally, six saturated fatty acids (SFA) (7.99%) and three unsaturated fatty acids (UFA) (8.91%) were characterized. Palmitic acid (C16:0) was detected as the major saturated fatty acid with the concentration of 3.08%, while oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) were the principal detected unsaturated fatty acids which constituted 4.69, 2.46, &1.76%, respectively.

Furthermore, the sterols profile in olive leaves lipoidal matter is studied. β-Sitosterol (1.53%), stigmasterol (1.02%),Δ^5-avenasterol (0.96%) and campesterol (0.84%) have the greatest values, while 3-methoxy-28-norolean-17-ene (0.45%) and oleanolic acid (0.32%) were found in minor amounts. In the last few years, the sterols composition of the lipoidal matter of olive leaves has attracted specific interest due to its nutritious and health-promoting benefits as antimicrobial, anti-inflammatory and cancer-fighting properties [25]**,** as well as its ability to lower plasma total cholesterol and low-density lipoprotein (LDL) [26].

It is interesting to note that the investigation outcomes are consistent with published researches **[27, 28]**, supporting the positive impacts of the lipoidal matter extracted from olive leaves on human health for lowering the risk of cardiovascular disease

because of its characteristic fatty acid composition and phytosterols [29].

Table 2. GC/MS analysis of petroleum ether extract of Egyptian olive leaves.

Chemical class	Compound	BP	Molecular weight	Molecular formula	Area %	Total area %
Unsaturated hydrocarbons	3-Dodecene	41	168	$C_{12}H_{24}$	0.93	**24.77**
	4-Tetradecene	43	196	$C_{14}H_{28}$	1.49	
	Pentadecene	43	210	$C_{15}H_{30}$	3.42	
	9-Octadecene	43	252	$C_{18}H_{36}$	2.87	
	Heptadec-8-ene	55	238	$C_{17}H_{34}$	1.95	
	5-Octadecene	55	252	$C_{18}H_{36}$	5.02	
	1-Nonadecene	55	266	$C_{19}H_{38}$	3.21	
	5-Eicosene	55	280	$C_{20}H_{40}$	1.04	
	10-Heneicosene	55	294	$C_{21}H_{42}$	4.08	
	1-Tetracosene	43	336	$C_{24}H_{48}$	0.76	
Saturated hydrocarbons	Pentadecane	57	212	$C_{15}H_{32}$	3.39	**16.15**
	2,6,10-Trimethyl	57	226	$C_{16}H_{34}$	0.57	
	2-Methylheptadecane	57	254	$C_{18}H_{38}$	2.31	
	2-Methyloctadecane	57	268	$C_{19}H_{40}$	1.34	
	Docosane	43	310	$C_{22}H_{46}$	4.05	
	Tetracosane	57	338	$C_{24}H_{50}$	1.63	
	2-Cyclohexyleicosane	82	364	$C_{26}H_{52}$	0.87	
	Hentriacontane	57	436	$C_{31}H_{64}$	1.58	
	Dotriacontane	57	450	$C_{32}H_{66}$	0.41	
Fatty alcohol	1-Octen-4-ol	69	128	$C_8H_{16}O$	1.30	**22.42**
	Tetradecanol	55	214	$C_{14}H_{30}O$	1.49	
	Hexadecanol	43	240	$C_{16}H_{16}O_2$	3.42	
	Heptadecanol	55	256	$C_{17}H_{36}O$	4.08	
	Nonadecanol	55	284	$C_{19}H_{40}O$	2.37	
	Eicosanol	83	298	$C_{20}H_{42}O$	4.79	
	Tetracosanol	55	354	$C_{24}H_{50}O$	1.54	
	Hexacosanol	43	382	$C_{26}H_{54}O$	0.96	
	Triacontanol	43	438	$C_{30}H_{62}O$	2.47	
Fatty acid methyl esters	Nonanoic acid methyl ester(C9:0)	74	172	$C_{10}H_{20}O_2$	1.20	**16.9**
	Myristic acid, methyl ester (C14:0)	74	242	$C_{15}H_{30}O_2$	0.94	
	Methyl pentadecanoic acid methyl ester	74	256	$C_{16}H_{32}O_2$	1.23	
	Palmitic acid, methyl ester (C 16:0)	74	270	$C_{17}H_{34}O_2$	3.08	
	Linolenic acid, methyl ester (C18:3)	67	292	$C_{19}H_{32}O_2$	1.76	
	Linoleic acid, methyl ester (C18:2)	67	294	$C_{19}H_{34}O_2$	2.46	
	Oleic acid methyl ester (C18:1)	55	296	$C_{19}H_{36}O_2$	4.69	
	nonadecanoic acid methyl ester (C19:0)	74	312	$C_{20}H_{40}O_2$	0.83	
	Triacontanoic acid methyl ester (C30:0)	74	466	$C_{31}H_{62}O_2$	0.71	
Phytosterols	β-sitosterol	43	414	$C_{29}H_{50}O$	1.53	**5.12**
	Δ^5-avenasterol	55	412	C29H48O	0.96	
	Campesterol	43	400	$C_{28}H_{48}O$	0.84	
	Stigmasterol	55	412	$C_{29}H_{48}O$	1.02	

	3-Methoxy-28-norolean-17-ene	191	426	$C_{30}H_{50}O$	0.45	
	Oleanolicacid(3b-hydroxy-5a-olean-12-en-28-oicacid)	203	456	$C_{30}H_{48}O_3$	0.32	

1.3. Identification of the major phenolics and flavonoids

Due to the numerous positive impacts of olive leaves on human health, many research investigations have recently focused on correlation of the observed biological activities with their chemical characterization [7].

To chemically identify the phenolic and flavonoid constituents in olive leaves, UPLC-MS/MS negative ion mode analysis was performed. A variety of compounds were recorded as phenolic acids, flavonoid aglycones, flavonoid glycosides and seco-iridoids via chemical classification as illustrated in table 3. These chemical compounds have a wide range of health impacts [30]**.** Therefore, the positive health effects of olive leaves could be ascribed to the grouping of these bioactive constituents [31]. Worthily, the phenolic constituents found in olive leaves are responsible for the majority of bioactivities **[7]**. **Kabbash [32]** earlier characterized many flavonoidal compounds in olive leaves comprising flavonolsas quercetin andrutin, flavones as luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin, beside to flavan-3-ols as catechin**.**

In the present research study, 3 phenolic acids, 4 flavonoid aglycones, 8 flavonoid monoglycosides, 3 flavonoid diglycosidesand two seco-iridoids were identified in the methanol extract of olive leaves. The phenolic acids were caffeic acid, dihydrocaffeic acid, and quinic acid with m/z 179, 181,and 191, respectively were detected in the negative ionization mode. In addition to characterization of the flavonoid aglycones (apigenin, luteolin, chrysoeriol and quercetin) with their mono and di glycosides. Moreover, the two seco-iridoids were identified as oleuropein and oleuropein-3'-glucoside with molecular weight $[M - H]^-$ at 539 and 701, respectively. The results of this investigation line up with previously conducted studies [33-35].

Table 3. Identification of the main phenolics and flavonoids in olive leaves by UPLC- MS/MS negative ion mode analysis.

Class	Name	$[M - H]^-$	Molecular	Main fragments
Phenolic acids	Caffeic acid	179	$C_9H_8O_4$	107, 135
	Dihydrocaffeic acid	181	$C_9H_{10}O_4$	109, 121, 137
	Quinic acid	191	$C_7H_{12}O_6$	109, 127, 160
Flavonoid aglycones	Apigenin	269	$C_{15}H_{10}O_5$	117,151, 227
	Luteolin	285	$C_{15}H_{10}O_6$	147, 285
	Chrysoeriol	299	$C_{16}H_{12}O_6$	227, 256, 284
	Quercetin	301	$C_{15}H_{10}O_7$	121, 151, 178
Flavonoid mono-glycosides	Apigenin-7-O-glucoside	431	$C_{21}H_{20}O_{10}$	239, 269
	Apigenin-7-O-rutinoside	577	$C_{27}H_{30}O_{14}$	269, 433
	Quercetin-3-O-rhamnoside	447	$C_{21}H_{20}O_{11}$	257, 301
	Quercetin-3-Glucuronide	477	$C_{21}H_{18}O_{13}$	151,301
	Quercetin-3-Arabinoside	433	$C_{20}H_{18}O_{11}$	271, 301
	Rutin	609	$C_{27}H_{30}O_{16}$	271, 301
	Luteolin-7-O-glucoside	447	$C_{21}H_{20}O_{11}$	285, 412
	Luteolin-7-O-rutinoside	593	$C_{27}H_{30}O_{15}$	285, 383, 412
Flavonoid di-glycosides	Apigenin 6,8-di-C-glucoside	593	$C_{27}H_{30}O_{15}$	117, 269
	Luteolin-3', 7-di-O-	609	$C_{27}H_{30}O_{16}$	285, 447
	Quercetin 3,4'-diglucoside	625	$C_{27}H_{30}O_{17}$	301, 463
Seco-iridoids	Oleuropein	539	$C_{25}H_{32}O_{13}$	223, 307, 377
	Oleuropein-3'-glucoside	701	$C_{31}H_{42}O_{18}$	135, 315, 469

1.3. Structure elucidation of the principal flavonoids

Apigenin-7-O-glucoside was isolated as yellowish crystals, m.p.180 ºC. It produced a dark purple color under short UV light and turned to yellow with $AlCl_3$ spray reagent with R_f of 0.89, UV-λmax nm; MeOH (263, 338) for flavonoid skeleton, MeOH+NaOMe (240sh,270,305sh,389) gave bathochromic shift in band I with increased intensity confirming the presence of polyhydroxyl groups. MeOH+$AlCl_3$ (270,304, 350,387) bathochromic shift indicative for free OH group at C-3 or C-5; MeOH+$AlCl_3$/HCl (273,300, 348,386) no hypsochromic shift was observed; indicating absence of *ortho*-dihydroxyl groups. MeOH+NaOAc (265, 352,388) no change in UV absorbance of band II indicating substitution of 7-OH group, while bathochromic shift was observed in band I indicating presence of a free 4`hydroxyl group. MeOH+NaOAc/ H_3BO_3 (266,350) no change in UV absorbance was observed; indicating absence of *ortho*-dihydroxyl groups. ^{1}H NMR (400 MHz, DMSO, δ ppm): 6.98 (s, H-3),6.51 (d, J 2.3 Hz, H-6), 6.87 (d, J 2.3 Hz, H-8), 8.14 (d, J 8.5 Hz, H-2′,6′), 7.35 (d, J 8.5 Hz, H-3′, 5′), 5.12 (d, J 7.1 Hz, H-1″), 3.24-3.81 (m, sugar protons). The achieved spectral data matched to Sezen Karaoğlan, [36]**.**

quercetin-7-O-glucoside was isolated as yellow crystals with melting point 248 °C. It produced brown spot under UV light and converted to yellow color with $AlCl_3$ reagent with R_f of 0.83, UV-λmax nm; MeOH (254,268sh, 370) with absorbance more than 350 nm indicating presence of flavonol nucleus; MeOH+NaOMe (246sh,290, 365,455) producing bathochromic shift confirming the presence of polyhydroxyl groups. MeOH+$AlCl_3$ (259sh,274, 339,457) bathochromic shift indicating presence of hydroxyl group at C-3 or C-5 or C-3 and C-5 or *ortho*-dihydroxyl groups; MeOH+$AlCl_3$/HCl (260,302sh, 342,458) keeping UV absorbance was observed in band I and II indicating presence of hydroxyl group at C-3 and C-5. MeOH+NaOAc (285,378,427sh) showing bathochromic shift of band I s ascribable to free 4`-OH group; MeOH+NaOAc/ $H_3BO_3$ (261,290sh,452) with bathochromic shift of band I indicates the presence of 3`, 4`dihydroxy group. ^{1}H-NMR (500 MHz, CD_3OD) δ / ppm: 6.39(1H, d, *J*=2.4Hz ,H-6), 6.78(1H, d, *J*=2.4Hz ,H-8), 7.88(1H, d, *J*=2.1Hz ,H-2'), 6.94(1H, d, *J*=8.3Hz ,H-5'), 7.62(1H, dd, *J*=2.1 , 8.3Hz , H-6'), 5.03 (H-1``). ^{13}C-NMR (125 MHz, CD_3OD) δ / ppm: 156.85 **(C-2)**, 127.30 **(C-3)**, 180.25 **(C-4),** 161.46 **(C-5),** 94.63 **(C-6),** 164.85 **(C-7),** 95.21 **(C-8),** 162.59 **(C-9),** 99. 74 **(C-10),** 121.36**(C-1`),** 106.56 **(C-2`),** 145.26 **(C-3`),** 159.31 **(C-4`),** 104.12 **(C-5`),** 116.81 **(C-6`),** 102.41 **(C-1``),** 74.51 **(C-2``),** 77.37 **(C-3``),** 68.53 **(C-4``),** 78.93**(C-5``),** 61.77**(C-6``).** The aforementioned spectroscopical data were in agreement with that reported by Legault [37].

quercetin-3,7-diglucopyranoside was isolated as yellow crystals with melting point 225 °C. It produced purple color under UV light and changed to yellow color after spraying $AlCl_3$ with R_f of 0.78, UV-λmax (MeOH) nm: 255,267sh, 359 with absorbance more than 350 nm pointing to presence of flavonol nucleus; MeOH+NaOMe (269,299sh,398) producing bathochromic shift directing to existence of polyhydroxyl groups. MeOH+$AlCl_3$ (271,299sh, 337,443) gave bathochromic shift signifying occurrence of hydroxyl group at C-3 or C-5 or C-3 and C-5 or *ortho*-dihydroxyl groups; MeOH+$AlCl_3$/HCl (270,300sh, 340,440) with stable UV absorbance in band I and II representing presence of hydroxyl group at C-3 and C-5. MeOH+NaOAc (261,295sh, 376,424sh) displaying bathochromic shift of band I for free 4`-OH group; MeOH+NaOAc/ $H_3BO_3$ (260, 435) with bathochromic shift of band I directs the presence of 3`, 4`dihydroxy group. ^{1}H-NMR (500 MHz, CD_3OD) δ / ppm: 6.34(1H, d, *J*=2.2Hz, H-6), 6.59(1H, d, *J*=2.2Hz, H-8), 7.63(1H, d, *J*=3.4Hz, H-2'), 6.85(1H, d, *J*=8.5Hz, H-5'), 7.54(1H, dd, *J*=3.4 , 8.5Hz, H-6'), 5.01 (H-1``), 5.17 (H-1```). ^{13}C-NMR (125 MHz, CD_3OD) δ / ppm: 157.12 **(C-2)**, 134.42 **(C-3)**, 177.82 **(C-4),** 161.32 **(C-5),** 98.78 **(C-6),** 163.61 **(C-7),** 95.85 **(C-8),** 158.01 **(C-9),** 106.25 **(C-10),** 123.41 **(C-1`),** 115.78 **(C-2`),** 145.31 **(C-3`),** 148.41 **(C-4`),** 117.84 **(C-5`),** 122.89 **(C-6`),** 101.33 **(C-1``),** 71.65 **(C-2``),** 72.51**(C-3``),** 68.34 **(C-4``),** 77.81 **(C-5``),** 60.54 **(C-6``),**101.32 **(C-1```),** 70.69 **(C-2```),** 73.22 **(C-3```),** 66.65**(C-4```),** 76.45 **(C-5```),** 61.76 **(C-6```).** The spectral data matched to Al-Taweel [38].

2. *In Vitro* Biological Activities

2.1. Antioxidant Activity

Many prevalent diseases including cancer, atherosclerosis, rheumatoid arthritis and aging-related degenerative processes, are thought to entail excessive lipid oxidation and inflammation. The principal approach for avoiding as well as treating such conditions may be through reducing this extra oxidation processes by exogenous consumption of natural antioxidants such as olive leaves products for example **[7]**. Both petroleum ether and methanol extracts of Egyptian olive leaves proved remarkable total antioxidant capacity (TAC), iron reducing power (IRP), and free radical scavenging activities against DPPH and ABTS, with significant effect of methanol extract more than that of petroleum ether as illustrated in table 4.

Anter [39] reported that olive leaves were beneficial in cell protecting against the oxidative

injury produced by hydrogen peroxide without genotoxication and therefore, they can be utilized to enrich human health. Additionally, it has been established that the chemical structure of the phenolic compounds plays a significant role in their antioxidant properties; therefore, It has been speculated that the great antioxidant and scavenging ability of olive leaves extracts could be related to the abundance of phenolics and flavonoids with high degree of hydroxylation in their skeletons **[6]**, which interact with the free radicals to produce more stable products **[40]**. Additionally, Kermanshah [7], reported greater antioxidant effects of olive leaves oil than butylated hydroxyl toluene (BHT) due to various sterols and fatty acid composition.

2.2. Anti-diabetic Activity

The *in vitro* anti-diabetic activity of the petroleum ether and methanol extracts of Egyptian olive leaves was assessed in comparison with acarbose as a standard drug. The study verified that both extracts inhibited α-amylase and α-glucosidase by 36.13 & 26.95% for petroleum ether extract and by 36.57 & 26.54% for methanol extract in a remarkable value when compared with acarbose (inhibition percentage= 67.33 & 54.33%, respectively) as illustrated in table 5. The study's findings coincided with those published by Eidi [41], who examined the antidiabetic influence of alcoholic olive leaves extract in normal and streptozotocin-induced diabetic rats for 14 days and proved the significant decrease in serum glucose level. Oleuropoeside is one of the main active constituents that manage this action. According to Gonzalez [42], this compound's hypoglycemic effect may be caused by two different mechanisms; either by stimulation of sugar-induced insulin secretion or by improving the peripheral glucose intake.

2.3. Anti-Alzheimer's Activity

The *in vitro* anti-Alzheimer activity of the petroleum ether and methanol extracts of Egyptian olive leaves was evaluated in comparing with donepezil as a standard drug. The study proved that methanol extract have a significant inhibition of acetylcholinesterase by 51.88% in a value near to that of donepezil (68.34%) as shown in table 6. The activity may be attributed to the various phenolic and flavonoids constituents which play in a synergism mode [43].

Despite the paucity of studies concerning treatment of Alzheimer disease by olive leaves, a recent record proposed that olive leaves consuming boosts autophagy and recovers proteostasis, which is evident in decreased harmful protein aggregation in Alzheimer disease. As consequently, olive active constituents could be used as useful supplement in the treatment of Alzheimer disease [44].The reduction of neuroinflammation and oxidative stress through NF-B and Nrf2 regulation, respectively, might represent the mechanism driving those beneficial actions [45]**.**

2.4. Anti-arthritic Activity

The anti-arthiritic effect of the petroleum ether and methanol extracts of Egyptian olive leaves was examined in comparison with diclofenac sodium as a standard drug. The investigation proved that both extracts have similar remarkable proteinase denaturation percentage by 34.88% & 34.60%, respectively and proteinase inhibition by 31.31 & 31.03%, respectively as presented in table 7.

The study outcomes are in consistence with that achieved by Rosillo [46] who assessed the anti-inflammatory impact of olive oil polyphenolic components in mice with collagen-induced arthritis. Kaneko [47] additionally investigated into how olive leaves extracts affected the reduction of cytokine production, and stated that using olive leaves extract for treatment of inflammation could reduce the release of pro-inflammatory cytokines and the expression of the NF-B p65 protein in human placenta cell cultures. The statistical correlations among the different *in vitro* biological activities of the tested extracts were illustrated in table 8.

Table 4. Antioxidant capacity and scavenging activity of petroleum ether and methanol extracts of Egyptian olive leaves.

Tested group	Antioxidant Activity		Scavenging Activity	
	TAC (mg gallic acid/gm)	**IRP** (µg/mL)	**DPPH** (IC_{50} µg/ml)	**ABTS** (%)
Petroleum ether extract	85.97 ± 0.14	50.15 ± 0.08	11.63 ± 0.02	20.47 ± 0.03
Methanol extract	**256.01 ± 0.60**	**149.34 ± 0.35**	**3.91 ± 0.01**	**60.96 ± 0.14**
Ascorbic Acid (standard)	-	-	**3.58 ± 0.01**	**41.75 ± 0.10**

Values were calculated from three replicates and expressed as mean ± SE.

Table 5. The *in vitro* anti-diabetic activity of the petroleum ether and methanol extracts of Egyptian olive leaves.

Tested group	Inhibition (%)	
	α-amylase	α-Glucosidase
Petroleum ether extract	36.13 ± 0.06	26.95 ± 0.07
Methanol extract	36.57 ± 0.09	26.54 ± 0.09
Acarbose (standard)	**67.33 ± 0.16**	**54.33 ± 0.01**

Values were calculated from three replicates and expressed as mean ± SE.

Table 6. Anti-Alzheimer effect of petroleum ether and methanol extracts of Egyptian olive leaves.

Tested group	AChE inhibition %
Petroleum ether extract	8.86 ± 0.02
Methanol extract	**51.88 ± 0.12**
Donepezil (standard)	**68.34 ± 0.16**

Values were calculated from three replicates and expressed as mean ± SE.

Table 7. Anti-arthiritic effect of petroleum ether and methanol extracts of Egyptian olive leaves.

Tested group	Percentage (%)	
	Proteinase Denaturation	Inhibition of Proteinase
Petroleum ether extract	34.88 ± 0.08	31.31 ± 0.08
Methanol extract	34.60 ± 0.08	31.03 ± 0.08
Diclofenac Sodium (standard)	**45.92 ± 0.11**	**43.72 ± 0.11**

Values were calculated from three replicates and expressed as mean ± SE.

Table 8: The statistical correlations among the different *in vitro* biological activities of the tested extracts.

		Phyto-constituents		Antioxidant Activity		Scavenging Activity		Inhibition (%)			Anti-arthritic activity	
								Anti-diabetic Activity		Anti-Alzheimer Activity		
		TPC	TCT	TAC	IRP	DPPH	ABTS	α-amylase	α-Glucosidase	ACE	Proteinase Denaturation	Inhibition of Proteinase
Phyto-constituents	TPC	-	0.000**	0.000**	0.000**	-0.000**	0.000**	0.012*	0.022*	0.001**	0.071	0.070
	TCT	0.000**	-	0.000**	0.000**	-0.000**	0.000**	0.012*	0.022*	0.000**	0.071	0.070
Antioxidant	TAC	0.000**	0.000**	-	0.000**	-0.000**	0.000**	0.012*	0.022*	0.000**	0.071	0.070
	IRP	0.000**	0.000**	0.000**	-	-0.000**	0.000**	0.012*	0.022*	0.000**	0.071	0.070
Scavenging	DPPH	-0.000**	-0.000**	-0.000**	-0.000**	-	0.000**	0.012*	0.022*	0.000**	0.071	0.070
	ABTS	0.000**	0.000**	0.000**	0.000**	0.000**	-	0.012*	0.022*	0.000**	0.071	0.070
Inhibition (%) Anti-diabetic	α-amylase	0.012*	0.012*	0.012*	0.012*	0.012*	0.012*	-	0.209	0.012*	0.373	0.370
	α-Glucosidase	0.022*	0.022*	0.022*	0.022*	0.022*	0.022*	0.209	-	0.022*	0.001**	0.001**
Inhibition (%) Anti-Alzheimer	ACE	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.012*	0.022*	-	0.070	0.070
Anti-arthritic	Proteinase Denaturation	0.071	0.071	0.071	0.071	0.071	0.071	0.373	0.001**	0.070	-	0.001**
	Inhibition of Proteinase	0.070	0.070	0.070	0.070	0.070	0.070	0.370	0.001**	0.070	0.001**	-

Conclusion

The study verified that Egyptian olive leaves are considered as a promising medicinal plant with antioxidant, anti-diabetic, anti-Alzheimer and anti-arthritic. These biological activities could be attributed to the combination of various lipoidal and flavonoidal constituents, encouraging the scientists to use this plant leaves in an economical and accessible means.

Conflict of interest

The authors clarify that they have no conflicts of interest in this research paper.

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