A new terpenoid from *Olea europaea* L. leaves and potential aptitude of different leaf extracts as anti-inflammatory agents

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Background
The olive tree (*Olea europaea* L.) has played a significant role in medical history, being used to relieve a wide range of illnesses in traditional medicine. The value of olive leaves came from their medicinal and nutritional properties. In recent times, it has been proven that there is a potential role of leaves in cancer prevention and inflammatory illnesses.
Objective
The purpose of this study was to look at the chemical makeup of methylene chloride fraction and also to evaluate the chemopreventive properties and the anti-inflammatory activity of different olive leave fractions using hepa1c1c7 cells and RAW264.7 macrophages activated by lipopolysaccharides, respectively.
Materials and methods
Air-dried powdered olive leaves were defatted with hexane, and the marc was then soaked in 80% methanol and successively extracted with CH_2Cl_2, EtOAc, and n-BuOH. Phytochemical investigation of CH_2Cl_2 fraction was done. The chemopreventive effect was determined *in vitro* using a hepa1c1c7 human carcinoma cell line, and the anti-inflammatory was carried out using RAW264.7 macrophages.
Results and conclusion
One novel compound, identified and given the name oleuropeinone (**1**), was obtained together with two known compounds, blumenol A (**2**) and megaritolactonol (**3**). Spectroscopic data such as ^1H and ^{13}C NMR were used to identify their chemical structures. The CH_2Cl_2 fraction is the most effective fraction to inhibit inflammatory markers inducible nitric oxide synthase and COX-2 protein expression in RAW264.7 macrophages. Moreover, it has a potent effect in inducing the chemopreventive marker NQO1 protein expression.

Keywords:
anti-inflammatory, chemopreventive activity, olive leaves, terpenoid lactone

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Introduction

The olive tree (*Olea europaea* L.) is considered one of the oldest cultivated plants that exist in the Mediterranean basin (monoecious plant). It belongs to the Oleaceae family and is well known for its edible fruits [1]. Olive leaves and fruits are used in traditional medicine to treat diarrhea, diabetes, inflammation, asthma, hypertension, and respiratory and urinary tract diseases [2]. Recently, olive leaves have received more attention owing to their therapeutic value because of the existence of several compounds such as flavonoids, secoiridoids, triterpenes, and polyphenols [3]. These constituents possess many interesting biological activities such as antimicrobial, carcinogenesis, antioxidant, anti-inflammatory, and reducing mutagenesis (*in vitro* and *in vivo*) [4]. In addition, many recent studies reported the ability of phenolic, secoiridoid, and triterpene compounds to inhibit specific enzymes involved in the inflammatory process [5] by acting as an assailant of signal transduction pathways and modulating the expression of the pro-inflammatory gene [6]. It is well evidenced that the inflammation process leads to excessive production and accumulation of arachidonic acid metabolites, oxygen, and reactive nitrogen species. These metabolites play a significant role in cancer occurrence [7,8]. Recent studies illustrate that polyphenols and sesquiterpenes isolated from olive leaves possess the ability to inhibit pro-inflammatory

enzymes, such as nitric oxide synthase (iNOS) [9,10], COX-2, lipoxygenase, and suppression tyrosine kinases, NF-κB, phosphoinositide 3-kinase, and AP-1 [11,12]. Therefore, they prevent the occurrence of some chronic diseases like cancer [8]. In our previous study [13], different leaf extracts, excluding CH_2Cl_2 fraction, were evaluated chemically and biologically for their phenolics and flavonoids as well as for microelements and macroelements in addition to the fatty acids. Moreover, antioxidant and antimicrobial activities were carried out. In the present study, we investigated the phytochemical composition of CH_2Cl_2 fraction as well as anti-inflammatory activities, and chemopreventive properties of different extracts from *O. europaea* L. leaves.

Materials and methods

Plant material

Olive leaves (*O. europaea* L.) were harvested from a farm located in Ein-Helwan, Cairo, Egypt in August 2016 (plant samples were kept in the deep freeze) and kindly authenticated by Professor Dr Mona Marzouk, Department of Phytochemistry and Plant Systematic, National Research Center, Egypt.

Experimental

General experimental procedures

A reversed-phase high-performance liquid chromatography using an Agilent pump system operated with an Agilent-G1200 with a refractive index detector was used. Precoated silica gel plates (Kiesel gel 0.25 mm, 60 F_{254}) were used for the thin-layer chromatography technique and envisioned by heating after spraying with 10% vanillin-H_2SO_4.

One and two-dimensional-NMR were performed were performed in $CDCl_3$ on an Ultra Shield Plus system operating at 700 MHz for ^1H and at 175 MHz for ^{13}C (Bruker Biospin GmbH, Rheinstetten, Germany). Chemical shifts (δ) in ppm and coupling constants (*J*) (Hz) were recorded. An internal standard Tetramethylsilane (TMS) was used. ESI-MS negative and positive ion acquisition mode was performed on a XEVO TQD triple quadruple instrument (Waters Corporation, Milford, Massachusetts, USA). Column flow rate was 0.2 ml/min. Acquity Uplc – Beh C^{18} 1.7 µm – 2.1×50 mm column was used, with the following solvent systems: solvent system I: water containing 0.1% formic acid and solvent system II: methanol containing 0.1% formic acid.

Extraction and isolation

Olive leaves were washed with cold water to remove any dust and impurities, dried in the oven at 40°C, and powdered with an electric mill. Approximately 620 g of the dried powdered leaves were extracted with the liquid–liquid method starting with hexane (3×1 L) using an ultrasonic bath (UP400S, 220 V, and 20 Hz) at 25°C. The solvent was removed using a rotary evaporator Bushi (BUCHI Labortechnik AG. B01MR0RG55.Switzerland) at 40°C to give a dark greenish residue (~12.26 g). The dried marc was extracted with 80% methanol (3×1 l). The extracts were pooled and evaporated under reduced pressure at 47°C to produce a dark brown remnant (~154 g), which was dissolved in 200 ml of distilled water and then successively extracted with CH_2Cl_2, EtOAc, and n-BuOH (3×500 ml). All fractions were evaporated under reduced pressure at 45°C to give 2, 4.5, and 18.5 g, respectively. CH_2Cl_2 fraction was subjected to isolation and purification by high-performance liquid chromatography on a reversed-phase C^{18} column (5 µm, 250×10 mm inner diameter) using a mobile phase composed of methanol/water (30 : 70, v/v), at a flow rate of 1 ml/min with a refractive index detector. Three compounds [**1** (15 mg), **2** (8 mg), and **3** (10 mg)] were isolated and identified. The preparation of saponified and unsaponified fractions was carried out according to our previous work [13].

Biological investigation

Cell culture

For anti-inflammatory activity, we employed murine macrophage cell RAW264.7 cells (ATCC®) were maintained in Dulbecco's Modified Eagle's Medium (DMEM from Lonza, Verviers, Belgium) supplemented with 10% FBS, penicillin (100 U/ml), streptomycin sulphate (100 µg/ml) and 4 mM L-glutamine. For chemopreventive activity, murine hepatoma cell line Hepa-1c1c7 was maintained as monolayer culture in α- modified Minimum Essential Medium Eagle (α-MEME) supplemented with 10% (v/v) heat-and charcoal–inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin sulphate. Both cells were maintained and incubated in a humidified 5% CO_2 incubator.

Anti-inflammatory activity

Overnight 6-well plate culture of RAW264.7 (initially seeded as 1.5×10^6 cells/well) was subjected to different olive leaf fractions [80% methanol, ether, methylene dichloride, ethyl acetate, n-butanol, saponified and unsaponified fractions (100 µg/ml), or indomethacin (250 µM) in the absence or the presence of 100 ng/ml of lipopolysaccharides (LPS) (LPS– or LPS+, respectively)]. The relative protein expression of the pro-inflammatory marker iNOS was assessed according to Hamed *et al.* [14]. Following 24 h of treatment, RAW264.7 cells were washed using ice-

cold PBS and scrapped in RIPA lysis buffer followed by sonication and centrifugation (15,000 × *g* for 10 min). Supernatants were assessed for iNOS protein expression as described later.

Cancer chemopreventive activity (NQO1 induction)

The induction of the chemopreventive marker NAD (P)H quinone dehydrogenase 1 (NQO1) in the murine hepatoma cell line was assessed. In brief, Hepa1C1C7 cells (1.5×10^5 cells/ml) were seeded onto 6-well plates and incubated overnight to form semiconfluent monolayers. Monolayers were treated with either vehicle (final concentration 0.1% v/v DMSO) or olive leaf fractions (100 µg/ml) for supplemental 24 h. In parallel, 4'-bromoflavone was used as a positive control for NQO1 induction. After aspiration of treatment media, the treated monolayers were washed with ice-cold Dulbecco's PBS (2 ml/well). Then, cells were then scrapped in ice-cold lysis buffer (25 mMTris-Cl, pH 7.4, 250 mM sucrose and 5 µM FAD) and transferred to labeled microcentrifuge tubes. Cell suspensions were then sonicated on ice for 5 s (20% amplitude). Sonicates were then centrifuged (15,000 × g for 10 min) and the supernatants were used for protein expression by Western blotting.

Western blotting analysis for inducible nitric oxide synthase and NQO1

Proteins that exist in cell lysates (20–30 µg) were separated on 10% PAGE gel (Cleaver Scientific TETRAD System, Warwickshire, UK) and transmitted onto nitrocellulose membrane using a Trans-blot mini-module (Bio-Rad, California, USA). The nitrocellulose membrane was blocked using 5% skim milk for 1 h at room temperature, followed by overnight incubation at 4°C with either iNOS primary antibody (1 : 1000; Ontario, Canada), NQO1 (1:1000, Elabscience, Texas, USA), or β-actin (1 : 2000, Thermofisher Scientific, Massachusetts, USA). Following three times 5 min washes using Tris buffer saline Tween 20, the membranes were incubated with 1:10,000 dilution of corresponding Horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. After three Tris buffer saline Tween 20 washes, membrane proteins were revealed using the enzyme chemiluminescence western blotting detection substrate. Protein bands were imaged using a UVP Bio spectrum imaging system (Analytik Jena, UK).

Result and discussion

The methylene chloride fraction of *O. europaea* leaves subjected to intensive chromatographic tools of fractionation and purification afforded a new compound identified as oleuropeinone (**1**) in addition to the two known compounds: blumenol A (**2**) [15] and megaritolactonol **(3)** [16].

The structure elucidation of all isolated compounds was performed based on different spectroscopic methods (^{1}H NMR, ^{13}C NMR, HMQC, and HMBC), in addition to the differentiation of their chromatographic characteristics with data previously published. Compound (**1**) was separated as a yellowish syrup, using a solvent system (mobile phase) of 30% MeOH/H_2O. It exhibited a molecular ion peak at m/z 305.3451 daltons $[M-H]^-$ determined by ESI-MS corresponding to a molecular formula $C_{17}H_{22}O_5$, which possesses seven unsaturated bonds. The ^{13}C NMR spectrum displayed 17 carbon signals that were categorized into four quaternary carbons (comprising three olfinic carbon at δ_C130.6, 145.4, and 143.8, and keto groups at δ_C 174.1), seven methines (3 olefinic at δ_C115.8, 116.7, and 119.8), five methylenes, and one methyl. The ^{1}H NMR of compound **1** showed the presence of an ABX spin system (two doublets at δ_H 6.77 (d, *J*=7 Hz, 1H) and δ_H 6.69 (d, *J*=1.75 Hz, 1H) and one doublet of a doublet at δ_H 6.59 (*J*=7 Hz) as well as two triplet signals at δ_H 3.50 (t, 2H) and δ_H 2.57 (t, *J*=7 Hz, 2H). From all characterized ^{1}H and ^{13}C NMR (Table 1) results, the presence of hydroxytyrosol moiety was indicated [17–19]. ^{1}H- and ^{13}C NMR signals observed from compound **1** suggested data revealed the presence of boonein moiety. A functional ester was detected, based on the ^{13}C NMR signal at δ_C 174.3 ppm, linked to a -CH_2-

Table 1 Delta H and delta C data of compound 1 in CD_3OD (700 and 175 MHz δ in ppm, *J* in Hz)

Position	^{1}H NMR shifts (ppm)	^{13}C NMR shifts (ppm)
1	3.50 (2H, t)	63.11
2	2.57(1H, t, *J*=7 Hz)	40.5
3	–	130.6
4	6.69(1H, d, *J*=1.75 Hz)	116.7
5	–	145.4
6	–	143.8
7	6.77(1H, d, *J*=7 Hz)	115.8
8	6.59(1H, dd, *J*=7 Hz)	119.8
1'	–	174.13
3'	4.27 (2H, m, *J*=4.6 Hz) 4.16(2H,m, *J*=7 Hz)	66.8
4'	1.47 (2H, m, H'a), 1.20 (2H, m, H-'b)	29.1
5'	2.44 (1H, m, *J*=5.6 Hz)	45.7
6'	2.08 (2H, m, *J*=14 Hz)	31.5
7'	3.98 (1H, m)	77.1
8'	1.88 (1H, q, *J*=7 Hz)	38.9
9'	2.55 (1H, t, *J*=8.4 Hz)	47.3
10'	1.06 (3H, d, *J*=7 Hz)	17.8

group at δ_C 66.8 (t) ppm δ_H 4.16 (2H, m, *J*=7 Hz)/ 4.27 (2H, m, *J*=4.6 Hz). Two additional -CH_2- groups were present at δC 29.1 and 31.5 (δH 2.08 and 1.47, respectively), and four -CH- groups at δ_C 38.9, 77.1, 45.7, and 47.3, corresponding to ^{1}H NMR signals at 1.88 (1H, q, *J*=7 Hz), 3.98 (1H, m), 2.44 (1H, m, *J*=5.6 Hz) and 2.55 (1H, t, *J*=8.4 Hz), respectively. The signal at δ_C 77.1/δ_H 3.98 (1H, m) proposed an oxygenated carbon, besides the detection of the methyl group at δ_C 17.8/δ_H 1.06 (3H, d, *J*=7 Hz). A previous literature study by Marini-Bettolo *et al.* [20] obtained similar spectral data.

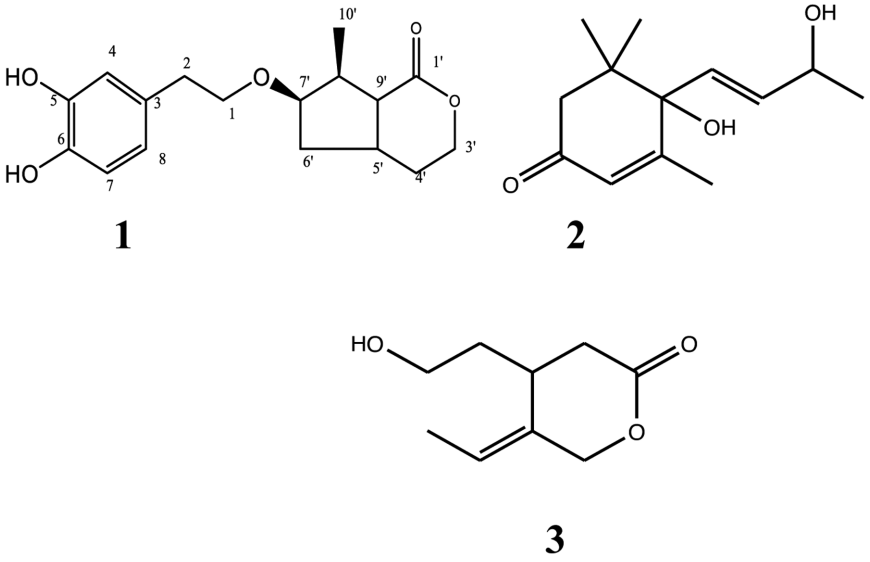
Additionally, a characteristic methyl signal at δ_H 1.06 (3H, d, *J*=7 Hz) for H-10' showed an HMBC correlation with oxygenated methine carbon at δ_C 77.1 (C-7'), 38.9 (C-8') and 47.3 (C-9'), oxygenated methine proton at δ_H 3.98 (1H, m, H-7') revealed HMBC correlations with carbons at δ_C 45.7 (C-5'), 31.5 (C-6'), 47.3 (9'), and methylene protons at 3.50 (2H, t, H-1) showed HMBC correlations with carbons at δ_C 40.5 (C-2), 130.6 (C-3) and 77.1 (C-7'). Therefore, the hydroxytyrosol moiety was placed at C-7' (Fig. 2). The relative configuration of compound **1** was confirmed based on the coupling constant. Therefore, compound **1** was characterized as a new compound with the name oleuropeinone.

Assessment of anti-inflammatory potential

The most powerful anti-inflammatory fraction (inhibition of LPS-induced iNOS protein expression in RAW264.7 macrophages) is CH_2Cl_2 fraction followed by hexane extract and finally a total of 80% MeOH extract of the plant leaves. Our results agree with the previous studies that showed the potency of olive leaf extracts to inhibit iNOS expression [10].

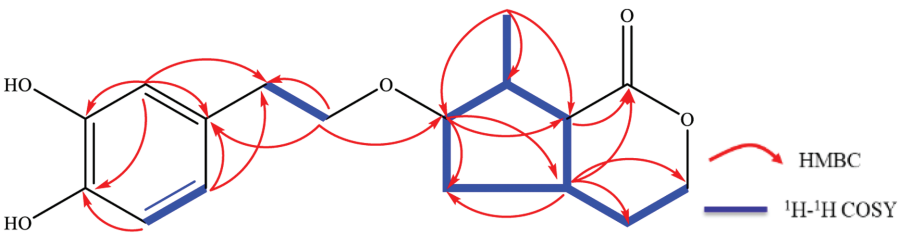
Treatment of RAW264.7 cells with increasing concentrations of M3 extract (6.25, 25, 100 µg/ml) showed concentration-dependent inhibition of both iNOS and COX-2 protein expression (Fig. 4). The highest inhibition of these inflammatory markers was recorded at a 100 µg/ml concentration of the extract.

Figure 1



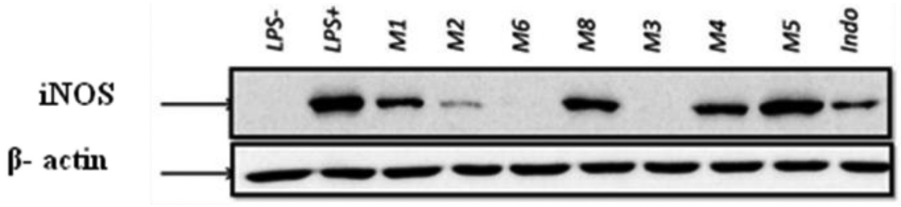
The structure of the isolated compounds from olive leaves.

Figure 2



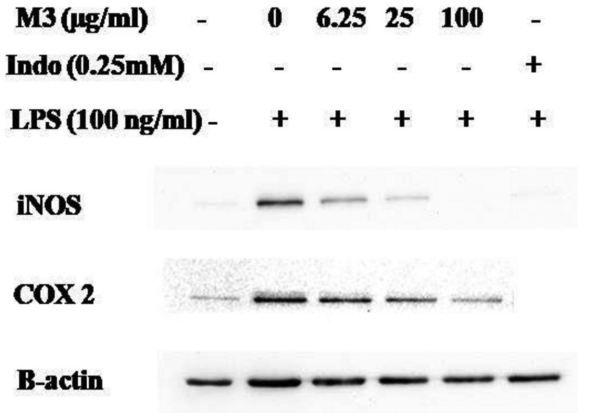
Selected ^{1}H-^{1}H COSY (—) and HMBC. (↷) correlations of compound **1**.

Figure 3



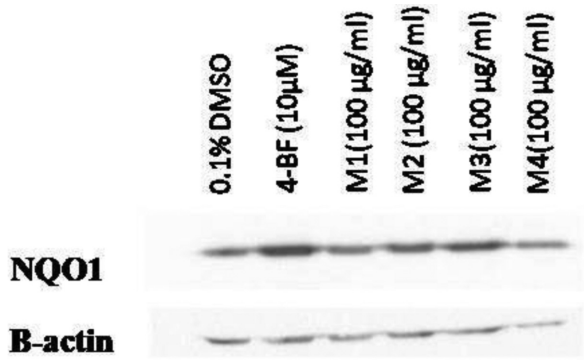
Assessment of the anti-inflammatory potential by Western blot analysis of iNOS inhibition in RAW264.7 macrophages (M1 = 80% MeOH, M2=hexane, M3=CH_2CL_2, M4=ethyl acetate, M5=n-butanol, M6= ether, M8=SAP).

Figure 4



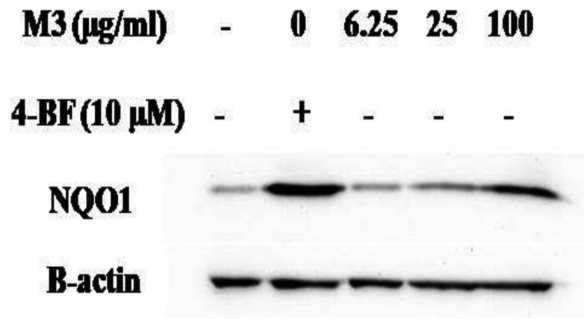
Western blotting of the potential of M3 to inhibit inflammatory markers inducible nitric oxide synthase and cyclooxygenase-2 protein expression in RAW264.7 macrophages.

Figure 5



Estimation of the cancer chemopreventive potency by Western blot analysis of the NQO1 protein expression for M1–M4.

Figure 6



Assessment of cancer chemoprevention methylene chloride fraction conc-dependent effect Western blot analysis of the NQO1 protein expression.

Cancer chemoprevention assessment

Treatment of Hepa1c1c7 cells with prescreen concentration of extracts M1–M6 (100 µg/ml) (M1=80% MeOH, M2=Hexane, M3= CH_2Cl_2, M4=Ethyl acetate, M5=n-Butanol, M6=ether) revealed the potency of M3 to induce the chemopreventive marker NQO1 protein expression as revealed by western blotting analysis (Fig. 5). The highest induction of this marker was achieved at a 100 µg/ml concentration of the extract.

Treatment of hepa1c1c7 cells with increasing concentrations of methylene chloride fraction (M3) (6.25, 25, and 100 µg/ml) showed a concentration-dependent induction of chemopreventive marker NQO1 protein expression as revealed by western blotting analysis (Fig. 6). The highest induction of this marker was achieved at a 100 µg/ml concentration of the extract.

Discussion

Inflammation is a natural defense action in the human body against external threats. A low level of chronic inflammation is correlated to several age-associated diseases and health complications such as cancer, neurodegenerative, cardiovascular, immune-inflammatory, and aging-related diseases. During inflammation, activated macrophages produce different types of inflammatory mediators such as TNF-α, PGE2, NO, and IL-6 and regulatory enzymes (COX-2 and iNOS). In the present study, the LPS-stimulated increase of iNOS and COX-2 in RAW264.7 macrophages was inhibited by treating

macrophages with CH_2Cl_2 fraction, implying that CH_2Cl_2 fraction has strong anti-inflammatory activity in LPS-stimulated RAW264.7 macrophages. These data agreed with previous literature studies that showed the efficacy of olive leaf extract and its components especially oleuropein as an anti-inflammatory agent, which caused a down-regulation of nitric oxide (NO) and cyclooxygenase-2 (COX-2) in LPS-stimulated RAW264.7 macrophages [21,22], as well as the ability of the olive leaf extract to interact with NF-κB pathways [23], which play a central role in the inflammatory response. Recent cellular studies proved the ability of the olive extract to make a down-regulation of nuclear transcription factor-kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK) activations as well as up-regulation of heme-oxygenase-1 (HO-1) protein expressions through nuclear factor E2-related factor 2 (Nrf2), which performs an important role in regulating the expression of antioxidant proteins that protect against oxidative damage and in mice fed with diets contain olive leaves [24]. Martínez-Huélamo *et al.*, 2017 illustrated that polyphenols found in the leaves initiate the endogenously antioxidant defense mechanisms through amendment transcription factors as the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) which is considered a negative regulator to iNOS and activator of the chemopreventive NQO1 [27]. The activity of CH_2Cl_2 fraction as an anti-inflammatory and chemopreventive agent could be attributed to the presence of two important anti-inflammatory compounds: blumenol A **(2)** and megaritolactonol **(3)**. Megaritolactonol can suppress the production of NO production and decrease its levels in the LPS-stimulated murine microglia BV-2 cells [16]. Blumenol A **(2)** shows a cytotoxic effect against cancer cell lines such as MFC-7 and A-549 [25] and a suppression effect on NO production in RAW264.7 cells [26].

Conclusion

Olive leaves have been used to prevent and treat some diseases in traditional medicine for centuries. Many studies have been performed and reported the positive effects of leaf extracts on diabetes, cardiovascular, as well as inflammation parameters. However, some questions need to be answered, and this requires more research. Overall, our findings provide insights into the anti-inflammatory and cancer chemopreventive activities of different extracts of olive leaves. The data show promising results, which agree with the previous ones. Further pharmacological studies are essential and significant. In addition, we identified a new compound that was isolated here for the first time in nature.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Diaba MA, Hadad GM, Ibrahim AK. Review article on chemical constituents and biological activity of *Olea europaea*. Rec. Pharm Biomed Sci 2020; 4:36–45.

2 Hashmi MA, Khan A, Hanif M, Farooq U, Perveen S. Review article traditional uses, phytochemistry, and pharmacology of *Olea europaea* (Olive). Evid Based Complement Alternat Med 2015; 29:541–591.

3 Obied HK. Biography of biophenols: past, present, and future. Funct Foods Health Dis 2013; 3:230–241.

4 Carrera-Gonzalez MP, Ramirez-Exposito MJ, Mayas MD, Martinez-Martos JM. Protective role of oleuropein and its metabolite hydroxytyrosol on cancer. Trends Food Sci Technol 2013; 31:92–99.

5 Abdelgawad SM, El Hassab MA, Abourehab MAS, Elkaeed EB, Eldehna WM. Olive leaves as a potential phytotherapy in the treatment of COVID-19 disease. Front Pharmacol 2022; 13:879118.

6 Santangelo C, Varì R, Scazzocchio B, Di Benedetto R, Filesi C, Masella R. Polyphenols, intracellular signaling, and inflammation. Ann Dell Istit Super Di Sanita 2007; 43:394–405.

7 Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene 1999; 18:7908.

8 Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD. The origins of the age-related proinflammatory state. Blood 2005; 105:2294–2299.

9 Ghanam J, Laaboudi W, Benlemlih M. Effects of rich polyphenols olive tree extract on inflammation and pain in patients with rheumatoid arthritis: an 8-weeks randomized, double-blind, placebo-controlled clinical trial. Int J Biol Pharm Res 2015; 2:51–61.

10 De Cicco P, Maisto M, Tenore GC, Ianaro A. Olive leaf extract, from *Olea europaea* L., reduces palmitate-induced inflammation via regulation of murine macrophages polarization. Nutrients 2020; 12:3663.

11 Muscoli C, Lauro F, Dagostino C, D'Agostino C, Ilari S, Giancotti LA. *Olea europea*-derived phenolic products attenuate antinociceptive morphine tolerance: an innovative strategic approach to treat cancer pain. J Biol Regul Homeostatic Agents 2014; 28:105–116.

12 Obied HK, Prenzler PD, Omar SH, Ismael R, Servili M, Esposto S, *et al.* Pharmacology of olive biophenols. Adv Mol Toxicol 2012; 6:195–242.

13 Mona Ghanem TM, Tawfik WA, Mahdy ESM, Abdelgawad ME, Abdel-Azim NS, El-Missiry MM. Chemical and biological evaluation of olive leaves as a waste by-product of the olive oil industry. Egypt Pharma J 2019; 18:172–177.

14 Hamed AR, Barakat AZ, Bassuiny RI, Abdelâ Aty AM, Mohamed SA. Date palm and saw palmetto seeds functional properties: antioxidant, anti-inflammatory and antimicrobial activities. J Food Measure Characterizat 2020; 14:1064–1072.

15 Chen M, Wu J, Zhang XX, Wang Q, Yan SH, Wang HD, *et al.* Anticancer activity of sesquiterpenoids extracted from solanum lyratum via the induction of mitochondria-mediated apoptosis. Oncol Lett 2017; 13:370–376.

16 Mousouri E, Melliou E, Magiatis P. Isolation of megaritolactones and other bioactive metabolites from 'Megaritiki' table olives and debittering water. J Agric Food Chem 2014; 62:660–667.

17 Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Identification of lignans as major components in the phenolic fraction of olive oil. Clin Chem 2000; 46:7976–7988.

18 Stella Christophoridou Photis Dais. Detection and quantification of phenolic compounds in olive oil by high resolution ^{1}H nuclear magnetic resonance spectroscopy. Anal Chim Acta 2009; 633:283–292.

19 Francesca L, Pannucci E, Clemente M, Grande E, Urciuoli S, Romani A, *et al.* Hydroxytyrosol and oleuropein-enriched extracts obtained from olive oil wastes and by-products as active antioxidant ingredients for poly (vinyl alcohol)-based films. Molecules 2021; 26: 2104.

20 Marini-Bettolo GB, Nicoletti M, Messana I, Patamia M, Galeffi C, Oguakwa JU, *et al.* Research on African medicinal plants boonein, a new c-9 terpenoid lactone from *Alstonza boonez*: a possible precursor in the Indole alkaloid biogenesis. Tetrahedron 1983; 33:323.

21 Mao X, Xia B, Zheng M, Zhou Z. Assessment of the anti-inflammatory, analgesic, and sedative effects of oleuropein from *Olea europaea* L. Cell Mol Biol (Noisy-le-grand) 2019; 65:52–55.

22 Boss A, Bishop KS, Marlow G, Barnett MPG, Ferguson LR. Evidence to support the anti-cancer effect of olive leaf extract and future directions. Nutrients 2016; 8:513.

23 Ryu S-J., Choi H-S., Yoon K-Y., Lee O-H., Kim K-J., Lee B-Y. Oleuropein suppresses LPS-induced inflammatory responses in R AW264. 7 cell and zebrafish. J Agric Food Chem 2015; 63:2098–2105.

24 Castejón ML, Alarcón-de-la-Lastra C, Ángeles Rosillo M, Montoya T, Fernández-Bolaños JG, González-Benjumea A, Sánchez-Hidalgo M. A new peracetylated oleuropein derivative ameliorates joint inflammation and destruction in a murine collagen-induced arthritis model via activation of the Nrf-2/Ho-1 antioxidant pathway and suppression of MAPKs and NF-κB activation. Nutrients 2021; 13:311.

25 Liu X, Tian F, Zhang HB, Pilarinouo E, Jerry L. Biologically active Blumenol A from the leaves of Annona Glabra. Natl Prod 1999; 14:77–78.

26 Bai N, He K, Roller M, Lai CS, Shao X, Pan MH, *et al.* Flavonoid glycosides from *Microtea debilis* and their cytotoxic and anti-inflammatory effects. Fitoterapia 2011; 82:168–172.

27 Martínez-Huélamo M, Rodríguez-Morató J, Boronat A, de la Torre R. Antioxidants, 2017;6:73.