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Original Article

The anti-aging, anti-tuberculosis and antioxidant potential benefits of Saudi Arabia *Olea-Europaea Leaves* extracts

Potenciais benefícios antienvelhecimento, antituberculose e antioxidantes dos extratos de folhas de *Olea-Europaea* da Arábia Saudita

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Abstract

The olive leaf extract and olive leaf indicated a high potential for application in food additives and foodstuffs. It could be these bio-products useful and important in condition therapy related with oxidative stress and can use it to develop functional foods and to improve the food's shelf life. The olive leaf chemical composition of *Oleaeuropaea* L. grown from eljouf in Saudi Arabia, using solvents of increasing polarity cyclohexane, dichloromethane, chloroform, ethyl acetate, methanol and ethanol was determined using by GC/MS. Furthermore, the antioxidant activity (diphenylpicrylhydrazyl (DPPH), anti-aging, and anti-tuberculosis of olive leaf extracts were evaluated. The results indicated that extract of *Oleaeuropaea L.* has a considerable contains in polyphenols (hydroxytyrosol, oleuropein and their derivatives) regarding its antioxidant effects, the major components were detected by GC/MS in Olea dichloromethane extract are Hexadecanoic acid (15.82%), 7(4Dimethylaminophenyl)3,3,12trimethyl3,12dihyd ro6 Hpyrano[2,3c]acridin 6 one (11.21%), and in Olea chloroform extract are Hexatriacontane (12.68%), nTetratr iacontane (10.95%). The results concluded that the plant extract of chloroform showed no anti-aging activities and the lower anti-aging activities for cyclohexane extract, while, the Olea dichloromethane extract was the most active extract. The obtained data confirmed that the most active extract of anti-tubercolisis was for chloroform and ethyl acetate extract, while, anti-tubercolisis activity of ethanolic extract was the lower. The extract amount as well as the solvent polarity influence the inhibitory activity. A favorable connection was demonstrated inter alia the leaf extracts antioxidant activity and the content of total phenol.

Keywords: anti-aging, anti-tuberculosis, antioxidant, extracts, *Oleaeuropaea L.* leaves, phenolic content.

Resumo

O extrato de folha de oliveira e a folha de oliveira indicaram alto potencial para aplicação em aditivos alimentares e alimentos. Esses bioprodutos podem ser úteis e importantes na terapia de condições relacionadas ao estresse oxidativo e podem ser utilizados para desenvolver alimentos funcionais e melhorar a vida útil dos alimentos. A composição química da folha de oliveira de *Olea europaea L.* cultivada em Eljouf na Arábia Saudita, usando solventes de polaridade crescente ciclohexano, diclorometano, acetato de etil clorofórmio, metanol e etanol foi determinada usando GC/MS. Além disso, foi avaliada a atividade antioxidante (difenilpicrilhidrazil – DPPH) antienvelhecimento e antituberculose de extratos de folha de oliveira. Os resultados indicaram que o extrato de *Olea europaea L.* que consideravelmente possui polifenois (hidroxitirosol, oleuropeína e seus derivados) quanto aos seus efeitos antioxidantes, os componentes majoritários detectados por GC/MS no extrato diclorometânico de Olea são o ácido hexadecanoico (15,82%), 7-(4-Dimetilaminofenil)-3,3,12-trimetil-3,12-dihidro-6H-pirano[2,3c]acridin-6-ona (11,21%) e no extrato de clorofórmio de *Olea* são Hexatriacontane (12,68%), nTetratr iacontane (10,95%). Os resultados concluíram que o extrato vegetal de clorofórmio não apresentou atividades antienvelhecimento e as atividades antienvelhecimento mais baixas para o extrato de cicloexanona, enquanto o extrato de *Olea* diclorometano foi o extrato mais ativo. Os dados obtidos confirmaram que o extrato mais ativo de antituberculose foi para clorofórmio e extrato de acetato de etila, enquanto a atividade antituberculose de extrato etanoico foi menor. A quantidade de extrato, bem como a polaridade do solvente influenciam a atividade inibitória, atividade e o teor de fenol total.

Palavras-chave: antienvelhecimento, antituberculose, antioxidante, extratos, folhas de *Olea europaea L.*, conteúdo fenólico.

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1. Introduction

In Mediterranean especially region olive tree (*Olea Europaea, Oleaceae*) leaves in an inclusive used in traditional herbal medicine with the purpose of banning and handling several diseases (Boss et al., 2016). The phenolic compounds of olive oil and its leaves have been known especially in medicine and a healthy diet as essential components (Visioli et al., 2002). In the past, olive leaves have been broadly used for the treatment of fever and other diseases. Olive leaf is the essential origin of phenolic compounds. The powerful components in the olive leaf are known to be oleuropein and its derivatives such as caffeic acid, hydroxytyrosol and tyrosol, vanillic acid, p-coumaric acid, vanillin, rutin, and, leuteolin, diameter, and the 7-glucoside of the three components luteolin-, apigenin, and diosmetin (Farag et al., 2003). The extract of olive leaf contains many potentially bioactive compounds exhibiting a number of health benefits, such as antiarrhythmic, hypocholesterolemic, anti-inflammatory, spasmolytic, cardio protective, hypotensive, immune- stimulant, anti-hypoglycemic, antioxidant, anti-thrombic, and anti-aging properties (Morteza et al., 2012; Pereira et al., 2007; Hassen et al., 2015), because it contains triterpenes, flavonoids, chalcones and tannins (Kontogianni and Gerothanassis, 2012). Olive leaf extract is ideally suited as an ingredient for all kind of personal care products and toiletries, since it plays a double role as a preserving and active ingredient. Further, it is a 100% natural vegetable product, non-toxic, non-hazardous and completely safe and biodegradable (Morteza et al., 2012; Pereira et al., 2007), Oleuropein, and hydroxytyrosol (Bulotta et al., 2014). *Oleaeuropaea* L. and is particularly abundant in unprocessed olive leaves, with concentrations up to 60–90 mg g-1 (Le Tutour and Guedon, 1992). Skin aging call attention to an original sin wherever cells are incapable to be reformed or task properly as an effect of cell absence or functional deterioration (Jones and Rando, 2011). Pigment spot in the skin is one of the most popular cosmetic problems, mostly for the aged. It is investigated that pigmentation is induced and aggravate by ultraviolet (UV) light, and the reactive oxygen species (ROS) produced stimulate melanocytes and promote melanogenesis (Jimbow et al., 1974). Therefore, to prevent skin pigmentation, it is important to obstruct the production of ROS in order. Oleu present at high levels in olive leaf extract and acts as a skin protectant, as free radical scavenger at the skin level, as well as anti-aging by decreasing levels of reactive oxygen species (ROS) and eventually delaying appearance of senescence in cells (Morteza et al., 2012; Pereira et al., 2007). For this considers, in various cellular models of oxidative stress, that HT and OLE contribute to maintaining the redox homeostasis (Ergin et al., 2013). HT and OLE regulate different indicating roads linked to the cellular redox status, that conduct to a cytoprotective effect and get better cell functionality (Cumaoğlu et al., 2011). In worldwide, infectious diseases are the most important induce of death (Parekh and Chanda, 2007). There is an important global health problem attributable to tuberculosis (TB) diseases, which are complex due to drug resistance (Zhang et al., 2006). Tuberculosis is an infectious bacterial disease, that generally affects the lungs. Even though considerable pains have been made to control TB, about one-third of the world's population is infected with M. tuberculosis, every year eight million people develop tuberculosis disease, while two million people die (Dye et al., 1999). The terrible rise of MDR TB cases requires the imperative expansion of novel, more active and safer anti-tuberculosis (anti-TB) drugs. However, no research has been conducted on olive leaves extract as anti-aging and anti-tuberculosis. Therefore, the aim of this work is to valorize Olea leaves extract collected from Aljouf region in Saudi Arabia. By the determination of its compounds chemical structure by gas chromatography mass spectrometry (GC–MS) analysis, and total phenolic content, the antioxidant activity and the study of the anti-aging and anti-tuberculosis screening.

2. Experimental Conditions

2.1. Extracts of plant preparation

First, we collected the plant materials and grinded after sliced into small pieces. The powdered materials were extracted using dichloromethane, cyclohexane, chloroform, ethyl acetate, methanol and ethanol at room temperature for 24 h. What-man filter paper No. 1 was used to filtrate the extracts and a rotary evaporator was used to concentrate the extracts at 40 °C. The glassy desiccator was used to dry the extracts, then, the residues of powdered were stored pending analysis.

2.2. Determination of total phenolic content

Total phenolic content (TPC) in extracts was quantified using Folin–Ciocalteu method (Singleton et al., 1999). For comparison, gallic acid was used as a standard and the results are expressed in milligrams of Gallic Acid Equivalent per 100 g sample (mg GAE/100). g).

2.3. Antioxidants activity (DPPH %)

The free radical scavenging effect of plant extracts was assessed by the discoloration of ethanol solution of DPPH radical 0.2 aromatic in ethanol according to Aromatic et al., (2013).

2.4. Anti-aging activity: anti-collagenase assay

To check for shifts and interference prior to screening in all assays, the Cary 300 UV-visible spectrophotometer in the lambda max was used to record all extracts. The spectrophotometric methods according to Thring et al., (2009) were used in a microplate reader with some modifications as a basis for the assay. Tricine buffer (50 mM) (10 mM $CaCl_2$ and 400 mM NaCl with pH 7.5) was used to perform the assay. According to the activity data of supplier's, the buffer was used to dissolve collagenase from Clostridium histolyticum (ChC – EC.3.4.23.3) for use at 0.8 units/mL an initial concentration. Then, used Tricine buffer to dissolve the synthetic substrate N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) to 2 mM. Before adding substrate to the enzyme was incubated with

samples (1000-7.81 µg/ ml) for 15 min in buffer to start the reaction. Water was used to perform negative controls. After adding substrate immediately, the absorbance was measured at 335 nm and then continuously for 20 minutes using a Microplate Reader. The positive control was EGCG (Equation 1)

$$\textit{The percentage of elastase inhibition}\ (\%) = \left(1 - \frac{S}{C}\right) \times 100 \quad (1)$$

Where: S = the corrected absorbance of the samples containing collagenase inhibitor (the enzyme activity in the presence of the samples). C = the corrected absorbance of controls (the enzyme activity in the absence of the samples). The IC_{50} value was defined as the sample concentration to inhibit 50% of collagenase under the assay conditions.

2.5. Anti-mycobacterial activity Mycobacterial cultures

M. tuberculosis (ATCC 25177) standard cultures were procured from American Type Culture Collection (ATCC), Manassas, VA, USA. Dubos medium was supplemented with sodium nitrate (50 mM) to grow M. tuberculosis. The cultures were grown at 37 °C/150 rpm under aerobic conditions to log phase optical density (OD595 =1). The water bath sonicator (Ultrasonic, Freeport, IL, USA) was used to sonicate the aggregated clumps of grown mycobacteria for 2 minutes. Minimum inhibitory concentration assay (MIC). The microplate alamar blue assay (MABA) (Aromatic et al., 2013) was used to determine MIC against M. tuberculosis. Positive controls were isoniazid and RFP. The concentrations of final testing range and compound stock solutions were 0.003 to 1000 µg/mL, respectively. M. tuberculosis was grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 Broth (Seebio) supplemented with glycerol (0.2% vol/vol), Tween 80 (0.05%), and albumin-dextrose-catalase (10% vol/vol) (Seebio).7H9-ADC-TG in a volume of 96-well clear-bottom microplates (BD) was used to prepare the compounds twofold dilutions. Then added M. tuberculosis (100 µL containing 2 × 105 CFU) to get final yield 200 µL as final testing volume. For incubation 37 °C was used to incubate the plates on day 7, then added alamar blue (20 µL) and 20%Tween 80 (12.5 µL) to all wells. The fluorescence was read at an emission of 590 nm and an excitation of 530 nm after incubation for 16 to 24 h at 37 °C. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacterium-only controls (Lu et al., 2011).

2.6. Gas Chromatography–Mass Spectrometry (GC-MS) analysis

The GC/MS analysis was performed using the method according Zaghloul et al., (2015).

3. Result and Dissection

3.1. Total phenolic and free radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl)

Table 1 shows the total phenolic content of extracts obtained from olive leaves and the antioxidant activity

Table 1. Quantitative estimation of total phenolic and Anti-oxidant activity of olive leaves extracts

Extracts	Total phenolic content (mg GAE/100 g)	DPPH radical scavenging activity (%)
Methanol	255.20± 1.92	53.6 ± 0.96
Ethanol	217.4± 2.31	39.4 ± 0.68
Ethyl acetate	302± 2.04	22.72 ± 0. 91
Chloroform	315.10± 3.16	64.78 ± 1.01
Cyclohexane	248.12± 3.97	42.38 ± 0.87
Dichloromethane	398.08± 2.59	69.81 ± 0.99

All determinations were carried out in triplicate manner and values are expressed as the mean ± SD.

of the olive leaves extracts radical scavenging activity (expressed as absorbance percentage) and IC_{50} of olive leaf. The values of the total phenolic components obtained ranged from 217.4± 2.31 to 398.08± 2.59 mg GAE/100 g of extract. The highest total phenolic compounds contents were observed in the extracts of dichloromethane and chloroform (398.08± 2.59 and 315.10± 3.16, respectively), may be due to some phenolic compounds soluble in dichloromethane and chloroform than other extracts. It must be noted that these results are in line with those obtained for other authors (Jaski et al., 2019) they reported that the total phenolic components of ethanol extract were in the range of 10.5 to 12.8 mg GAE g-1. The highest values of 231.98 and 230.61 mg GAE/100 g were obtained for 90 vol% methanol and 90 vol% ethanol, respectively, in comparison to distilled water (Won-Young et al., 2020). The extraction by maceration using ethanol 80% gave the highest contents of both total phenolic contents (101.5 ± 1.27 mg/g; $p < 0.01$) in comparison to distilled water and, acetonitrile (Ghomari et al., 2019). Chloroform extract had 288 µg/mg of leaves of O. ferruginea (Mehmood and Murtaza, 2018). Olive leaves, particularly *Oleaeuropaea* L., are rich in phenolic compounds including substituted phenols, catechin, flavonols and flavones. Polyphenols determination of plants is of great interest because these compounds have natural antioxidant activity (Rahmanian et al., 2015).

The antioxidant capacity results, are shown in Table 1. The DPPH radical scavenging activity values obtained demonstrate the good antioxidant capacity of the extracts, varying from 22.72 ± 0. 91 to 69.81 ± 0.99%. The highest antioxidant capacity was observed in the extracts of dichloromethane and chloroform (69.81 ± 0.99 and 64.78 ± 1.01, respectively). Antioxidant activity was in good correlation with total phenolic except ethyl acetate extract. Dichloromethane and chloroform extract total phenolic content were higher compared to other extracts. These results confirm the highest antioxidant activity of dichloromethane and chloroform, where bioactive compounds like phenols corresponded to DPPH free radical scavenging activity. Free radicals such hydroxyl, superoxide, singly oxygen and peroxyl play a necessary part for numerous malady conditions. Grassy drugs, comprising free radical scavengers, are getting prominence

in remediation of this diseases. The antioxidant properties of many plants due to their phytochemicals including phenolic compounds (Larson, 1988). Oleuropein has been shown to be free radical formation prevention and potent antioxidant may be due to its ability to chelate metal ions, which catalyze free radical generation reactions, e.g. Cu and Fe (Andrikopoulos et al., 2002) also, its ability to inhibit many enzymes of inflammatory e.g. lipoxygenases, without affecting the cyclo-oxygenase pathway (Visioli et al., 2002). Depending on the habitat, olive leaves extract has a different composition (either wild or cultivated). For both the wild and cultivated leaf extracts, the ethanolic extract had a high phenolic content (21.3 to 22.6 mg GA/gdw) and antioxidant activity (71% to 57%) (Ghalia, 2022).

3.2. Anti-aging activity:

The skin has collagen as the major component, it is degraded by collagenase enzyme. Collagenase activity inhibition delays the forming pre-college fibers process and subsequently the process of wrinkling (Mukherjee et al., 2011). Five extracts inhibited the enzyme by more than 60%, with two of these inhibiting the enzyme by more than 75% (Table 2, Figure 1a). The dichloromethane extract of olive leaves with the highest concentration of 1000 µg/ml possessed an excellent collagenase inhibition percentage (86.32 ± 0.63%), IC_{50} = 34.71 than the other extracts, while, the EGCG slandered solution showed 92.24 ± 1.50%, IC_{50} =24.70 as shown in Table 2 and Figure 1a. On the other hand, chloroform extract of olive leaves hadn't activity against collagenase. The cyclohexane extract had the lowest effect (60.14±1.50%), IC_{50} =200.37 against collagenase activity than other extracts except for chloroform extract. Also, the results showed that Dichloromethane extract > Ethyl acetate extract >Ethanol extract > Methanol extract > Cyclohexane extract > Chloroform extract. From the present results, we can suggest that olive leaves' dichloromethane extract is capable of inhibiting collagenase enzymes nearly equally strong as the standard EGCG, depicting its potential as a good source of anti-aging agent. The studied plant extracts anti-collagenase activity has not been reported previously. No information was found regarding the collagenase inhibition activity of the olive leaves' dichloromethane extract. These results may be due to polyphenol in olive leaves and agree with Choi et al., (2008), they told that olive leaves are considered as the best polyphenol complex with balanced super polyphenols such as tyrosol, hydroxytyrosol, oleuropein and caffeic acid. The extracts of olive leaf help skin with the presence of oleanolic acid and flavonoids, which stimulate the connective tissue components and regularize the tissue thereby boosting the skin health protecting it from aging (Sabry, 2014).

The fibroblasts bear replication antiaging scheduled for living organisms genetic and environmental factors. The proteasome, a multi catalytic nonlysosomal protease, has impaired function during aging, while its increased utterance delays deterioration with age in human fibroblasts. Katsiki et al., (2007) give explanation that oleuropein improve proteasome activities in vitro more successfully than other known chemical activators, probably out of conformational changes of the proteasome. In addition, continuous handling of soon transit human genetic fibroblasts with oleuropein reduces the amount of oxidized proteins through increased proteasome-mediated degradation average and retains proteasome function over replication process deterioration with age and decreases the intracellular levels of interactive oxygen species. Importantly, oleuropein-treated cultures display a retard in the showing of deterioration with age morphology, and their life spread is extended around 15% Katsiki et al., (2007). Ancora et al. (2004) demonstrated that oleuropein, who acts as a free radical scavenger at the skin level and especially the antioxidant activity of phenol components of olive oil has a doing activity on skin.

3.3. Anti-tuberculosis activity:

The anti-mycobacterial activity of olive leaf extract was further characterized by bioactivity guided fractionization using different solvents like dichloromethane, cyclohexane, chloroform, ethyl acetate, methanol and ethanol against M. tuberculosis were recorded in the Table 3 and Figure 1b. Among the six collected fractions of olive leaves, four fractions (chloroform, ethyl acetate, cyclohexane and methanol extracts) showed activity against *M. tuberculosis*. The chloroform extract of olive leaf was found to be highly effective at several concentrations from 0.24 to 125µg, MIC = 15.63, MIC90 = 6.6 (based on the general values of our unpublished data). The second extract after chloroform

Table 2. Anti-aging (Collagenase inhibitory %) inhibitory of olive leaves extracts.

conc. (µg) / Samples	0	7.81	15.63	31.25	62.5	125	250	500	1000	IC_{50}
EGCG	0.00±0.00	22.34±1.20	46.25±0.58	52.69±0.72	59.21±1.50	64.98±1.30	72.35±1.20	84.22±0.58	92.24±1.50	24.70
Dichloromethane	0.00±0.00	37.81±1.20	40.75±1.50	48.84±1.20	59.31±1.50	60.17±0.63	69.37±1.20	80.14±0.58	86.32±0.63	34.71
Cyclohexane	0.00±0.00	9.25±1.30	15.36±0.72	23.32±0.95	32.15±1.20	46.34±1.50	52.41±0.63	55.63±1.20	60.14±1.50	200.37
Chloroform	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-
Ethyl acetate	5.34±1.50	12.37±0.58	26.15±1.60	42.32±1.50	53.32±1.30	60.13±0.63	66.62±1.20	70.22±0.63	75.14±1.20	53.06
Methanol	0.00±0.00	7.31±0.58	10.63±1.50	17.35±1.20	29.14±0.63	46.92±1.90	53.16±0.72	58.91±1.20	63.58±1.50	186.69
Ethanol	0.00±0.00	12.15±0.92	26.14±1.30	42.17±2.10	50.14±1.50	56.24±1.30	60.14±0.63	66.08±1.20	71.35±0.58	61.90

All placement was carried out in three manner and values are expressed as the mean ± SD. The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed condition.

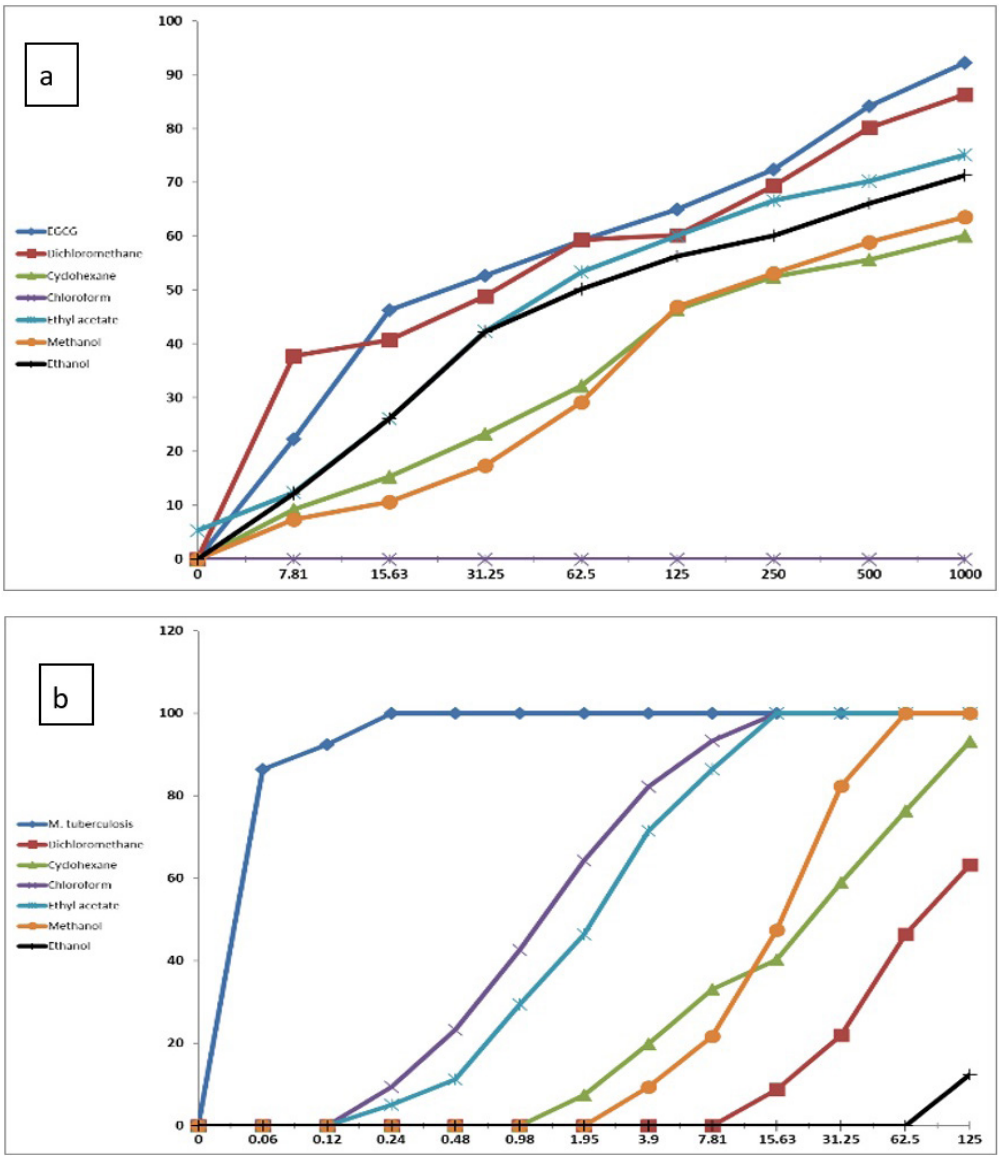


Figure 1. a) Anti-aging (Collagenase inhibitory%) inhibitory of olive leaves extracts and b) Anti-tuberculosis activity of olive leaves extracts.

was ethyl acetate extract, it was also, gave a good effect at several concentrations from 0.24 to 125µg, MIC = 15.63, MIC90 = 9.8. Methanol extract of olive leaf showed a good result at 31.25 – 125 µg MIC = 62.5, MIC90 = 44.7, cyclohexane at 125 µg, MIC = >125, MIC90 = 113.1.

On other hand, ethanol and dichloromethane extract showed less effect on M. Tuberculosisas Anti-tuberculosis activity (Table 3 and Figure 1b). These results indicate that ethanol extract has lower activity compared to chloroform extract, may be due to the bioactive substances of chloroform olive leaf extract. Leaves of olive are rich in bioactive substances such as triterpenic acids, sugars and phenolic compounds (Romero et al., 2017). Among polyphenols, oleuropein was clearly the major compound of total phenolic compounds, it representing more than 88–94%. Oleanolic acid and maslinic acid both compounds with well-known health properties, oleanolic acid was the major triterpene (up to 79–89%), then maslinic acid (14–20%) (Medina et al., 2019).

3.4. Chemical composition

All extracts of olive leaves were analyzed. The constituents were identified by the familiar GC-MS technique. Chemical constituents of dichloromethane and chloroform extract of olea leaves in Table 4, Table 5, Figures 2A and 2B has been mentioned for their anti-aging and anti-tubercolisis major effect, respectively. Every GC-MS chromatogram showed 30 peaks corresponding to the leaves extract compounds which were characterized by comparing their analogous reported by NIST library with their mass spectra (Table 4 and 5). In this GC-MS result is the maximum value present in the sample followed by

Table 3. Anti-tuberculosis activity of olive leaves extracts.

conc. (µg) / Samples	0	0.06	0.12	0.24	0.48	0.98	1.95	3.9	7.81	15.63	31.25	62.5	125	MIC90	MIC
Isoniazid	0.00	86.34	92.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	------	----	**0.40**	**0.24**
Dichloromethane	0.00	------	----	0.00	0.00	0.00	0.00	0.00	0.00	8.69	21.88	46.34	63.21	**>125**	**>125**
Cyclohexane	0.00	------	----	0.00	0.00	0.00	7.43	19.85	33.01	40.17	58.95	76.34	93.21	**113.10**	**>125**
Chloroform	0.00	------	----	9.32	23.21	42.62	64.31	82.14	93.25	100.00	100.00	100.00	100.00	**6.60**	**15.63**
Ethyl acetate	0.00	------	----	5.04	11.16	29.35	46.34	71.46	86.35	100.00	100.00	100.00	100.00	**9.80**	**15.63**
Methanol	0.00	------	----	0.00	0.00	0.00	0.00	9.36	21.63	47.34	82.36	100.00	100.00	**44.70**	**62.50**
Ethanol	0.00	------	----	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.36	**>125**	**>125**

All determinations were carried out in triplicate manner and values are expressed as the mean ± SD.

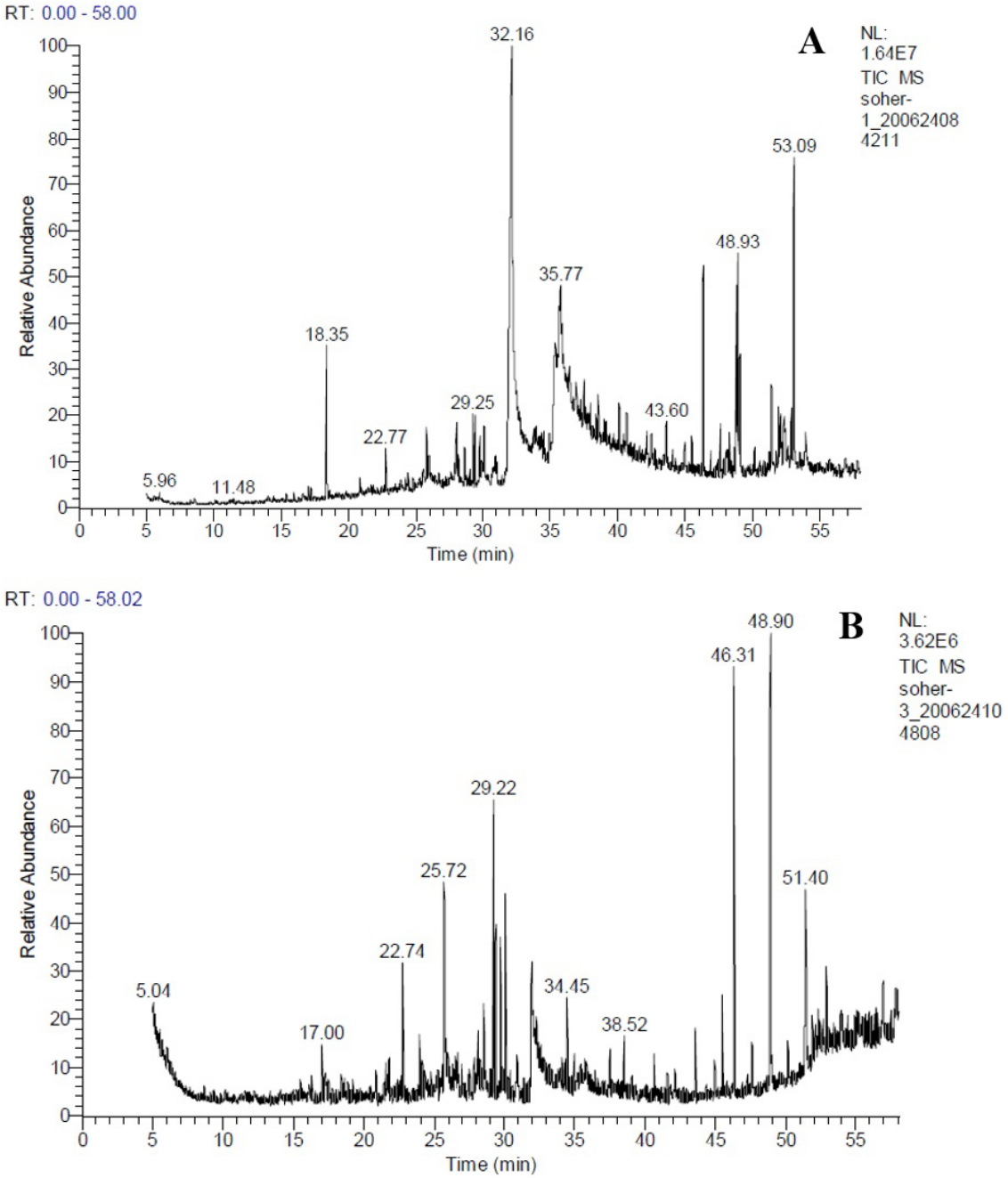


Figure 2. GC.MS analysis result of (A) dichloromethane and (B) chloroform olea leaves.

15.82 for dichloromethane extract and 12.68 for chloroform extract. This would provide researchers with a more real-world analysis of an industrially relevant sample. There are 59 compounds were separated by peak value (Table 4, 5 Figures 2A and 2B). The main constituents of the essential compounds obtained from the olive leaves were various organic compounds, and it must be separated into its component fraction: aliphatic, aromatic and polar fractions. Fatty acids, methyl and ethyl ester, flavonoids and Phytol etc. Thymol, carvacrol, tetratriacontane, and palmitic acid were found to be the main and most important components of the *T. vulgaris EO*, chloroform, and dichloromethane, according to the GC-MS study (Omar et al., 2021). Further researches are in need to determine the compounds which are responsible for the biological activity previously reported for this plant species.

Table 4. GC.MS analysis result of dichloromethane olea leaves.

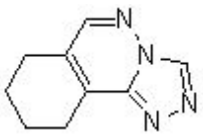
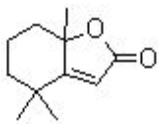
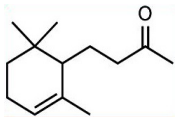
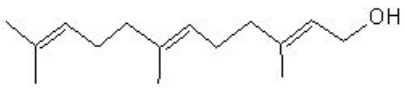
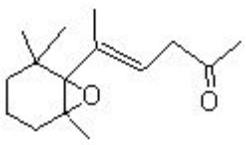
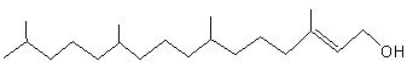
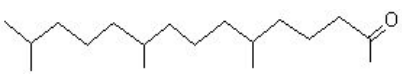
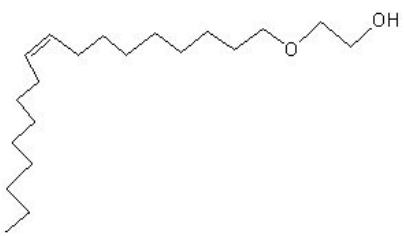
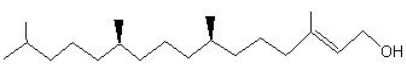
Compound identified	Compound structure	Rt	M.W	M.F	Area (%)
7,8,9,10Tetrahydrostriazolo(3,4a) phthalazine		18.36	174	$C_9H_{10}N_4$,	4.24
2(4H)Benzofuranone, 5,6,7,7atetrah ydro4,4,7atrimethyl®		22.77	180	$C_{11}H_{16}O_2$	1.41
Dihydroαionone, 2Butanone, 4(2,6,6trimethyl2cyclohexen1yl)		25.79	194	$C_{13}H_{22}O$	1.69
Farnesol, 3,7,11trimethyl -2,6,10Dodecatrien1ol,		25.79	222	$C_{15}H_{26}O$	1.69
Tetradecanoic acid, Myristic acid		28.02	228	$C_{14}H_{28}O_2$	1.88
Acetic acid, 2(2,2,6trimethyl7oxabicyclo[4.1.0] hept1yl)propenyl ester		28.62	208	$C_{13}H_{20}O_2$	1.87
3,7,11,15-Tet ramethyl-2-hexadecen-1-ol or Neophytadiene		29.25	296	$C_{20}H_{40}O$	1.82
			278	$C_{20}H_{38}$	
6,10,14 Trimethyl 2 pentadecanone		29.40	268	$C_{18}H_{36}O$	1.65
2 cis 9 Oct adecenyloxyethanol		29.74	312	$C_{20}H_{40}O_2$	1.33
2Hexadecen1ol, 3,7,11,15tetramethyl, Phytol		30.09	296	$C_{20}H_{40}O$	1.37

Table 4. Continued...

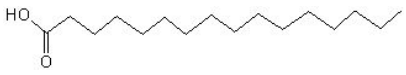
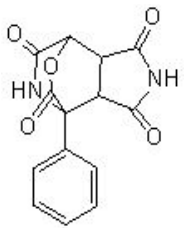
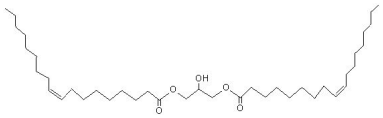
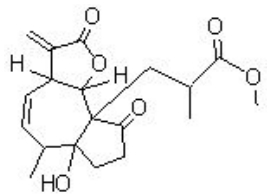
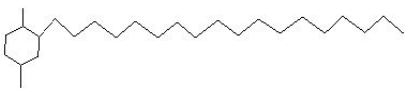
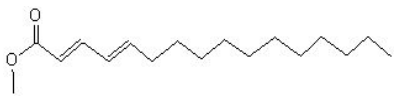
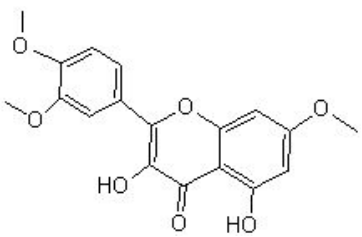
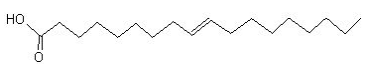
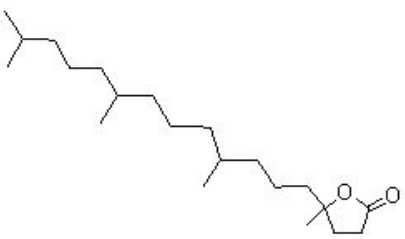
Compound identified	Compound structure	Rt	M.W	M.F	Area (%)
Hexadecanoic acid Palmitic acid or Endo7Methyl1phenyl8oxa4,10diaz atricyclo[5.2.2.0(2,6)]undeca3,5,9,1 1tetraone	 	32.15	256 300	$C_{16}H_{32}O_2$ $C_{15}H_{12}N_2O_5$	15.82
t rans13-Octadecenoic acid		35.32	282	$C_{18}H_{34}O_2$	2.88
Di(9octadecenoyl)glycerol Isochiapin B	 	35.69	620 346	$C_{39}H_{72}O_5$ $C_{19}H_{22}O_6$	3.40
Cyclohexane, 1,4dimethyl2octadecyl		35.77	364	$C_{26}H_{52}$	2.86
Hexadecadienoic acid,methyl ester		36.43	266	$C_{17}H_{30}O_2$	1.43
QUERCETIN or Oleic acid	 	36.91	344 282	$C_{18}H_{16}O_7$ $C_{18}H_{34}O_2$	1.45
5Methyl5(4,8,12trimethyltridecyl) dihydro2(3H)furanone		38.55	324	$C_{21}H_{40}O_2$	2.01

Table 4. Continued...

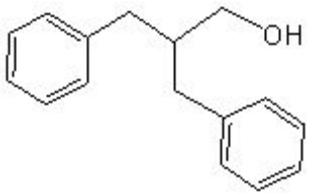
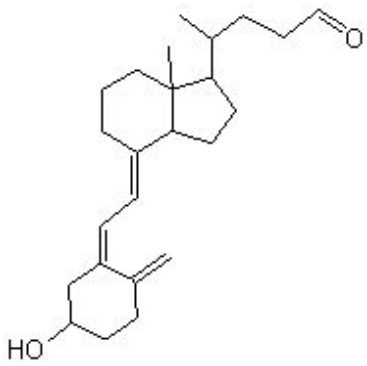
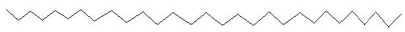
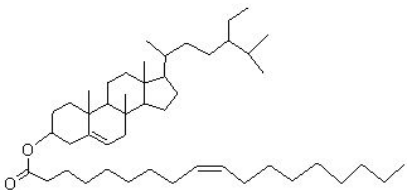
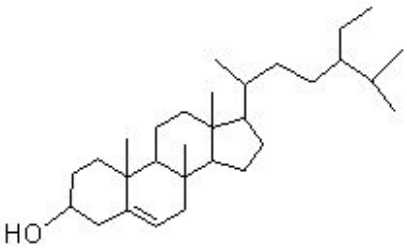
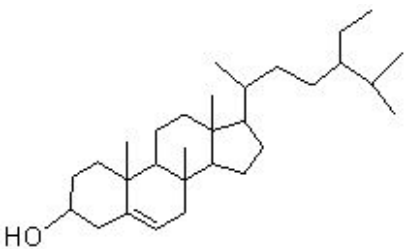
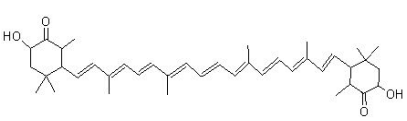
Compound identified	Compound structure	Rt	M.W	M.F	Area (%)
2,2Dibenzylethanol		40.12	226	$C_{16}H_{18}O$	1.47
Pentatriacontane		46.33	492	$C_{35}H_{72}$	5.78
9,10Secochola5,7,10(19trien24al		48.82	356	$C_{24}H_{36}O_2$	5.14
Nonacosane		48.92	408	$C_{29}H_{60}$	6.58
Clionasterol or Stigmast-5-en-3-ol, oleate		49.08	678	$C_{47}H_{82}O$	4.84
			414	$C_{29}H_{50}O$	
Stigmast5en3ol, (3á,24S)		51.94	414	$C_{29}H_{50}O$	2.11
Astaxanthin 5 á,áCarotene4,4'dione, 3,3'dihydroxy, (3S,3'S)		52.10	596	$C_{40}H_{52}O_4$	1.58

Table 4. Continued...

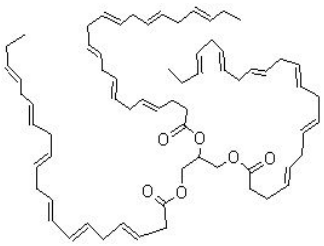
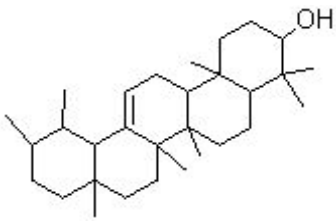
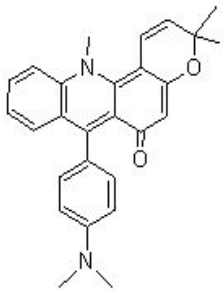
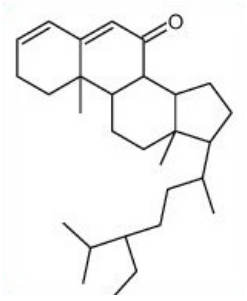
Compound identified	Compound structure	Rt	M.W	M.F	Area (%)
Docosahexaenoic acid 1,2,3-propanetriyl ester Tridocosahexaenoyl-glycerol		52.36	1022	$C_{69}H_{98}O_6$	3.05
α-Amyrin		52.91	426	$C_{30}H_{50}O$	2.11
7(4Dimethylaminophenyl)3,3,12trimethyl3,12dihydro6Hpyrano[2,3c] acridin6one or Stigmasta3,5dien7one		53.09	410	$C_{27}H_{26}N_2O_2$	11.21
			410	$C_{29}H_{46}O$	

Table 5. GC.MS analysis result of chloroform olea leaves.

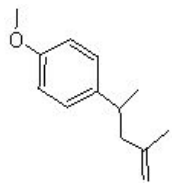
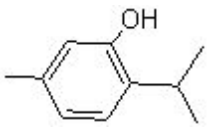
Compound identified	Compound structure	Rt	M.W	M.F	Area %
4 (1,3 Dimet hyl 3 butenyl) phenyl methyl ether		17.01	190	$C_{13}H_{18}O$	1.90
Phenol, 5methyl2(1methylethyl Thymol		17.25	150	$C_{10}H_{14}O$	1.35

Table 5. Continued...

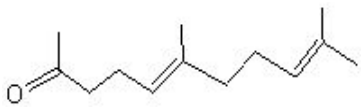
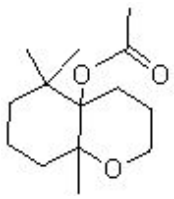
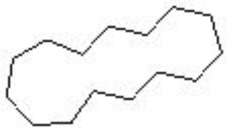
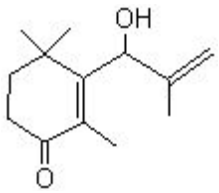
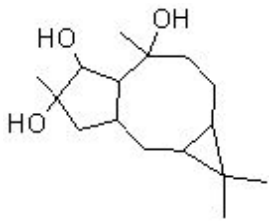
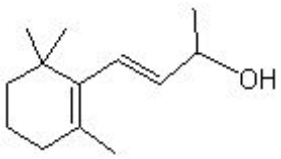
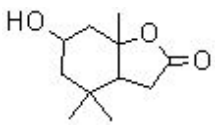
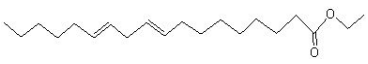
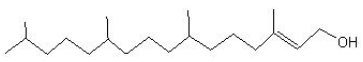
Compound identified	Compound structure	Rt	M.W	M.F	Area %
6,10dimethyl, 5,9Undecadien2one, (E) Geranylacetone		20.86	194	$C_{13}H_{22}O$	1.20
PENTADECANE		21.73	212	$C_{15}H_{32}$	1.23
5,5,8aTrimet hylhexahydr o 2H chromen4a(5H) yl acetate		22.75	240	$C_{14}H_{24}O_3$	4.44
cyclohexadecane		23.97	224	$C_{16}H_{32}$	2.03
3(1Hydroxy2methyl2propenyl)2,4,4trime thyl2cyclohexen1one		24.16	208	$C_{13}H_{20}O_2$	1.41
1,1,4,6 Tetramethyldecahydro 1H cyclopropa[e] azulene4,5,6triol		24.33	254	$C_{15}H_{26}O_3$	1.14
3Buten2ol, 4(2,6,6trimethyl1cyclohexen1yl) αIonol		25.72	194	$C_{13}H_{22}O$	5.45
2(4H)Benzofuranone, 5,6,7,7atetrahydro6 hydroxy4,4,7atrimethyl.		28.12	196	$C_{11}H_{16}O_3$	1.85
Linoleic acid, ethyl ester		28.54	308	$C_{20}H_{36}O_2$	2.55
3,7,11,15Tetramethyl2hexadecen1ol		29.23	296	$C_{20}H_{40}O$	6.79
6,10,14 Trimethyl 2 pentadecanone		29.38	268	$C_{18}H_{36}O$	4.03

Table 5. Continued...

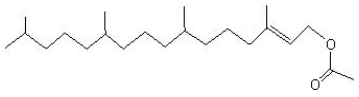
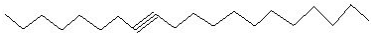
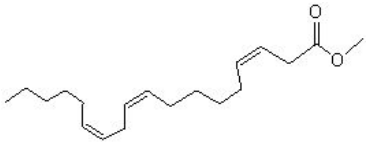
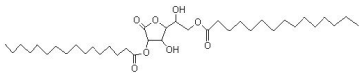
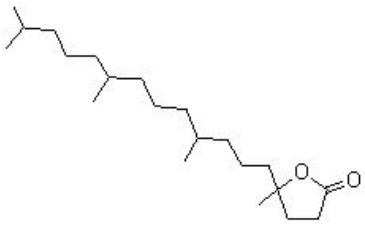
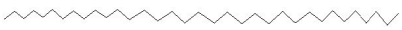
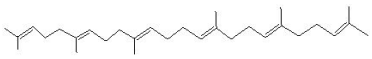
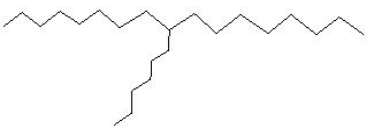
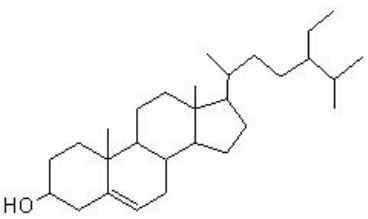
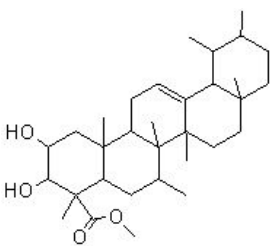
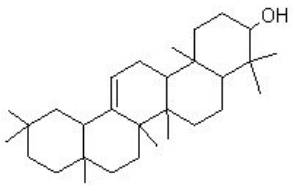
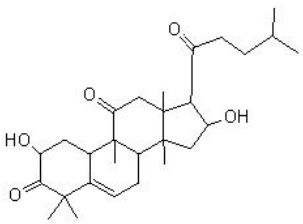
Compound identified	Compound structure	Rt	M.W	M.F	Area %
Phytol, acetate		29.72	338	$C_{22}H_{42}O_2$	3.44
9Eicosyne		30.07	278	$C_{20}H_{38}$	5.08
3cis,9cis,12cisoctadec atrienoate		30.90	292	$C_{19}H_{32}O_2$	1.94
l (+) Ascorbic aci d 2,6 di hexadecanoate		31.94	652	$C_{38}H_{68}O_8$	4.95
5Methyl5(4,8,12trimethyltridecyl) dihydro2(3H)furanone		38.51	324	$C_{21}H_{40}O_2$	1.94
nNonacosane		43.57	408	$C_{29}H_{60}$	1.76
2,6,10,15,19,23hexamethyl2,6,10,14,18,22tetracosahexaene		45.48	410	$C_{30}H_{50}$	2.26
nTetratr iacontane		46.31	478	$C_{34}H_{70}$	10.95
9hexyl heptadecane		47.62	324	$C_{23}H_{48}$	1.56
Hexatriacontane		48.90	506	$C_{36}H_{74}$	12.68
Docosane		50.15	310	$C_{22}H_{46}$	1.57
Tetratetracontane		51.41	618	$C_{44}H_{90}$	6.87
Stigmast5en3ol		51.89	414	$C_{29}H_{50}O$	1.43
Methyl Commate D		52.32	486	$C_{31}H_{50}O_4$	1.80

Table 5. Continued...

Compound identified	Compound structure	Rt	M.W	M.F	Area %
Olean12en3ol		52.89	426	$C_{30}H_{50}O$	2.68
2,16Dihydroxy17(1hydroxy1,5dimethyl2 oxohexyl)4,4,9,14tetramethylestr5ene3, 11dione		54.86	502	$C_{30}H_{46}O_6$	1.12

4. Conclusion

The Olea leaves beneficial properties have been attributed to their composition, especially to their content in triterpenic acids, phenolic compounds, sugars and flavonoids shown by GC–MS analysis. The present study showed that the solvent polarity of the extract influenced the inhibitory activity. The complexity and great diversity of different olive leaf extracts compositions render comparison of their antioxidant activities difficult. The concentration of phenols obtained from the varied polarity of solvent extraction showed that the highest amount of phenols was for dichloromethane extract considered as semi-polar solvents. Dichloromethane extract, and chloroform extract of Olea leaves were the most active extract for anti-aging and anti-tuberculosis activity, respectively. The results obtained in this study encourage the researchers to carry out more studies on olive leaves. We conclude that for this study it has not been possible to assign the chemical compound in charge of anti-aging and anti-tuberculosis activities, but it has been confirmed that the entire plant is recommended as a noble source of inhibitors.

References

ANCORA, C., ROMA, C. and VETTOR, M., 2004. Evaluation of cosmetic efficacy of oleoeuropein. In: *Symposium on the New Frontiers of Dermo-cosmetology: Efficacy, Stability and Safety*, Rome, Italy, November 4-6. Rome: International Society of Cosmetic Dermatology.

ANDRIKOPOULOS, N.K., KALIORA, A.C., ASSIMOPOULOU, A.N. and PAPAGEORGIOU, V.P., 2002. Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil againstin vitro lowdensity lipoprotein oxidation. *Journal of Medicinal Food*, vol. 5, no. 1, pp. 1-7. http://dx.doi.org/10.1089/109662002753723160. PMid:12511107.

AROMATIC, F.A., ELMHDWI, M.F., AROMATIC, F. and AROMATIC, O.O., 2013. Estimation of antioxidant activities of fixed and volatile oils extracted from Aromatic aromatic (clove). *Der. Chemica Sinica*, vol. 4, no. 3, pp. 120-125.

BOSS, A., BISHOP, K.S., MARLOW, G., BARNETT, M.P. and FERGUSON, L.R., 2016. Evidence to support the anti- cancer effect of olive leaf extract and future directions. *Nutrients*, vol. 8, no. 8, pp. 513. http://dx.doi.org/10.3390/nu8080513. PMid:27548217.

BULOTTA, S., CELANO, M., LEPORE, S.M., MONTALCINI, T., PUJIA, A. and RUSSO, D., 2014. Beneficial effects of the olive oil phenolic components oleuropein and hydroxytyrosol: focus on protection against cardiovascular and metabolic diseases. *Journal of Translational Medicine*, vol. 12, no. 1, pp. 219. http://dx.doi.org/10.1186/s12967-014-0219-9. PMid:25086598.

CHOI, N.Y., LEE, J.H. and SHIN, H.S., 2008. Antioxidant activity and nitrite scavenging ability of olive leaf (*Oleaeuropaea* L.) fractions. *Korean J. Food Sci. Thechnology*, vol. 40, pp. 257-264.

CUMAOĞLU, A., RACKOVA, L., STEFEK, M., KARTAL, M., MAECHLER, P. and KARASU, C., 2011. Effects of olive leaf polyphenols against H_2O_2 toxicity in insulin secreting β-cells. *Acta Biochimica Polonica*, vol. 58, no. 1, pp. 45-50. http://dx.doi.org/10.18388/abp.2011_2284. PMid:21383995.

DYE, C., SCHEELE, S., DOLIN, P., PATHANIA, V. and RAVIGLIONE, M.C., 1999. Consensus statement. Global burdenof tuberculosis: estimated incidence, prevalence, and mortality by country. WHO global surveillance and monitoring project. *JAMA*, vol. 282, no. 7, pp. 677-686. http://dx.doi.org/10.1001/jama.282.7.677. PMid:10517722.

ERGIN, V., HARIRY, R.E. and KARASU, Ç., 2013. Carbonyl stress in aging process: role of vitamins and phytochemicals as redox regulators. *Aging and Disease*, vol. 4, no. 5, pp. 276-294. http://dx.doi.org/10.14336/AD.2013.0400276. PMid:24124633.

FARAG, R.S., EL-BAROTY, G.S. and BASUNY, A.M., 2003. Safety evaluation of olive phenolic compounds as natural antioxidants. *International Journal of Food Sciences and Nutrition*, vol. 54, no. 3, pp. 159-174. http://dx.doi.org/10.1080/0963748031000136306. PMid:12775365.

GHALIA, S.A., 2022. Evaluation of phytochemical, antimicrobial, and antioxidant properties of wild versus cultivated olive leaves. *Nature and Science*, vol. 14, no. 10, pp. 448-461.

GHOMARI, O., SOUNNI, F., MASSAOUDI, Y., GHANAM, J., DRISSI KAITOUNI, L.B., MERZOUKI, M. and BENLEMLIH, M., 2019. Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. *Biotechnology*

Reports (Amsterdam, Netherlands), vol. 23, pp. e00347. http://dx.doi.org/10.1016/j.btre.2019.e00347. PMid:31193889.

HASSEN, I., CASABIANCA, H. and HOSNI, K., 2015. Biological activities of the natural antioxidant oleuropein: exceeding the expectation. A mini review. *Journal of Functional Foods*, vol. 18, pp. 926-940. http://dx.doi.org/10.1016/j.jff.2014.09.001.

JASKI, J.M., PIMENTEL, T.C., BARAO, C.E., PONTE, M.S., MARIUTTI, M., BRAGAGNOLO, N., FEIHRMANN, A.C. and CARDOZO-FILHO, L., 2019. Extraction and characterization of the phenolic compounds from leaves of Olea Europaea L. via Ple. *Chemical Engineering Transactions*, vol. 74, pp. 1543-1548. http://dx.doi.org/10.3303/CET1974258.

JIMBOW, K., KAIDBEY, K.H., PATHAK, M.A., PARRISH, J.A., KLIGMAN, A.M. and FITZPATRICK, T.B., 1974. Melanin pigmentation stimulated by UV-B, UV-A and psoralen. *The Journal of Investigative Dermatology*, vol. 62, no. 5, pp. 548.

JONES, D.L. and RANDO, T.A., 2011. Emerging models and paradigms for stem cell ageing. *Nature Cell Biology*, vol. 13, no. 5, pp. 506-512. http://dx.doi.org/10.1038/ncb0511-506. PMid:21540846.

KATSIKI, M., CHONDROGIANNI, N., CHINOU, I., RIVETT, A.J. and GONOS, E.S., 2007. The olive constituent oleuropein exhibits proteasome stimulatory properties in vitro and confers life span extension of human embryonic fibroblasts. *Rejuvenation Research*, vol. 10, no. 2, pp. 157-172. http://dx.doi.org/10.1089/rej.2006.0513. PMid:17518699.

KONTOGIANNI, V.G. and GEROTHANASSIS, I.P., 2012. Phenolic compounds and antioxidant activity of olive extracts. *Natural Product Research*, vol. 26, no. 2, pp. 186-189. http://dx.doi.org/10.1080/14786419.2011.582842. PMid:22060136.

LARSON, R.A., 1988. The antioxidants of higher plants. *Phytochemistry*, vol. 27, no. 4, pp. 969-978. http://dx.doi.org/10.1016/0031-9422(88)80254-1.

LE TUTOUR, B. and GUEDON, D., 1992. Antioxidative activities of Oleaeuropaea leaves and related phenolic compounds. *Phytochemistry*, vol. 31, no. 4, pp. 1173-1178. http://dx.doi.org/10.1016/0031-9422(92)80255-D.

LU, Y., ZHENG, M., WANG, B., FU, L., ZHAO, W., LI, P., XU, J., ZHU, H., JIN, H., YIN, D., HUANG, H., UPTON, A.M. and MA, Z., 2011. Clofazimine analogs with efficacy against experimental tuberculosis and reduced potential for accumulation. *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 11, pp. 5185-5193. http://dx.doi.org/10.1128/AAC.00699-11. PMid:21844321.

MEDINA, E., ROMERO, C., GARCÍA, P. and BRENES, M., 2019. Characterization of bioactive compounds in commercial olive leaf extracts, and olive leaves and their infusions. *Food & Function*, vol. 10, no. 8, pp. 4716-4724. http://dx.doi.org/10.1039/C9FO00698B. PMid:31304950.

MEHMOOD, A. and MURTAZA, G., 2018. Phenolic contents, antimicrobial and antioxidant activity of *Olea ferruginea* Royle (Oleaceae). *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, pp. 173. http://dx.doi.org/10.1186/s12906-018-2239-0. PMid:29866091.

MORTEZA, A.A., REZA, K.D., MAHDIYEH, L.R., MARYAM, N. and GOLNAZ, R., 2012. Antimicrobial activity of olive leaf aqueous extract. *Annals of Biological Research*, vol. 3, no. 8, pp. 4189-4191.

MUKHERJEE, P.K., MAITY, N., NEMA, N.K. and SARKAR, B.K., 2011. Bioactive compounds from natural resources against skin aging. *Phytomedicine*, vol. 19, no. 1, pp. 64-73. http://dx.doi.org/10.1016/j.phymed.2011.10.003. PMid:22115797.

OMAR, H.S., ABD EL-RAHMAN, S.N., ALGHANNAM, S.M., REYAD, N.E.A. and SEDEEK, M.S., 2021. Antifungal evaluation and molecular docking studies of olea europaea leaf extract, thymus vulgaris and boswellia carteri essential oil as prospective fungal inhibitor candidates. *Molecules (Basel, Switzerland)*, vol. 26, no. 20, pp. 6118. http://dx.doi.org/10.3390/molecules26206118. PMid:34684700.

PAREKH, J. and CHANDA, S.V., 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*, vol. 31, pp. 53-58.

PEREIRA, A.P., FERREIRA, C.F.R.I., MARCELINO, F., VALENTAO, P., ANDRADE, P.B., SEABRA, R., ESTEVINHO, L., BENTO, A. and PEREIRA, J.A., 2007. Phenolic compounds and antimicrobial activity of olive (*Oleaeuropaea L. CvCobrancosa*) Leaves. *Molecules (Basel, Switzerland)*, vol. 12, no. 5, pp. 1153-1162. http://dx.doi.org/10.3390/12051153. PMid:17873849.

RAHMANIAN, N., JAFARI, S.M. and WANI, T.A., 2015. Bioactive profile, dehydration, extraction and application of the bioactive components of olive leaves. *Trends in Food Science & Technology*, vol. 42, no. 2, pp. 150-172. http://dx.doi.org/10.1016/j.tifs.2014.12.009.

ROMERO, C., MEDINA, E., MATEO, M.A. and BRENES, M., 2017. Quantification of bioactive compounds in Picual and Arbequina olive leaves and fruit. *Journal of the Science of Food and Agriculture*, vol. 97, no. 6, pp. 1725-1732. http://dx.doi.org/10.1002/jsfa.7920. PMid:27447942.

SABRY, O.M.M., 2014. Review: beneficial health effect of olive leaves extracts. *J Nat Sci Res.*, vol. 4, pp. 1-9.

SINGLETON, V.L., ORTHOFER, R. and LAMUELA-RAVENTOS, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. *Methods in Enzymology*, vol. 299, pp. 152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

THRING, T.S., HILI, P. and NAUGHTON, D.P., 2009. Anticollagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine*, vol. 9, no. 1, pp. 27. http://dx.doi.org/10.1186/1472-6882-9-27. PMid:19653897.

VISIOLI, F., POLI, A. and GALL, C., 2002. Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews*, vol. 22, no. 1, pp. 65-75. http://dx.doi.org/10.1002/med.1028. PMid:11746176.

WON-YOUNG, C., DA-HEE, K., HA-JUNG, L., SU-JUNG, Y. and CHI-HO, L., 2020. Journal of food quality evaluation of effect of extraction solvent on selected properties of olive leaf extract. *Journal of Food Quality*, vol. 2020, pp. 3013649.

ZAGHLOUL, S.S., AZZAM, S.M., EID, H.H., HASSAN, H.A. and SLEEM, A.A., 2015. Chemical and biological investigation of essential oil of *Oroxylumindicum* L. leaves cultivated in Egypt. *International Journal of Pharmacognosy and Phytochemical Research*, vol. 7, no. 3, pp. 570-575.

ZHANG, Y., POST-MARTENS, K. and DENKIN, S., 2006. New drug candidates and therapeutic targets for tuberculosis therapy. *Drug Discovery Today*, vol. 11, no. 1-2, pp. 21-27. http://dx.doi.org/10.1016/S1359-6446(05)03626-3. PMid:16478687.