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Evaluation of the Antioxidant Activity of Fig Leaf Extract and Olive Leaf Extract

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Abstract :

This study aimed to investigate the antioxidant properties of fig leaf and olive leaf aqueous extracts. The components of extracts were characterized using GC-MS analysis, which identified the presence of active compounds like polyphenols and flavonoids. Antioxidant activity was assessed using the DPPH assay at different concentrations. Both fig and olive extracts demonstrated significant antioxidant properties. Notably, the olive extract exhibited higher scavenging activity compared to the fig extract, indicating its potential as a powerful natural antioxidant source. These results open up possibilities for developing antioxidant products and exploring their potential applications in the food and pharmaceutical industries.

Key words: Fig leaf extract, Olive leaf extract, Antioxidant, DPPH assay, GC-MS analysis .

تقييم النشاط المضاد للأكسدة لمستخلص أوراق التين ومستخلص أوراق الزيتون

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مستخلص:

هدفت هذه الدراسة إلى التعرف على الخصائص المضادة للأكسدة في المستخلصات المائية لأوراق التين وأوراق الزيتون. تم تمييز مكونات المستخلصات باستخدام تحليل GC-MS الذي حدد وجود مركبات فعالة مثل البوليفينول والفلافونويد. تم تقييم الفعالية المضادة للأكسدة باستخدام إختبار DPPH باستخدام تراكيز مختلفة من المستخلصات. أظهر كل من مستخلصي التين والزيتون فعالية مضادة للأكسدة. والجدير بالذكر أن مستخلص الزيتون أظهر نشاطًا أعلى في كسح الجذور الحرة مقارنة بمستخلص التين، مما يشير إلى قدرته كمصدر طبيعي قوي كمضاد للأكسدة. تفتح هذه النتائج إمكانيات لتطوير منتجات مضادات الأكسدة واستكشاف تطبيقاتها المحتملة في الصناعات الغذائية والصيدلانية.

الكلمات المفتاحية: مستخلص أوراق التين، مستخلص أوراق الزيتون، مضادات الأكسدة، فحص DPPH ، تحليل GC-MS .

Introduction

Oxidation reactions are vital for life but can also harm organisms. To counteract this, plants and animals have intricate antioxidant systems. Insufficient levels of antioxidants or antioxidant enzymes can lead to oxidative stress, resulting in cell damage or death (Kabel, 2014).

Free radicals contribute to various diseases, including cancer, atherosclerosis, diabetes, neurodegenerative disorders, and aging. Research has demonstrated that certain natural bioactive compounds derived from plants, such as phenols and flavonoids, possess antioxidant properties. These compounds can effectively neutralize free radicals and regulate oxidative stress (Fodouop *et al*., 2015).

Secondary metabolites, like flavonoids, present in numerous plants, hold significant importance for humans due to their ability to interact with multiple cellular targets. These compounds exhibit beneficial properties, including antioxidants and potential anticancer effects (Abood *et al*., 2021; Al-Juraisy, 2022).

The *Ficus carica*, a small tree belonging to the Moraceae family, is native to the Mediterranean, West Asia, and South Asia. It is now grown worldwide (Eisen, 1901; Ercisli *et al*., 2012). Different parts of the fig tree have been used in traditional medicine for treating ailments like anemia, cancer, diabetes, leprosy, liver diseases, paralysis, skin diseases, and ulcers. It shows promise in pharmaceutical biology for developing new drugs and future clinical applications (Badgujar *et al*., 2014; Kebal *et al*., 2022). Fig leaves, rich in bioactive compounds and antioxidants such as phenolic acids, flavonoids, vitamins, and fatty acids, offer various health benefits (Bashir *et al*., 2023; Kiralan *et al*., 2023).

The *Olea europaea*, a small tree or shrub of the Oleaceae family, is traditionally found in the Mediterranean basin (Green, 2002). The olive tree is known for its bioactive molecules, including oleuropein, hydroxytyrosol, tyrosol, caffeic acid, and ligstroside, which are associated with disease prevention. The leaves, in particular, are highly effective (Guinda *et al*., 2015). Olive leaves contain a diverse range of bioactive compounds and have been widely used in the treatment of various

diseases. Studies on olive leaf extracts and their constituents have demonstrated hypotensive, hypoglycemic, vasodilator, antimicrobial, antiviral, antioxidant, anti-tumor, and anti-inflammatory activities (Micol *et al*., 2005; Scheffler *et al*., 2008; Grawish *et al*., 2011; Wainstein *et al*., 2012; Abd El-Rahman, 2016). The active components of olives exhibit pharmacological properties, such as antiarrhythmic, spasmolytic, immune-stimulant, cardioprotective, anticancer, hypotensive, anti-inflammatory, antioxidant, and antithrombotic effects (El & Karakaya, 2009; Hassen *et al*., 2015).

Materials and Methods

1. Preparation of the extracts

Both the aqueous leaf extracts of fig and olive were prepared as described by Martin-Vertedor *et al*., (2016) by following these steps:

- Weighing 100 g of powder of leaves and putting it in a special beaker.
- 1000ml of distilled water was added to the powdered leaves and mixed by a glass rod, and the flask was sealed tightly with a cork stopper.
- The mixture was left in the water bath for 4 hours at a temperature of 65-70°C.
- Then the mixture was filtered first by using many layers of medical gauze to remove the large plant parts.
- Secondly, the mixture was filtered using Whatman No.1 filter paper.
- The filtrate was poured into glass dishes and placed in an oven at a temperature of 38 °C for 72 hours to obtain the extract.

The dried extract was placed in clean plastic containers covered with a light-proof layer and then kept in the refrigerator at (-4) °C until use.

2. Gas Chromatography-Mass Spectrum analysis (GC-MS)

A GC-MS analysis is done in the ministry of science and technology by using Shimadzu gc-2010 plus coupled with Shimadzu gcms-q2010 ultra. Capillary column (inert cap 1ms, 0.25mm, 30m, 0.25µm, gl sciences, Japan). The carrier gas is helium; constant flow rate 1 ml/min; auto-injector: aoc-20i, shimadzu. Injection volume: 5 µl. Coldumn oven temperature: 100°c.

Oven temperature set as: 100 °c for 3 min.; 240 °c for 9 min.; 280 °c for 5 min.; and 300 °c for 2min. The rate was 15.

Identification of chemical components is a direct comparison of the retention times and mass spectral data with computer matching with the) NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 2008).

3. Determination of antioxidant activity

The measurement of radical scavenging capacity was carried out according to (Braca *et al*., 2002) by mixing 1 ml of each plant extract concentration with 1 mL of DPPH solution. After 30 min of incubation in the dark, we measured the absorbance at 517 nm with an ultraviolet-visible (UV/VIS) spectrophotometer. Then we were calculating the percentage of free radical scavenging capacity by using the following equation (Su & Li, 2020):

Radical scavenging capacity% = (blank) – (extract) / (blank) x 100%

where: A (blank) = the absorbance of DPPH with methanol

An (extract) = the absorbance of DPPH mixed with plant extracts.

Results and Discussion

1- Gas Chromatography-Mass Spectrometry Analysis

GC-MS was employed in the detection of phytochemical compounds present in the aqueous leaf extracts of fig and olive. These compounds were characterized and listed based on their retention time (RT) and their peak area (Area %).

1.1 GC-MS analysis of fig leaf extract

The results of GC-MS analysis on fig leaf extract exhibited 25 peaks, which are presented in Figure 1 and Table 1. The obtained compounds are identified and listed:

Hexadecanoic acid, and methyl ester had the highest value (16.20%) among the active compounds followed by 5-(4-Bromo-phenyl carbamoyl)-3-methyl-3H-imidazole-4-carboxylic acid (7.48%), cis-13-Octadecenoic acid, methyl ester (7.34%), Heptadecanoic acid, 16-methyl-methyl ester (7.07%), Tricyclo (5.96%) and the remaining compounds were present in smaller amounts.

The hexadecanoic acid methyl ester which has 16.20% in the extract,

also known as Methyl palmitate, is an aliphatic acid ester reported to cause growth inhibition and apoptosis induction in human gastric cancer cells (Gugssa *et al*., 2011).

The 5-(4-Bromo-phenyl carbamoyl)-3-methyl-3H-imidazole-4-carboxylic acid which has 7.48% percent in the extract, has a high effect as an antioxidant and has a potent reduction in viral replicative capacity of HIV-1 *in vitro* (Serrao *et al*., 2013).

The 3,4-dimethylphenol which has 5.78% in the extract, has a high anticancer activity, and it showed cytotoxicity to MCF-7 cells in a study performed by Xin and his colleagues, (2020).

Psoralen (3.70%) is a furocoumarin compound with potent pharmacological action. Figs are one of the top sources of psoralen (Abdel-Aty *et al*., 2019). Psoralen exhibits a wide range of biological properties and has been demonstrated as an antioxidant, antidepressant, anticancer especially breast cancer as well antibacterial, and antiviral agent (Wu *et al*., 2013).

Tocopheryl acetate (vitamin E) with 1.70% in the extract has antioxidant activity (Ergul *et al*., 2019).

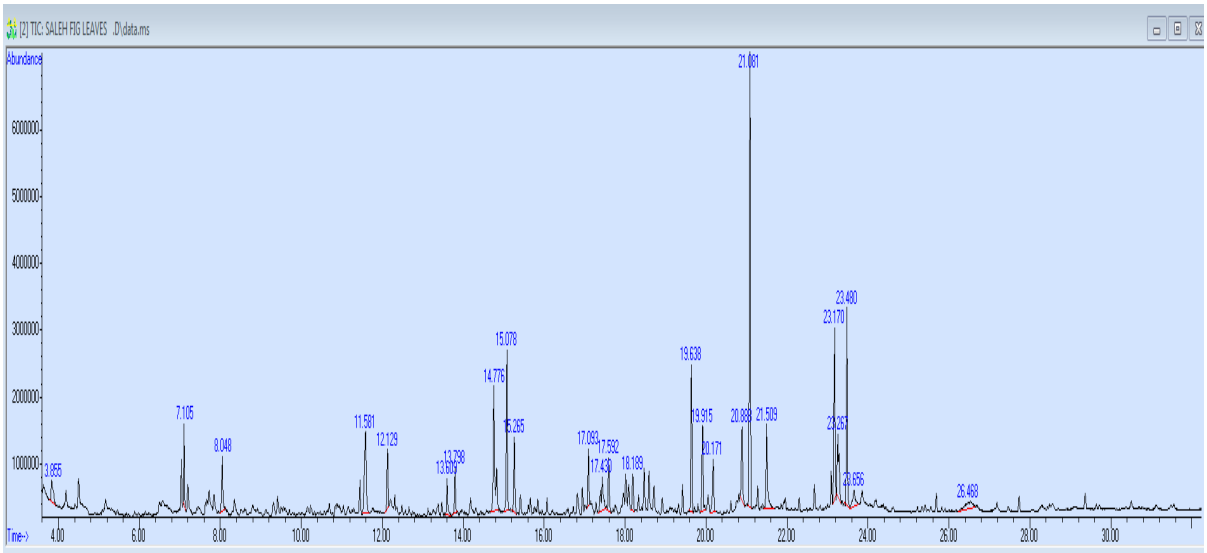

Figure 1. GC-MS analysis chromatogram of fig leaf extract

Table 1. The chemical compounds of fig leaf extract in GC-MS analysis.

	Components	RT	Area %	Molecular weight	Formula
1	Acetic acid, hydroxy-, ethyl ester	3.855	1.52	104.1045	$C_4H_8O_3$
2	2-Pentanol, 4-methyl-	7.102	2.25	102.1748	$C_6H_{14}O$
3	Heptanol	8.048	2.48	116.2013	$C_7H_{16}O$
4	Caprolactam	11.583	5.66	113.1576	$C_6H_{11}NO$
5	Anethole	12.131	2.51	148.2017	$C_{10}H_{12}O$
6	Acetaldehyde	13.611	1.54	44.0526	C_2H_4O
7	Dichloroxylenol	13.800	1.70	191.05	$C_8H_8Cl_2O$
8	5-(4-Bromo-phenylcarbamoyl)-3-methyl-3H-imidazole-4-carboxylic acid	14.779	7.48	324.13	$C_{12}H_{10}BrN_3O_3$
9	Tricyclo[4.3.1.1(3,8)]undecane, 1-methoxy-	15.079	5.96	180.29	$C_{12}H_{20}O$
10	3-Oxo-. alpha. -ionone	15.268	2.58	206.28	$C_{13}H_{18}O_2$
11	1-Cyclohexanol, 2-(3-methyl-1,3-butadienyl)-1,3,3-trimethyl-	17.094	1.99	204.38	$C_{14}H_{24}O$
12	4-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-butanone	17.433	2.77	192.30	$C_{13}H_{20}O$
13	1H-pyrrole-2-carboximidamide	17.590	2.08	109.13	$C_5H_7N_3$
14	beta. -Sitosterol	28.672	4.379	414.7	$C_{29}H_{50}O$
15	3,4-Dimethylphenol	19.638	5.78	122.16	$C_8H_{10}O$
16	Isopsoralen	19.918	3.70	186.16	$C_{11}H_6O_3$
17	propanoic acid 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)	20.172	2.68	170.17	$C_7H_{10}N_2O_3$
18	Tricyclo[6.3.0.0(2,7)]undecane-1,3,7-triol	20.890	3.34	266.31	$C_{13}H_{22}O_3$
19	Hexadecanoic acid, methyl ester	21.079	16.20	270.5	$C_{17}H_{34}O_2$
20	n-Hexadecanoic acid	21.509	4.48	256.4241	$C_{16}H_{32}O_2$
21	cis-13-Octadecenoic acid, methyl ester	23.173	7.34	296.4879	$C_{19}H_{36}O_2$
22	Octadecenoic acid, methyl ester	23.264	3.97	294.4721	$C_{19}H_{34}O_2$
23	Heptadecanoic acid, 16-methyl-, methyl ester	23.479	7.07	298.5038	$C_{19}H_{38}O_2$
24	Cyclododecanone, 2-(6-chloro-1-oxohexyl)-	23.655	1.57	314.9	$C_{18}H_{31}ClO_2$
25	Tocopheryl acetate	26.466	1.70	472.7	$C_{31}H_{52}O_3$

1.2 GC-MS analysis of olive leaf extract

The results of GC-MS analysis on olive leaf extract exhibited 25 peaks in the extract, which are presented in Figure 2 and Table 2. The obtained compounds are identified and listed:

Benzoic acid, 4-formyl-, methyl ester had the highest value (18.07%) among the other active compounds followed by n-Hexadecanoic acid (11.76%), Hexadecanoic acid, methyl ester (9.77%), Benzofuran, 2,3-dihydro (5.012%), 11-Octadecenoic acid, methyl ester (4.81%) and the remaining compounds were present in a smaller amount.

Benzoic acid (18.07%) and its derivatives play an important role in antifungal activity and other pharmacological activity. Two forms of hexadecanoic acid, which have (11.76% and 9.76%) in the extract, and also have a high percentage in the fig leaves extract, are reported to cause growth inhibition and apoptosis induction in human gastric cancer cells (Gugssa *et al*., 2011).

Benzofuran (5.012%) derivatives have various biological effects, including anti-inflammatory, antibacterial, antifungal, antihyperglycemic, analgesic, and antiparasitic, and have shown potential as therapeutic agents for breast cancer (Khodarahmi *et al*., 2015; Syed *et al.,* 2022).

Several forms of octadecadienoic acid (11-octadecenoic acid, methyl ester, octadecanoic acid and cis-13-octadecenoic acid, methyl ester) were found in the extract (4.81%, 4.003%, and 2.48%), octadecadienoic acid and its positional (c8, c10; c9, c11; c10, c12, and c11, c13) and geometric (cis, cis; cis, trans; trans, cis; and trans, trans) isomers of linoleic acid have been shown to have a variety of biological effects. Significant effects on health, such as anticancer, anti-oxidation, anti-atherosclerosis, and improving immuno-responses (Bergamo *et al*., 2014).

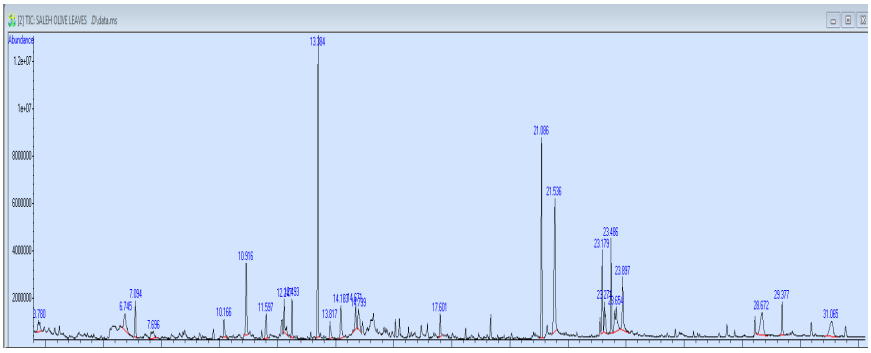

Figure 2. GC-MS analysis chromatogram of olive leaf extract

Table 2. The chemical compounds of olive leaf extract in GC-MS analysis

	Components	RT	Area %	Molecular weight	Formula
1	Ethyl ether	3.78	1.514	74.12	$C_4H_{10}O$
2	Propenamide, 2,2-dimethyl-	6.745	2.571	101.15	$C_5H_{11}NO$
3	Butane, 1-methoxy-2-methyl-	7.094	2.445	102.17	$C_6H_{14}O$
4	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester	7.696	1.504	238.19	$C_{11}H_{10}O_6$
5	2-Propenal, 3-(2-furanyl)	10.166	1.531	137.14	$C_7H_7NO_2$
6	Benzofuran, 2,3-dihydro	10.916	5.012	210.27	$C_{15}H_{14}O$
7	Caprolactam	11.597	2.057	113.16	$C_6H_{11}NO$
8	2-Pyridinamine, 4,6-dimethyl	12.27	2.309	122.167	$C_7H_{10}N_2$
9	Phenol, 3,5-diethyl	12.493	1.912	150.217	$C_{10}H_{14}O$
10	Benzoic acid, 4-formyl-, methyl ester	13.384	18.072	164.158	$C_9H_8O_3$
11	3,5-Dichloro-2,4-dimethylphenol	13.817	1.338	191.5	$C_8H_8Cl_2O$
12	Benzeneethanol, 4-hydroxy	14.183	2.77	207.27	$C_{12}H_{17}NO_2$
13	2-Propenoic acid, trimethylsilyl ester	14.671	2.039	144.24	$C_6H_{12}O_2Si$
14	Cycloheptasiloxane,tetradecamethyl-	14.799	2.159	519.07	$C_{14}H_{42}O_7Si_7$
15	1,5,5-Trimethyl-6-methylene-cyclhexene	17.601	1.417	136.23	$C_{10}H_{16}$
16	Hexadecanoic acid, methyl ester	21.086	9.768	270.5	$C_{17}H_{34}O_2$
17	n-Hexadecanoic acid	21.536	11.764	256.42	$C_{16}H_{32}O_2$
18	11-Octadecenoic acid, methyl ester	23.179	4.810	296.5	$C_{19}H_{36}O_2$
19	cis-13-Octadecenoic acid, methyl ester	23.271	2.487	296.5	$C_{19}H_{36}O_2$
20	Heptadecanoic acid, 16-methyl-, methyl ester	23.486	4.368	298.5	$C_{19}H_{38}O_2$
21	cis-Vaccenic acid	23.654	4.176	282.5	$C_{18}H_{34}O_2$
22	Octadecanoic acid	23.897	4.003	284.5	$C_{18}H_{36}O_2$
23	7-Octylidenebicyclo [4.1.0] heptane	18.190	1.65	206.37	$C_{15}H_{26}$
24	Tetracosane	29.377	1.883	338.7	$C_{24}H_{50}$
25	Taraxasterol	31.065	3.708	426.7	$C_{30}H_{50}O$

2- Determination of antioxidant activity

The free radical scavenging activity of fig and olive extracts was evaluated by DPPH assay. This method has been extensively used to predict antioxidant activities because of the relatively short time required for the analysis.

2.1 Antioxidant activity of fig leaf extract

The results obtained from using five concentrations of fig leaf extract showed free radical scavenging activity. The highest antioxidant potency of the extract was 84.04% when using a concentration of 100 µg/ml followed by a value of 68.49% when using a concentration of 50 µg/ml as shown in Table 3 and Figure 3.

Table 3. DPPH free radical scavenging activity of fig leaf extract

Concentrations (µg/mL)	Absorbance (A)	Radical scavenging capacity (%)
0 (control)	2.212	0
6.25	1.473	33.40
12.5	1.170	47.1
25	0.758	65.73
50	0.697	68.49
100	0.353	84.04

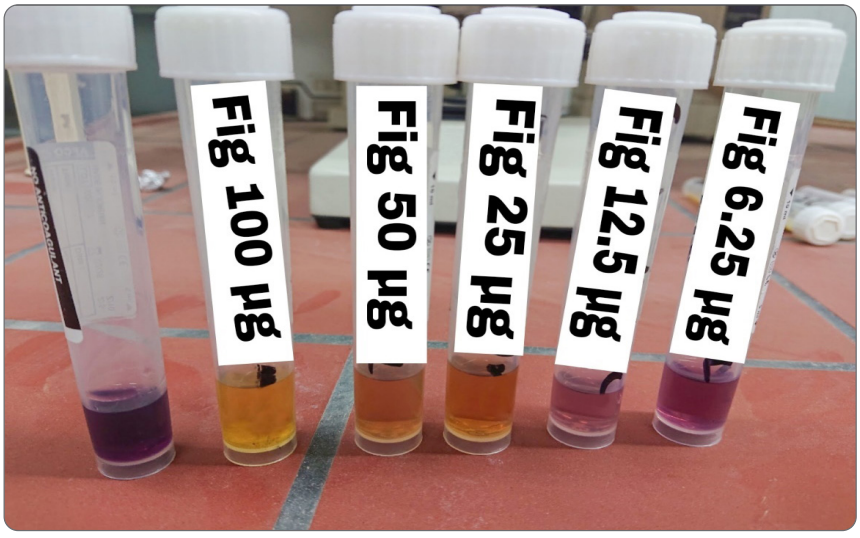

Figure 3. DPPH free radical scavenging activity for different concentrations of fig leaf extract

The results of the free radical scavenging activity of fig leaf extract agree with several studies that confirm that fig leaf extract has antioxidant activity because of the radical scavenging activity of phenols and their ability to quench free radicals because of their hydrogen-donating potential, thus reducing oxidative stress (Angelino *et al.,* 2017; Nadeem & Zeb, 2018).

The aqueous fig leaf extract showed an antioxidant capacity three times higher than the extract obtained with ethanol for conventional extraction and four times higher for ultrasound-assisted extraction; this might be attributed to the different polarity of water, thus modifying the solubility of the different target compounds (Alcantara *et al*., 2020)

The antioxidant activity of fig is probably due to the presence of plant secondary metabolites, which contain several bioactive compounds such as polyphenols, flavonoids, organic acids, vitamin E, and carotenoids that have the potency to inhibit oxidative mechanisms (Ayoub *et al*., 2019). These compounds are able to act as antioxidants in different ways, such as reducing agents, hydrogen donators, free radical scavengers, and singlet oxygen quenchers (Costa *et al*., 2009; Fattouch *et al*., 2007).

Fig leaves are phenolic sources that have antioxidant activity compared to the other plant parts; leaves were characterized by the presence of phenolic compounds such as flavone C-glycosides and hydroxybenzoic acid (Ammar *et al*., 2015).

2.2 Antioxidant activity of olive leaf extract

The results obtained in this study revealed that all concentrations of the olive leaf extract showed free radical scavenging activity by their electron transfer or hydrogen donating ability, where the highest antioxidant potency was 92.31% when using a concentration of 100 µg/ml of the extract, followed by a value of 90.91% when using a concentration of 50 µg/ml as shown in Table 4 and Figure 4.

Table 4. Free radical scavenging activity of olive leaf extract

Concentrations (µg/mL)	Absorbance (A)	Radical scavenging capacity (%)
0 (control)	2.212	0
6.25	0.735	66.77
12.5	0.411	81.14
25	0.252	88.6
50	0.201	90.91
100	0.170	92.31

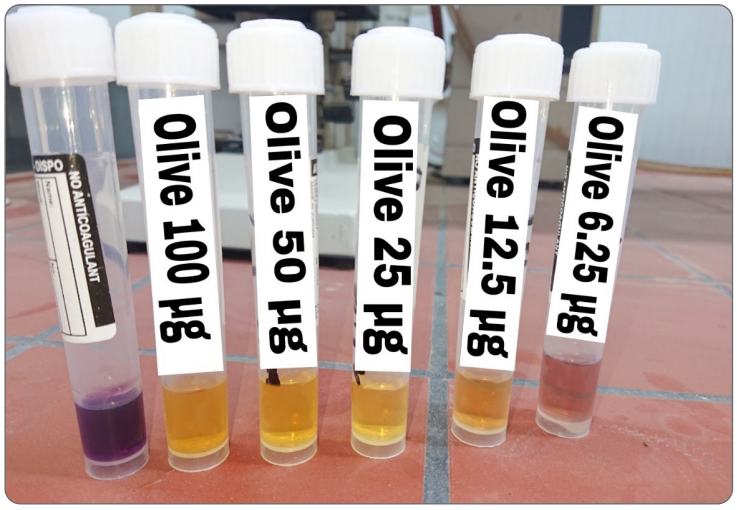

Figure 4. DPPH free radical scavenging activity for different concentrations of olive leaf extract

The results are consistent with several studies that indicated the antioxidant activity demonstrated for most olive leaf extract preparations, and the selection of the aqueous extract for analysis of its antioxidant potency, easiness of preparation and water solubility, which indicates water solubility for its active compounds, a very desirable physical property for potential drugs (Goulas *et al.,* 2009; Martinez-Navarro *et al*., 2023).

The compound derivatives of olive leaves have high antioxidant activity. The content of the total phenolic compounds (TPC) and dihydroxybenzoic had antioxidant activity (Xie *et al*., 2015; Yancheva *et al*., 2016).

Olive leaves have compounds such as phenols in several forms that are present in large quantities in mature olive leaves. These compounds con-

tain groups of phenolic compounds that serve as antioxidants by chelating metals like copper and iron, which catalyze free radical production reactions such as lipid oxidation (Ezz El-Din Ibrahim *et al.,* 2022).

In a study that was conducted, the antioxidant properties of the hot aqueous extract of olive leaves were identified that were used with foods, which have high effectiveness due to the presence of many biological active compounds like phenolic compounds (Cho *et al*., 2020).

In the results, when we compare the free radical scavenging activity of fig leaf extract and that of olive leaf extract, we find that the olive extract has a greater ability to scavenge free radicals in all five concentrations than the fig extract, as shown in Figure 5.

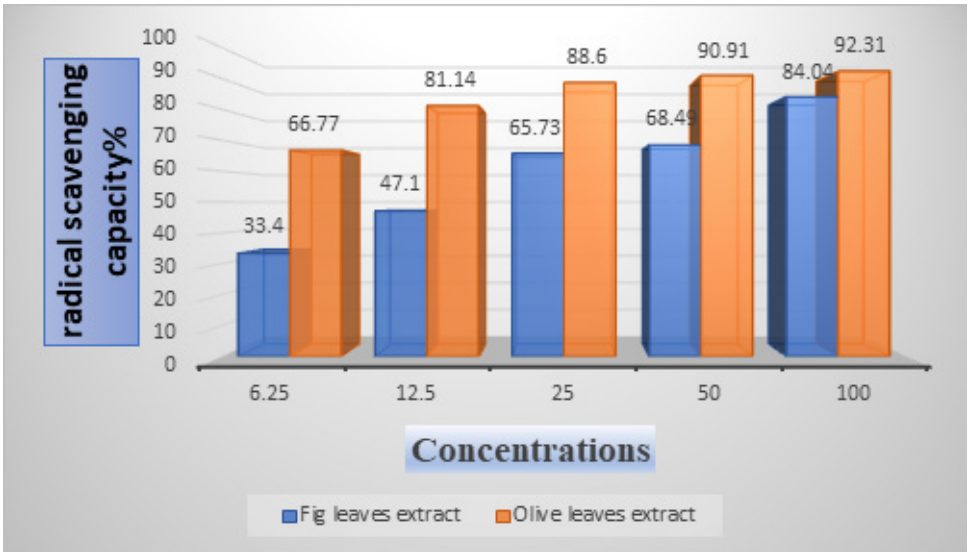

Figure 5. Comparison of free radical scavenging capacity of fig and olive leaf extracts

CONCLUSION

Cancer initiation and progression have been correlated to oxidative stress in the body by rising mutations, DNA damage, and genome instability. The fig leaf and olive leaf aqueous extracts contain various active components that may introduce antioxidant activity. The results of this paper ensure that both extracts have good anti-radical activity. The results suggest that fig and olive leaves can be used as a source of natural antioxidants, which can be used as nutraceuticals to promote health or as preservatives to delay the peroxidation of foods.

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