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An overview of chemical composition and fungicidal activity of Olive (Olea Europea L.) Leaf Extract.

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Abstract

Human has been dealing with olive tree (*Olea europea* L.) since the beginning of ages, relying on the oil of its fruits because of its great economic and nutritional importance. Attention began in recent decades for the leaves of the olive tree for its high medicinal importance. Several studies indicated that olive leaves have multiple vital useful components and can be used medically. Also, they were proven important in treating high blood pressure, high cholesterol and diabetes, as well as anti-oxidative food additives, antibacterial and antifungal. The chemical composition of olive leaf extract indicates that it is rich mainly in oleuropein. The present mini-review gives an overview on the different methods of olive leaf extraction, the chemical composition of the extracts and its use as an antifungal agents. All the results collected showed that the extract has an antifungal efficacy against a wide range of fungus. Olive leaf extracts can inhibit the growth of *Aspergillus flavus*, *Aspergillus Ochraceus*, *Penicillium sp*, *Fusarium moniliforme*, *Tricophyton rubrum*, *Mauginiella scaettae*, *Magnaporthe grisea*, *Candida dubliniensis*, *Penicillium digitatum*, *Penicillium italicum*, *Aspergillus niger*, *Aspergillus ochraceus*, *Phytophthora sp*, *Fusarium proliferatum* and *Candida albicans* strains with a good percentage of inhibition providing its fungicidal activity.

Keywords: Fungus, oleuropein, olive leaf extract, percentage of inhibition.

1. Introduction

Pathogenic microorganisms are a serious risk for human health by causing infectious diseases [1]. During the last three decades, the side effects of antimicrobial drugs increased with the development of certain antibiotics [2]. This problem raised the need to discover other alternatives which are intended to be beneficial for humanity. Exploring the benefits of plants can be an alternative to find new remedies against pathogenic diseases [3]. Olive tree is an important and traditional European and Mediterranean species, well known for its broad pharmacological benefits [4]. Generally, the main use of olives for many centuries was limited to consuming oil of fruits in food and medicine; however, leaves of this tree contain very significant chemical compounds of medicinal importance [5]. Looking at the web, researchers can find many commercial products from olive leaf extract, most of which focus on using the extract as an adjunctive treatment for patients with diabetes and high blood cholesterol [6].

The medicinal uses of *Olea europea* L. were highly cited in literature [7], especially, olive leaves have been used for a long time in traditional medicine in different forms against microbiological diseases [8]. Many scientific researchers have been focused on the study of the chemical composition of the olive leaves and their biological activities [9, 10]. Browsing literature, various studies reported that olive leaves were rich in polyphenols [11], particularly oleuropein [12], and described its health benefits [13] as well as

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antioxidant [14], hypoglycaemic [15], antihypertensive [16, 17], anticancer [18] and anti-inflammatory activities [19].

Moreover, olive leaves extract had antimicrobial behaviour [20] and may inhibit the growth of wide pathogens, including *Salmonella typhi*, *Escherichia Coli*, *Pseudomonas aeuroginosa*, *Bacillus subtilis* and *Staphylococcus aureus* [21, 22] .

Despite the large amount of research on the benefits of olive tree products and the abundance of these resources in the World Library, it was found that the benefit of olive leaves in the pharmaceutical fields did not have the appropriate reputation for the size of these benefits. Therefore, one of the most important goals of this work is to shed light on the pharmacological importance of olive leaf extract, in its use as an anti-fungus that annoys the human skin and sometimes it is an internal infection in people with weak immunity. Usually chemical compounds are used as anti-fungi that have unwanted side effects [23]. So the search for natural sources for the treatment of fungal diseases that affect living organisms, including humans, is an important matter from a therapeutic and clinical point of view.

We have reviewed the chemical composition of different extracts of olive leaves. Perhaps the reader will find a relationship between the chemical composition of olive leaves and their anti-fungal effects.

2. Preparation of olive leaf extracts

Extraction is a crucial step for the investigation of the desired plant. Chemical composition of the extract depends on the extraction procedure used. Olive leaf extract can be obtained even by aqueous and organic extractions.

2.1. Hot aqueous extraction

Olive's leaves collected then washed by distilled water and dried. 50 grams of leaf were put in Soxhlet apparatus and 250 ml of distilled water were added. After 8 hours, the apparatus was cooled and the water was evaporated by rotary. The obtained extract was refrigerated until analysis [24]. Hydrodistillation procedure was another alternative to obtain an aqueous extract from leaves [25, 26]. Also, the extract can be obtained by incubation of olive leaves in ultrapure water (1:10 w/v) for 3h at 60-65°C, followed by filtration and centrifugation [27].

2.2. Hot organic extraction

Olive leaves were collected then washed to remove impurities and dried in air oven for 3 days at 380°C. The dried leaves were grounded to obtain a homogenous powder. 10 grams were extracted by 200 mL of ethanol (70%) at 38°C for 2 hours by thermo-shaker. Ethanolic extract was obtained after centrifugation, and then the solvent was evaporated by rotary. The crude extracts were obtained and refrigerated until further use [28]. Powder of dried leaves can be extracted by Soxhlet apparatus using methanol for 7 h [29].

2.3. Cold aqueous extraction

Powder of olive leaves was added to a known volume of sterile distilled water for 15 days with shaking. The obtained solution was filtered, then lyophilized and refrigerated [30-32].

2.4. Cold organic extraction

Powder of olive leaves was added to known volume of 95% ethanol for 15 days with shaking. The obtained solution was filtered through cotton wool then filter paper to remove the coarse and fine particles. The obtained extract was evaporated and the concentrated extract was refrigerated [30, 33-35]. Also, ethyl acetate, acetone, methanol [36], chloroform [37, 38], Dimethyl sulfoxide (DMSO) [39], hexane and dichloromethane [40] could be good solvents for extraction.

3. Chemical composition of olive leaf extract (OLE)

Bioactive components from agriculture derivatives play a great role in food and pharmaceutical industries. Usually, they are used for food preservation because they are rich in polyphenolic compounds, flavonoids, vitamins, minerals and other components. Moreover, polyphenolic compounds and flavonoids were recognized by their antioxidant properties and their resistance against the development of considerable variety of microorganisms. The proximate physio-chemical composition of OLE is presented in Table1 [36].

Table 1: Physio-chemical parameters of methanolic olive leave extract: quantitative estimation [36].

Parameters	Percentage (%)
Ash	4.12±0.13
Moisture	52.92±0.12
Crude fiber	22.0±0.03
Crude fat	15.6±0.07
Brix	1.0±0.04
pH	7.17±0.05

Value = mean ±S.D of triplicate data

3.1. Determination of polyphenolic compounds in olive leaf extract (OLE)

OLE is an interest source of bio-phenols and is a mixture of different natural products divided into phenolic acids, phenolic alcohol, secoiridoid derivatives, flavonoids, hidrocinamic derivatives and flavanols (Table 2). The amount of total polyphenols depends on many parameters, including the cultivation zone, leaf age, leaf stage and other abiotic parameters [41]. Quantification of polyphenolic compounds in olive leaf extract was usually carried out by HPLC apparatus.

Oleuropein, secoiridoid derivative, is the major active compound (Fig.1). It is present in almost *olea europea.* L leaves extract (Table 2) and the content is approximately 33% to 53% of the total concentration of polyphenolic compounds depending on the cultivar. Oleuropein has exhibited many pharmacological effects including anti-oxidant [8], antimicrobial [11], anti-viral [44], neuroprotective, cardioprotective [45], hypoglycemic properties [46]. Furthermore, oleuropein also had anti-ischemic [47], anti-inflammatory [19] and hypolipidemic [48] effects.

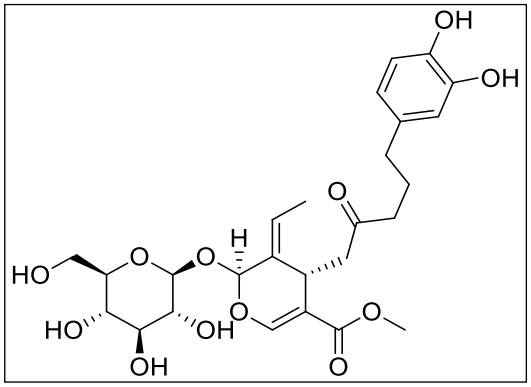

Fig. 1: Chemical structure of Oleuropein.

Table 2: Reported major constituent (phenolic compounds) of OLE from different countries.

Country	Major constituent	References
Croatia	Oleuropein (47.27%), hydroxytyrosol (1.28%), tyrosol (0.74%), pinoresinol (0.44%), protocatechuic acid (0.18%), Apigenin (0.12%) , non-identified phenolic compound (49.9%)	[31]
Italy	Oleuropein (33%), Luteolin-4-*O*-glucoside (5.94%), Luteolin-7-glucoside (14.19%), Verbacoside (1.65%), non-identified phenolic compound (45.21%)	[38]
Spain	Oleuropein (52.87%), hydroxytyrosol (25.62%), Luteolin-7-glucoside (8.77%), Apigenin -7-*O*- glucoside (5.03%), Tyrosol (3.94%), Verbacoside (2.14%), Rutin (0.88%), caffeic acid (0.47%), chlorogenic acid (0.072%), Quercetin -3-O-galactoside (0.071%), Epicatechin (0.05%), Quercetin -3-O-rutinoside (0.034%), Kaempferol (0.013%), gallic acid (0.004%), p-cumaric acid (0.0019%), vanillin (0.011%)	[27]
Tunisia	Oleuropein (53.77%), Luteolin-7-glucoside (9.11%), Luteolin-4-glucoside (5.88%), Apigenin (4.19%), Diosmetin (3.91%), 2-methoxyoleuropein (3.46%), Hydroxytyrosol (3.18%), Apigenin -7-glucoside (3.18%), Rutin (2.82%), Verbascoside (2.45%), Vanillin (2.27%), Luteolin (1.93%), Oleuropein aglycone (1.06%), Ligostride (0.99%), Ligastroside aglycone (0.33%), Hydroxytyrosol glucoside (0.26%), HydroxyD-oleuropein agylcon (0.16%), Tyrosol (0.14%), Agylcon oleuropein dialdehydic (0.10%), 10-hydroxy oleuropein aglycon (0.03%)	[42]
Iran	Oleuropein (40.50%), ferulic acid (11.80%), verbascoside (10.40%), cinnamic acid (4.40%), hydroxy tyrosol (4.10%), p-cumaric acid (4.00%), Rutin (3.10%), catechin (1.80%), caffeic acid (1.60%), vanillic acid (1.60%), Quercetin (1.60%), Luteolin (1.30%), Vanillin (1.00%), Tyrosol (0.90%), Gallic acid (0.90%), Apigenin (0.90%), Others (10.30%)	[43]

Halawi et al. (2015) [30] showed that the total polyphenolic content of olive leaf extract depend on the adopted procedure of extraction. Comparing water-based and organic-based extraction of olive leaves, cold ethanolic extraction provided a higher amount of bio-polyphenols on average 98 mg/g of extract (Fig.2). This finding was confirmed by Peršurić et al. (2019) [49], when high value of total polyphenol obtained in methanolic extract comparing with aqueous extract.

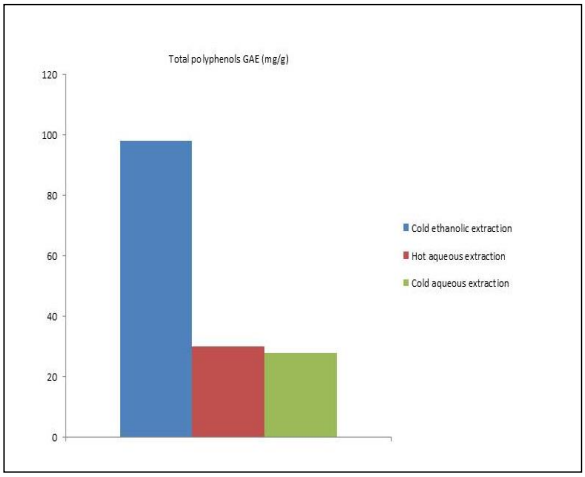

Fig. 2: Total polyphenols in OLE according to extraction process [30].

3.2. Determination of volatile compounds in olive leaf extract (OLE)

Identification of volatiles components is useful for food, pharmaceutical and cosmetics industries. Analysis of volatile compounds in olive leaf extract of different Tunisian cultivars was carried out by GC/MS apparatus (Table 3) [25].

Table 3: Volatiles compounds in OLE of three Tunisian varieties [25].

	Compound	**Fresh leaves extract Range (%)**	**Dried leaves extract Range (%)**
1	Furfural	0 - 0.8	-
2	(*E*,*Z*)-2.4-hexadienal	0.9 - 2.6	0.5 - 3.3
3	(*E*)-2-hexenol	<0.1	<0.1
4	(*E*)-3-hexenol	15.9 - 28	3.4 - 16.3
5	1-hexanol	0.8 - 11	0.4 - 5.2
6	(*Z*)-4-heptanal	0 - 0.4	0 - 0.6
7	Heptanal	0.4 - 0.5	0 - 1.2
8	(*E*,*E*)-2.4-hexadienal	0 - 1.1	0 - 0.8
9	2-acetylfurane	0.6 - 1	0 - 0.7
10	α-pinene	0 - 1.6	0 - 1.2
11	(*Z*)-2-heptenal	0 - 0.4	0 - 0.6
12	Benzaldehyde	1- 5.5	1.1 - 3.5
13	3-ethenylpyridine	2.2 - 18.1	2.2 - 5.5
14	Phenol	0.3 - 2.9	1.1 - 3.9
15	Hexanoic acid	0 - 0.8	-
16	3-Octanone	-	<0.1
17	6-methyl-5-hepten-2-one	-	0.3 - 0.7
18	Octanal	<0.1	0 - 0.9
19	(*E*,*E*) -2,4-heptadienal	0.8 - 1.9	0.6 - 1.6
20	(*E*,*Z*) -2,4-heptadienal	0.9 - 2.8	0 - 1.2
21	Benzyl alcohol	3.2 - 9.4	7.4 - 13.2
22	Phenyl acetaldehyde	0 - 1.3	0 - 5.5
23	(*E*)-2-octenal	0 - 0.4	0 - 0.1
24	1-octanol	0.8 - 2.7	0.9 - 1.6
25	*Cis*-linalool oxide	0 - 0.6	0 - 1.4
26	*Trans*-linalool oxide	0 - 0.4	0.5 - 1.6
27	Linalool	0 - 0.9	1.2 - 1.7
28	Nonanal	1.9 - 6.4	2.2 - 10
29	Phenyl ethyl alcohol	2.7 - 14.9	15.1 - 22.3
30	Methyl nicotinate	0 - 0.7	1.6 - 1.7
31	4-ketoisophorone	<0.1	-
32	(*E*,*Z*)- 2.6-nonadienal	0 - 0.4	0 - 0.4
33	(*E*)-2-nonenal	0.5 - 0.6	0.6 - 1.1
34	1-nonanol	0 - 0.7	0 - 0.7
35	*Trans*-linalool oxide (pyranoid)	-	0 - 0.7
36	*p*-cymen-8-ol	<0.1	0 - 0.4
37	α-terpineol	0 - 0.1	0.5 - 2
38	Methyl- salicylate	0 - 0.8	0 - 5.2
39	(*Z*)-4-decenal	0.3 - 1.3	0 - 1.9
40	Decanal	<0.1	0 - 1.2
41	2-ethylbenzaldehyde	0 - 0.7	0 - 0.4
42	benzothiazole	0 - 4.5	0 - 3.3
43	Geraniol	0 - 0.6	0 - 0.9

44	(*E*)-2-decenal	0.6 - 2.4	0.7 - 2.2
45	Salicylic alcohol	0 - 0.9	-
46	*p*-menth-1-en-7-al	0 - 0.4	0 - 0.6
47	1-tridecene	0 - 0.4	-
48	(*E,Z*)-2.4-decadienal	0 - 0.6	0 - 0.8
49	4-vinylguaiacol	1.7 - 6.8	0 - 6.2
50	(*E,E*)-2,4-decadienal	0 - 0.9	0 - 1.4
51	Eugenol	-	0 - 1.1
52	(*E*)-*β*-damascenone	3.3 - 14.7	1.7 - 13.7
53	*Cis*-*α*-bergamotene	0 - 3.9	0 - 3.7
54	(*Z,E*)-2.6-dodecadienal	0 - 0.6	0 - 3.3
55	*Trans*-*α*-bergamotene	-	0 - 0.4
56	(*E*)-isoeugenol	0 - 0.6	0 - 0.7
57	(*E*)-geranylacetone	0 - 0.8	0 - 5.4
58	(*E*)-β-ionone	0 - 1	0 - 1.4
59	Caryophyllene oxide	-	0 - 0.1

Fresh leaves samples contain mostly 3-ethynylpyridine ranging from 2.2 % to 18.1 % and (*E*)-3-hexenol ranging from 15.9 % to 28 %. Moreover, dried leaf samples were found to be rich in phenyl ethyl alcohol (15.1 % - 22.3 %), (*E*)-3-hexenol, (*E*)-β-damascenone and benzyl alcohol (Fig.3). Several minor compounds were detected in different amounts from fresh and dried leaves including (*E,Z*)-2,4-hexadienal (0.5-3.3 %), (*E,E*)-2,4-heptadienal (0.6-1.9 %), 1-octanol (0.8-2.7 %) and *E*-2-decenal (0.7-2.4%).

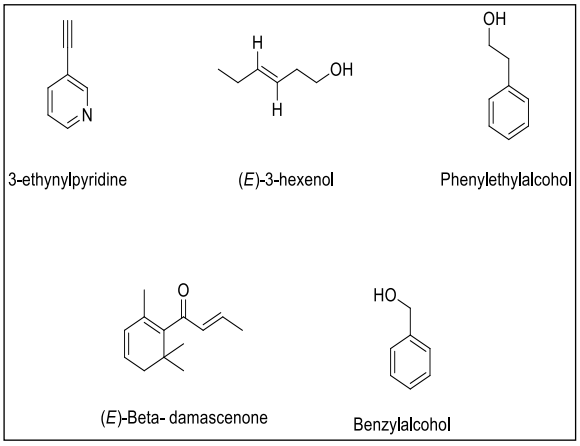

Fig.3: Major volatiles compounds identified in Tunisian OLE.

Battinelli et al. (2006) [50] reported antifungal activity of some aliphatic aldehydes from olive fruit [hexanal, nonanal, (*E*)-2-hexenal, (*E*)-2-heptenal, (*E*)-2- octenal, (*E*)-2-nonenal] to many human skin fungal pathogens. They proved the positive role of such compounds to suppress elastase, a virulence factor essential for the dermatophytes colonization, and their cytotoxicity on cultures of reconstructed human skin keratin cells. Aldehydes group inhibited the growth of many of the fungi tested in that study, especially the unsaturated aldehydes group. Additionally, Battinelli and his co-workers postulated that the anti-elastase activity played an important role as antimicrobial activity of these compounds. Subsequently, these aldehydes were not cytotoxicin to human reconstructed epidermis.

In the other hand, chemical composition of the volatile fractions in olive leaves from Gabon was completely different when half of the total volatile extract contains only two major compounds which are α-humulene (34.6%) and β-caryophyllene (14.9%) (Fig.4) [26].

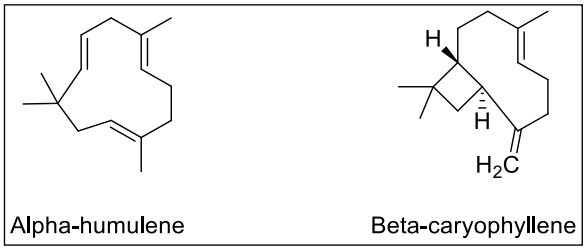

Fig.4: Major volatiles compounds identified in Gabon OLE.

4. Antifungal activities

Applications of olive leaves in food industries were largely described in the literature as shown by Souilem et al. (2016) [51]. The majority of studies confirmed the relationship between olive leaf extract and its biological activity. Additionally, antifungal effects of olive leaf extract have been already described in many studies. Subsequently, oleuropein was reported to be the major component of OLE that exhibited good antifungal activity [52].

Abdel-razek et al. (2017) [53] and Abd El-Aziz et al.(2012) [33] evaluated the efficacy of OLE against growth of aflatoxigenic fungi and its aflatoxins. These toxins could contaminate food products (Table 4). Two hundred mg/mL of OLE was sufficient to inhibit the growth of *Aspergillus flavus*, *A.*

Ochraceus, *Penicillium* sp. and *Fusarium moniliforme*.

Abdulhameed et al. (2016) [54] demonstrated that OLE had a good inhibition efficacy against *Tricophyton rubrum* developed in intestinal mice. Treatment by 0.5 ml of OLE (0.5mg/mL) per day during one month prevented the growth of *T. rubrum*. Hussain et al. (2015) [38] showed that OLE was effective against some fungi including *Fusarium moniliform*, *Mauginiella scaettae* and *Magnaporthe grisea*, while *Aspergillus flavus*, *Alternaria alternata*, *Trichothecium roseum*, *Saccharomyces cerevisiae* and *Botrytis cinerea* were resistant to OLE. Zorcić et al. (2016) [31] proved that the growth of *Candida dubliniensis* CBS 7987 could be inhibited by OLE. Tayel et al. (2015) [55] indicated that OLE could be a good antifungal agent against *Penicillium digitatum* and *Penicillium italicum* developed at the surface of citrus fruits. Similarly, OLE showed antifungal activity against *A. niger* with zone of inhibition 6±0.06 mm [36].

Olive leaf extract exhibited a growth inhibition zone against *Aspergillus ochraceus* and their minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) were 42.5 µg/mL and 45 µg/ml, respectively [34]. Moreover, fungi of *Phytophthora* spp. were abundant in soil and cause infection to different parts of plants. Due to richness of OLE by phenolic compounds, the growth of these fungi could be inhibited up to 49.6±3.1% [39]. Innocenzo et al. (2020) [38] evaluated that OLE could prevent the development of *Fusarium proliferatum* causing diseases to plants (percentage of inhibition ranging from 58.02±1.91% to 66.31±10.47%). Subsequently, free OLE or encapsulated in chitosan-tripolyphosphate nanoparticles could be used as bio-fungicides.

Commonly, *Candida albicans* is the main cause of many fungal infections. Various types of drugs used for the treatment of *candida* sp. [56] but they are subjected to problems of toxicity and resistance limiting they use [57]. OLE was tested for his efficacy against *C. albicans*, and potent results were reported.

Nosrallahi et al. (2015) [24], conducted that Iranian olive leaves aqueous extract could act as good antifungal agent against the development of *C. albicans* with MIC of 24 mg/ml, MFC of 48 mg/ml and inhibition zone diameter of 21 mm. Ethanolic extract didn't exhibit fungicidal activity against *C. albicans* [35]. Lebanon OLE was tested against the growth of *C. albicans*, and the best results obtained when ethanolic leaves extract was used comparing with hot and cold aqueous extracts. The largest inhibition zone diameter was obtained (18 to 27 mm) by using ethanolic OLE. However when hot aqueous extract and cold aqueous extract were used the inhibition zone diameter was ranging from 11 to 19 mm and 7-15 mm respectively [30]. Antifungal activity of OLE from Croatia was evaluated against *C. albicans* and the result showed that OLE inhibited the development of this fungus with MIC value was 46.875 mg/mL [31, 52]. Three Tunisian olive cultivars were tested against *C. albicans* and the results shown that the OLE from *Chemlali cultivar* did not exhibit antifungal activity. Moderate activity was obtained using OLE from *Neb Jmel cultivar* with MIC of 310 µg/ml. but OLE from the dried leaves of *Chemchali cultivar* proved high fungicidal activity (MIC=1250 µg/ml) [25]. OLE from Gabon displayed antifungal activity against *C. albicans* and inhibition zones were collected ranging from 22 to 35 mm [26].

It is worth to mention that, one of the most famous synthetic chemical compounds used in the treatment of fungal infections of human skin is the compound "miconazole". Miconazole is included in ointments and creams that are used to treat skin diseases in humans. Alerts increased for the danger of these compounds to human health, and even changed them as a compound included in the preparation of selective culture media for some microorganisms. Sometimes, synthetic chemical compounds are used to treat severe fungal infections through the blood, i.e. as systemic treatment. Therefore, it has become imperative and very necessary to search for safe sources to work as anti-fungi and treatments for skin fungi diseases for humans [58].

5. Conclusion

Due to the increase in serious human infections accorded to fungal species, divers' scientific studies were focused on the evaluation of the antifungal activity of *Olea europea* L. leaves extract against a large variety of fungal pathogens. Chemical analysis of the extract indicates that it is rich in polyphenols. Oleuropein is the major product, in addition to other derivatives whose contents vary depending on the species. Furthermore, the analysis of volatiles components showed the presence of 59 compounds. The richness of the chemical composition of the

extract has given it several fields of application. Research work has shown that olive leaf extract has antioxidant, anti-microbial, anti-viral and antifungal activities. Great interest is given to the antifungal properties of the extract due to its power to inhibit the development of a wide range of fungi, which gives it a broad range of applications. Therefore, further research work is needed to develop the use of the extract as an anti-fungal agent and to broaden its field of application.

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6. References

[1] Sarmah P., Dan M.M., Adapa D. and TK S., A review on common pathogenic microorganisms and their impact on human health. *Electronic j. boil.,* **14**, 50-58(2008).
[2] Singh R., Sripada L. and Singh R., Side effects of antibiotics during bacterial infection: Mitochondria, the main target in host cell. *Mitochondrion*, **16**, 50–54(2014).
[3] Abreu A.C., McBain A.J. and Simões M., Plants as sources of new antimicrobials and resistance-modifying agents. *Nat. Prod. Rep.,* **29**, 1007-1021(2012).
[4] Medina E., Brenes M., Romero C., García A. and De Castro A., Main antimicrobial compounds in table olives. *J. Agric. Food. Chem.,* **55**, 9817–9823(2007).
[5] Morton Walker D.P.M., Olive leaf extract: nature's antibiotic. Kensington Publishing Corp., New York, p.100-218(1997).
[6] Acar-Tek N. and Ağagündüz D., Olive Leaf (*Olea europea* L. folium): potential effects on glycemia and lipidemia. *Ann. Nutr. Metab.,* **76**, 10-15(2020).
[7] Khan Md.Y., Panchal S., Vyas N., Butani A. and Kumar V**.,** Olea europaea: A phyto-pharmacological review. *Phcog. Rev.,* **1**, 114-118(2007).
[8] Benavente-García O., Castillo J., Lorente J., Ortuño A. and Del Rio J.A., Antioxidant activity of phenolics extracted from Olea europaea L. leaves. *Food Chem.,* **68**, 457–462(2000).
[9] Sabry O.M.M., Review: beneficial health effects of olive leaves extracts. *J. Nat. Sci. Res.,* **4**, 1-10(2014).
[10] Özcan M.M. and Matthäus B., A review: benefit and bioactive properties of olive (Olea europaea L.) leaves. *Eur. Food Re. Technol.,* **243**, 89–99(2017).
[11] Pereira A.P., Ferreira I.C., Marcelino F., Valentão P., Andrade P.B., Seabra R., Estevinho L., Bento A. and Pereira J.A., Phenolic compounds and antimicrobial activity of olive (Olea europaea L. Cv. Cobrançosa) leaves. *Molecules,* **12**, 1153–1162(2007).
[12] Ortega-García F. and Peragón J., HPLC analysis of oleuropein, hydroxytyrosol, and tyrosol in stems and roots of *Olea europaea* L. cv. Picual during ripening: Phenol concentrations in stems and roots of olive tree during ripening. *J. Sci. Food Agric.,* **90**, 2295–2300(2010).
[13] Omar S.H., Oleuropein in olive and its pharmacological effects. *Sci. Pharm.,* **78**, 133–154(2010).
[14] Lee O.H. and Lee B.Y., Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. *Bioresour. Technol.,* **101**, 3751–3754(2010).
[15] Barbaro B., Toietta G., Maggio R., Arciello M., Tarocchi M., Galli A. and Balsano C., Effects of the olive-derived polyphenol oleuropein on human health. *Int. J. Mol. Sci.,* **15**, 18508–18524(2014).
[16] Perrinjaquet-Moccetti T., Busjahn A., Schmidlin C., Schmidt A., Bradl B. and Aydogan C., Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces blood pressure in borderline hypertensive monozygotic twins. *Phytother. Res.,* **22**, 1239–1242(2008).
[17] Khayyal M.T., El-Ghazaly M.A., Abdallah D.M., Nassar N.N., Okpanyi S.N. and Kreuter M.H., Blood pressure lowering effect of an olive leaf extract {Olea europea) in L-NAME induced hypertension in rats. *Arzneimittelforschung,* **52**, 797–802(2002).
[18] Anter J., Fernández-Bedmar Z., Villatoro-Pulido M., Demyda-Peyras S., Moreno-Millán M., Alonso-Moraga Á., Muñoz-Serrano A. and Luque De Castro M.D**.,** A pilot study on the DNA-protective, cytotoxic, and apoptosis-inducing properties of olive-leaf extracts. *Mutat. Res.*, **723**, 165–170(2011).
[19] Haloui E., Marzouk B., Marzouk Z., Bouraoui A. and Fenina N., Hydroxytyrosol and oleuropein from olive leaves: Potent anti-inflammatory and analgesic activities. *J. Food Agric. Environ.,* **9**, 128-133(2011).
[20] Sudjana A.N., D'Orazio C., Ryan V., Rasool N., Ng J., Islam N., Riley T.V. and Hammer K.A., Antimicrobial activity of commercial Olea europaea (olive) leaf extract. *Int. J. Antimicrob. Agents,* **33**, 461–463(2009).

[21] Markin D., Duek L.and Berdicevsky I., In vitro antimicrobial activity of olive leaves. *Mycoses*, **46**, 132–136(2003).
[22] Trong Le N., Viet Ho D., Quoc Doan T., Tuan Le A., Raal A., Usai D., Sanna G., Carta A., Rappelli P., Diaz N., Cappuccinelli P., Zanetti S., Nguyen H. and Donadu M.G., Biological Activities of Essential Oils from Leaves of *Paramignya trimera* (Oliv.) Guillaum and *Limnocitrus littoralis* (Miq.) Swingle. *Antibiotics*, **9**, 207-219(2020).
[23] Ravikant, Kaur T., Gupte S. and Kaur M**.,** A review on emerging fungal infections and their significance. *J. Bacteriol. Mycol. Open Access*, **1**, 39-41(2015).
[24] Nasrollahi Z.and Abolhasannezhad M., Evaluation of the antifungal activity of olive leaf aqueous extracts against *Candida albicans* PTCC-5027. *Curr. Med. Mycol.*, **1**, 37–39(2015).
[25] Brahmi F., Flamini G., Issaoui M., Dhibi M., Dabbou S., Mastouri M.and Hammami M., Chemical composition and biological activities of volatile fractions from three Tunisian cultivars of olive leaves. *Med. Chem. Res.*, **21**, 2863–2872(2012).
[26] Bikanga R., Makani T., Agnaniet H., Obame L.C., Abdoul-Latif F.M., Lebibi J. and Menut C., Chemical composition and biological activities of *Santiria trimera* (Burseraceae) essential oils from Gabon. *Nat. Prod. Commun.*, **5**, 961-964(2010).
[27] Martín-Vertedor D., Garrido M., Pariente J.A., Espino J. and Delgado-Adámez J., Bioavailability of Bioactive Molecules from Olive Leaf Extracts and its Functional Value. *Phytother. Res.*, **30**, 1172–1179(2016).
[28] Khalaf H.Y., Abdullah B.A. and Jassim N.A**.,** Study of the antifungal (*Candida Albicans*) activity of olive leaves extracted in the intestinal of mice. *Int. J. Adv. Res.*, **4**, 1016-1039(2016).
[29] Ferreira I.C.F.R., Barros L., Soares M.E., Bastos M.L. and Pereira J.A., Antioxidant activity and phenolic contents of Olea europaea L. leaves sprayed with different copper formulations. *Food Chem.*, **103**, 188–195(2007).
[30] Halawi M.H., Rahman S.M.A.and Yussef H., Comparative study of the antifungal activity of Olea europaea L. against some pathogenic *Candida albicans* isolates in Lebanon. *Int. J. Curr. Microbiol. App. Sci.*, **4**, 970-984(2015).
[31] Zorić N., Kopjar N., Kraljić K., Oršolić N., Tomić S.and Kosalec I., Olive leaf extract activity against *Candida albicans* and *C. dubliniensis* – the in vitro viability study. *Acta Pharm.*, **66**, 411–421(2016).
[32] Abd El-Aziz A.R.M., Al-Othman M.R., Al-Sohaibani S.A., Mahmoud M.A. and Murugan K., Prevention of aflatoxin contamination of maize by Aspergillus flavus through aqueous plant extracts in Saudi Arabia. *Afr. J. Microbiol. Res.*, **6**, 6931–6935(2012).
[33] Khattab R.A., Hosny A.E.D.S., Abdelkawy M.A., Fahmy R.H. and ElMenoufy N.A., Anti-HSV type-1 activity of olive leaf extract crude form acting as a microemulsion dosage form. *Afr. J. Microbiol. Res.*, **10**, 820–828(2016).
[34] Tayel A.A., Salem M.F., El-Tras W.F. and Brimer L., Exploration of islamic medicine plant extracts as powerful antifungals for the prevention of mycotoxigenic Aspergilli growth in organic silage. *J. Sci. Food Agric.*, **91**, 2160-2165(2011).
[35] Shialy Z., Zarrin M., Sadeghi Nejad B. and Yussef Naanaie S., In *vitro* antifungal properties of Pistacia atlantica and olive extracts on different fungal species. *Curr. Med. Mycol.*, **1**, 40–45(2015).
[36] Lutfullah G., Tila H., Hussain A. and Khan A.A**.,** Evaluation of plants extracts for proximate chemical composition, antimicrobial and antifungal activities. *Am-Euras J. Agric. & Environ. Sci.*, **14**, 964-970(2014).
[37] Hussain M.A., Khan M.Q., Ali I., Islam Dar M.E.U. and Habib T., Antifungal potential of different parts of *Olea europaea* and *Olea cuspidata* growing in Azad Jammu and Kashmir. *Pure Appl. Biol.*, **4**, 204–216(2015).
[38] Muzzalupo I., Badolati G., Chiappetta A., Picci N. and Muzzalupo R., In vitro antifungal activity of olive (*Olea europaea*) leaf extracts loaded in chitosan nanoparticles. *Front. Bioeng. Biotechnol.*, **8**, 151-161(2020).
[39] Del Río J.A., Báidez A.G., Botía J.M. and Ortuño A., Enhancement of phenolic compounds in olive plants (*Olea europaea L.)* and their influence on resistance against *Phytophthora sp. Food Chem.*, **83**, 75–78(2003).
[40] Wiart C., Hannah A., Yassim M., Hamimah H.and Sulaiman M., Antimicrobial activity of *Acalypha siamensis* Oliv. ex Gage. *J .Ethnopharmacol.*, **95**, 285–286(2004).
[41] Petridis A., Therios I., Samouris G. and Tananaki C., Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea L.)* and their relationship to

antioxidant activity. *Environ. Exp. Bot.*, **79**, 37–43(2012).
[42] Essafi H., Trabelsi N., Benincasa C., Tamaalli A., Perri E.and Zarrouk M., Phytochemical profile, antioxidant and antiproliferative activities of olive leaf extracts from autochthonous Tunisian cultivars. *Acta Aliment.*, **48**, 384–390(2019).
[43] Hukerdi Y.J., Nasri M.H.F., Rashidi L.and Ganjkhanlou M., The study of physicochemical properties and nutrient composition of *Mari* olive leaf cultivated in Iran. *Nutr. Food. Sci. Res.*, **5**, 39–46(2018).
[44] Micol V., Caturla N., Pérez-fons L., Más V., Pérez L. and Estepa A., The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). *Antiviral Res.*, **66**, 129–136(2005).
[45] Omar S.H., Cardioprotective and neuroprotective roles of oleuropein in olive. *Saudi Pharm. J.*, **18**, 111–121(2010).
[46] Al-Azzawie H.F. and Alhamdani M.S., Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sci.*, **78**, 1371–1377(2006).
[47] Andreadou I., Iliodromitis E.K., Mikros E., Constantinou M., Agalias A., Magiatis P., Skaltsounis A.L., Kamber E., Tsantili-Kakoulidou A. and Kremastinos D.T., The Olive Constituent Oleuropein Exhibits Anti-Ischemic, Antioxidative, and Hypolipidemic Effects in Anesthetized Rabbits. *J. Nutr.*, **136**, 2213–2219(2006).
[48] Jemai H., Bouaziz M., Fki I., El Feki A. and Sayadi S., Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chem. Biol. Interact.*, **176**, 88–98(2008).
[49] Peršurić Ž., Saftić L., Klisović D.and Pavelić S.K., Polyphenol-based design of functional olive leaf Infusions. *Food Technol. Biotechnol.*, **57**, 171–182(2019).
[50] Battinelli L., Daniele C., Cristiani G., Bisignano G., Saija A. and Mazzanti G., In vitro antifungal and anti-elastase activity of some aliphatic aldehydes from *Olea europea* L. fruit. *Phytomedecine*, **13**, 558-563(2006).
[51] Souilem S., Fki I., Kobayashi I., Khalid N., Neves M.A., Isoda H., Sayadi S. and Nakajima M., Emerging technologies for recovery of value-added components from olive leaves and their applications in Food/Feed industries. *Food Bioprocess Technol.*, **10**, 229–248(2017).
[52] Zorić N., Kopjar N., Bobnjarić I., Horvat I., Tomić S. and Kosalec I., Antifungal activity of oleuropein against *Candida albicans*—The in vitro study. *Molecules*, **21**, 1631-1640(2016).
[53] Abdel-Razek A.G., Badr A.N. and Shehata M.G., Characterization of olive oil by-products: antioxidant activity, its ability to reduce Aflatoxigenic fungi hazard and its Aflatoxins. *Annu. Res. Rev. Biol.*, **14**, 1–14(2017).
[54] Abdulhameed B., Younes H.and Suheel R., Study of the antifungal (Trichophyton rubrum) activity of olive leaves extracted in the intestinal of mice. *I M A J*, **3**, 164-177(2016).
[55] Tayel A.A., Moussa S.H., Salem M.F., Mazrou K.E.and El-Tras W.F., Control of citrus molds using bioactive coatings incorporated with fungal chitosan/plant extracts composite: Control of citrus molds using bioactive coatings. *J. Sci. Food Agric.*, **96**, 1306–1312(2016).
[56] De Aguiar M.M., De Albuquerque R.P., Marinho D.S., Braga B.R., Dornelas C.B., Oliveira A., De Sousa V.P., Torres S.R., Alviano D.S., Alviano C.S., Cabral L.M. and Holandino C., Oral sustained release nystatin tablets for the treatment of oral candidiasis: formulation development and validation of UV spectrophotometric analytical methodology for content determination. *Drug Dev. Ind. Pharm.*, **36**, 594–600(2010).
[57] Robinson R., Antifungal fluconazole induces aneuploidy, sowing the seeds of its own failure. *P.L.O.S. Biol.*, **12**, e1001816- e1001817(2014).
[58] Heel R.C., Brogden R.N., Pakes G.E., Speight T.M. and Avery G.S., Miconazole: A preliminary review of its therapeutic efficacy in systemic fungal infections. *Drugs*, **19**, 7-30(1980).