Physicochemical characterization and in vitro evaluation of the antioxidant, and anti-inflammatory activities of olive kernel essential oil

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Abstract
This study was carried out in order to assess the essential oil's antioxidant properties that were extracted from the kernel of *Olea europea*. The essential oil was extracted by hydrodistillation, and physicochemical examination and extraction yield determination came next. Then, the total antioxidant capacity and DPPH free radical scavenging techniques were used to assess the antioxidant activity. While the anti-inflammatory potential was assessed using protein denaturation inhibition method. The essential oil's content after extraction was 0.77 ± 0.01%. The essential oil of olive kernels was found to have acceptable quality based on an analytical evaluation of its physicochemical characteristics. The antioxidant activity evaluation's findings indicated that this essential oil possesses intriguing antiradical qualities. It showed up as a low IC50 value of 0.012 mg/ml. It was observed that the antioxidant capacity of olive kernel essential oil is approximately 21.48 ± 0.02 mg AAE/g. For the effect the anti-inflammatory, the essential oil of olives kernel at a concentration of 250 µg/ml showed inhibition of 29.49% of protein denaturation. This demonstrates how the phytochemicals and pharmacological potential of olive kernel essential oil would have a number of positive benefits and might be used in medication formulation.

Keywords: Olive's kernel, essential oil, Antioxidant, Anti-inflammatory.

Introduction

In the Mediterranean basin, the olive tree (*Olea europea* L.) has been cultivated since ancient times. It has great importance in the social, economic, cultural and nutritional fields, etc., for the populations of this region, which accounts for 95% of production and 85% of global consumption. It is made up of more than 2,600 different varieties (Muzzalupo et al., 2014). In Algeria, olive cultivation is a major fruit tree on a global scale and is a very important component of the sustainable development process. Olive growing is the most widespread fruit crop, with nearly 34,739,080 trees in our country, occupying 5.72% of the usable agricultural area (UAA) (Guechi et al., 2016). According to data from the Ministry of Agriculture, the olive-growing areas cultivated in Algeria have increased almost three times over the past seventeen years: from 170,000 hectares in 2000 to 487,000 hectares in 2018 (Oreggia and Marinelli, 2018). According to Chouki et al. (2006), there are more than 150 olive cultivars more or less cultivated, only 36 cultivars have been identified based on morphological and agronomic characteristics (Himour, 2016).
The olive tree (*Olea europaea* L.) is one of the most characteristic trees in the Mediterranean region. It is considered an aromatic and medicinal plant, a reservoir of natural compounds

with beneficial effects. Some compounds identified in leaf extracts, such as phenolic compounds, have very important biological activities (Bisignano et al., 1999). The olive tree is a plant that plays a very important role for humans, as it can be used in the production and extraction of olive oils, which are used in food, cosmetics, and therapeutic applications, etc. Moreover, the medicinal properties of the olive tree are also attributed to its kernels, which are currently the subject of numerous scientific studies. The olive industry generates, in addition to oil as its main product, large quantities of by-products (Molina et al., 2008). The recovery of these residues has become a dual ecological and economic necessity. It helps reduce increasingly significant pollution and contributes to improving the profitability of the olive sector.

Olive kernels can contain extractable components with high added value. However, in recent years, the use of oil from these kernels has grown worldwide, particularly in the cosmetic and pharmaceutical industries, due to their specific characteristics (Niazi et al., 2017). This oil is considered a valuable vegetable oil due to its richness in fatty acids, phenolic compounds, and antioxidants. Furthermore, it has multiple benefits for human health (Shahib and Marshal, 2003). It may also protect against UV rays, which are responsible for numerous cellular damages. Furthermore, oils derived from olive kernel may be a promising alternative to synthetic antioxidants due to their high antioxidant activity. Converting olive kernel into oil is a promising approach to reclaiming agricultural waste and promoting biotechnological solutions. Olive kernel, also known as olive stones or pomace, are a byproduct of olive oil production (Van der et al., 2007). They contain a high concentration of organic matter, making them a potential source of renewable energy. Oxidative stress is one of the most widespread pathologies, although its severity is often overlooked. Oxidative stress is responsible for many diseases such as diabetes, cancer, neuropathies, and infertility. The antioxidants present in our diet are not sufficient to neutralize the free radicals present in high concentrations in the body, hence the interest in studies on antioxidants of natural origin. The objective of this experimental study is to valorize a waste product from the agri-food industry, namely olive kernel, by extracting essential oils, and to evaluate the antioxidant and anti-inflammatory capacity of this oil.

Materials and methods

Sample Preparation

Sigoise olive kernel was collected from olive oil mills located in the Sig region, Mascara, in 2024. The kernel was washed with hot water, and then distilled water to remove any traces of pulp and impurities that might adhere to them. After washing, the kernel was dried in an oven at a temperature of 103ºC ± 2°C for 24 hours to facilitate grinding. Once dry, the kernel were ground into a fine powder using a mechanical grinder and an electric mixer. The resulting powder was then stored in airtight plastic jars and kept at room temperature.

Extraction of the essential oil

Using a Clevenger equipment, hydro-distillation was used to extract the essential oils. Olive powder (100 g) was heated. The distillate was gathered once the temperature had stabilized. The distillate was mixed with sodium chloride (NaCl). After that, the mixture was put in a separating funnel, and three consecutive Cyclohexane washes (10, 10, and 20 ml) were accomplished. To extract the essential oil and eliminate the cyclohexane, the organic phase was subjected to rotary evaporation following agitation. Following the yield computation, the essential oil was kept at +4°C (Kheyer et al., 2014).

Sensory properties and physicochemical indices

The sensory and physicochemical properties of olive powder essential oil (EO) were evaluated according to European Pharmacopoeia (AFNOR, 2000). Parameters measured included appearance, color, odor, solubility, relative density, refractive index, optical rotation, miscibility, acid index (AI), ester index (EI), and carbonyl index (CI). Density was measured with a pycnometer at 20 °C. Optical rotation was determined using a polarimeter with a sodium-vapor lamp (589.3 nm). Refractive index was measured using an ABBE refractometer (±0.0002). Miscibility was tested by adding ethanol to EO until a homogeneous solution formed. Acid index (AI) was calculated after titration of EO with 0.1 M KOH. Ester index (EI) was determined by refluxing EO with 0.5 M KOH, followed by titration with 0.5 M HCl, and calculated relative to AI. Carbonyl index (CI) was measured after reaction with hydroxyl ammonium chloride and KOH, followed by titration with 0.5 M HCl.

DPPH Free radical scavenging activity

The Sanchez-Moreno (2002) approach, which uses DPPH as a relatively free radical that absorbs in the visible at the wavelength λ of 517 nm, has been used to determine the antiradical activity of olive powder. 2.4 mg of DPPH were dissolved in 100 mL of 100% methanol to create the DPPH solution beforehand. 975 µl of DPPH is mixed with 25 µl of the essential oil at various doses. As a positive control, a standard antioxidant solution (ascorbic acid) was also made under the same circumstances. Methanol and DPPH served as the negative control. For half an hour, the mixture was left in the dark. A wavelength of 517 nm was used for the spectrophotometric test. The percentage of antiradical activity was estimated according to the equation:

$$\text{Anti-radical activity [\%]} = [(A1-A2) / A1] \times 100$$

A1: Absorbance of the negative control

A2: Absorbance in the presence of the extract

The concentration of the test sample needed to lower 50% of the DPPH radical was known as the Inhibitory Concentration 50 (IC50). Based on varying extract concentrations, the IC50 values were visually determined by inhibition % (Torres et al., 2006). Every test was run in triplicate for the duration of the experiment.

Total Antioxidant Capacity

Using the Prieto et al. (1999) approach, the Total Antioxidant Capacity (TAC) (Phosphomolybdate test) was assessed. A green to yellowish complex of phosphate/Mo (V) at acid pH was formed by reducing molybdenum Mo (VI), which was present as molybdate MoO_4^{2-}, to molybdenum Mo (V) MoO_2^{+} ions in the presence of essential oil. 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were combined with 0.3 mL of essential oil. After that, the tubes were incubated for 90 minutes at 95°C. Following cooling, the absorbance of the solutions was measured at 695 nm in comparison to the blank, which was incubated as a sample and included 3 mL of the reagent solution and 0.3 ml of methanol. The unit of measurement for total antioxidant capacity was milligrams of ascorbic acid equivalent per gram (mg AAE/g). The test was performed in triplicate.

Anti-inflammatory activity

Using a slightly modified version of Kandikattu's (2013) test of prevention of thermal denaturation of BSA (Bovine Serum Albumin) proteins, we assessed the anti-inflammatory properties of olive kernel essential oil. The essential oil works on the basis of preventing BSA from becoming denaturated by heat at 72°C. A range of essential oil concentrations, from 1 to 0.0312 mg/ml, are tested. 1 ml of the 0.2% BSA solution made in Tris HCl (0.05 M at pH 6.6) is mixed with 1 ml of each dilution. After that, the mixture is incubated for 15 minutes at 37°C then for 5 minutes at 72°C. After vortexing and quickly cooling the liquid at the end of

the incubation, a spectrophotometer is used to measure the turbidity at 660 nm. One milliliter of EO and one milliliter of Tris-HCl (0.05 M at pH 6.6) are combined to create a blank. The absorbance of EO and Tris-HCl is must be subtracted from the data using this blank (Wei, 2010). Diclofenac served as the reference anti-inflammatory in this test. The identical operating parameters that were used for the samples were used to evaluate its anti-inflammatory activity. The examinations are conducted twice. The percentage inhibition of bovine serum albumin (BSA) denaturation was determined using the following formula:

Percentage inhibition of denaturation = [(A control -A sample) / A control] × 100

A control: the absorbance of the negative control.

A sample: the absorbance of the essential oil or diclofenac

The concentration needed to lessen 50% of the BSA protein's thermal denaturation is known as the IC50. It is computed using the anti-inflammatory activity plotted against different amounts of diclofenac and the tested essential oil.

Statistical analysis

The mean ± standard deviation (Mean ± SD) was used to express the values. ANOVA single factor for multiple comparisons was used to examine the data. P values below the significance level ($p < 0.05$) were deemed statistically significant.

Results and discussion

The extraction yield of the olive kernel, which includes a significant amount of oil, is 0.77%. The content of an essential oil varied greatly, depending on a wide range of factors, whether they were intrinsic (genetic, vegetative stage), extrinsic (soil, temperature, latitude), or technological (Evans, 1998). The quality of an essential oil and the efficiency of its extraction were also affected by a number of factors, including temperature, relative humidity, and the length of insolation (Bruneton, 1993), as well as the method, operating pressure, and distillation time (Bennadja, 2013., Bagheri, 2014).

Sensory properties and physicochemical indices

The quantity of free fatty acids produced by the hydrolytic reactions of triglycerides was indicated by the acid index. It was thought to be a quality characteristic that allowed for the preservation of an essential oil; a high-grade essential oil should be low in acidity (Bourachouche, 2017).

Table 1. The physicochemical parameters of the essential oil of olive kernel.

	Properties	**Essential oil of olive kernel**
Chemical properties	Ester index	113.67± 0.11
	Carbonyl index	275.19±0.32
	Acid index	02.11 ± 0.08
	Saponification index	215.78 ± 0.13
	pH	4.4± 0.001
Physical properties	Mixibility	$V_{EO} / 2V_{Ethanol}$
	Refractive index	1.3913 ± 0.001
	Relative density	0.611 ± 0.03
	Rotary power	+3.2°± 0.04

A fatty material with a short carbon chain of fatty acids has a greater saponification index. In our instance, the carbonyl index for the kernal olive's essential oil had a value of 275.19 ± 0.32 and this index reached a value of 215.11 ± 0.13 (Table 1). Generally speaking, high values for both indices indicated high-quality essential oils. When assessing the quality of essential oils, physicochemical characteristics were helpful (Bey-Ould, 2016). The sensory and physicochemical characteristics of the olive kernel plant's essential oil were ascertained by its fractionation. The sensory characteristics of the essential oil revealed that it had a light

brown hue, a liquid appearance, and a strong, unusual, camphoric, fresh, striking, and lingering smell.

The pH estimate for the essential oil of olive kernels was 4.3 ± 0.01. This figure was found in the reference standard, which stated that essential oils must have a pH between 4 and 6. The presence of compounds and molecules with an acidic character likely connected this pH to the essential oil's chemical makeup. The density of essential oil was 0.611 ± 0.03. This index showed a value of +3.2°± 0.04 for the spinning power. It was discovered that this essential oil agreed with the AFNOR criteria for essential oils, which stipulated that the optical rotation value must fall between +0° and +10°. Essential oils were identified using the refractive index, which was regarded as a criterion of purity. Every material has a refractive index. In fact, essential oils were given a refractive index of 1.460 to 1.476 according to the AFNOR norm. This index in our instance came to 1.3913 ± 0.001 (Table 1). The amount of monoterpenes and oxygenated derivatives significantly altered the refractive indices. A higher index will result from a higher monoterpene content. Refractive index and density were unaffected by seasonal variations, according to Hussain et al. (2008). According to certain studies, essential oils with low refractive indices have a low refraction of light, which may encourage their usage in cosmetics (Bey-Ould; 2016).

DPPH Free radical scavenging activity

An absorbance measurement at 517 nm was carried out, which made it possible to evaluate the ability of olive kernel essential oil to trap the free radical DPPH at different concentrations. The results obtained led to the creation of curves representing the percentage of antiradical activity for olive kernel essential oil as well as that of vitamin C. The antiradical activity is dose-dependent, as shown in Figure 1, because it is proportional to the increase in the concentration of the essential oil. By absorbing an electron or a hydrogen atom provided by an antioxidant chemical, the DPPH radical was decolored in the presence of the antioxidant in this technique for trapping the DPPH radical (Apostolou). Therefore, this radical's great reducing power was caused by the essential oil's constituents' ability to donate hydrogen and electrons, which gives them a powerful antioxidant that inhibits free radicals.

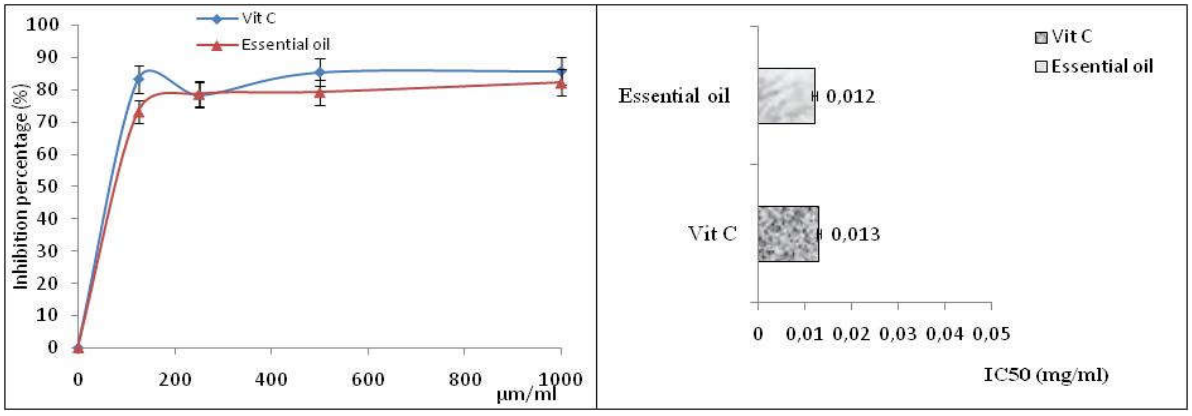

Figure 1. Inhibition percentage and IC50 of Essential oil of Kernal olives and ascorbic acid.

The 50% inhibitory concentration, or IC50 value, is calculated for both the standards and the essential oils. It stands for the amount of substrate needed to reduce DPPH activity by 50%, which is the same as lowering the absorbance of the original DPPH solution by 50%. A significant anti-radical impact is indicated by low IC50 values, which are inversely related to the scavenger effect (Villano et al., 2007). With a low IC50 value (0.012 mg/ml), the essential oil of olive kernel oil demonstrated an intriguing anti-free radical property. The essential oil of olive kernel oil demonstrated a substantially higher IC50 value (0.013 mg/ml) than ascorbic acid, indicating that it had a higher antioxidant potential (Figure 1).

Total Antioxidant Capacity

The antioxidant capacity of olive kernel essential oil was found to be approximately 21.48 ± 0.02 mg AAE/g. By lowering phosphomolybdate, this result demonstrated the potent antioxidant properties of olive kernel essential oil. Phenolic chemicals were identified as the source of the olive kernel essential oil's potent antioxidant effect. Numerous related polyphenols have been found to significantly contribute to phosphomolybdate scavenging activities in recent investigations. Additionally, flavonoids were in charge of effective free radical scavenging.

Inhibition of albumin denaturation

One of the reasons for inflammation is protein denaturation. Denaturation of proteins may be the cause of autoantigen formation in inflammatory disorders. The modification of electrostatic, hydrogen, hydrophobic, and disulfide linkages that preserve proteins' three-dimensional structure is one potential process of denaturation.

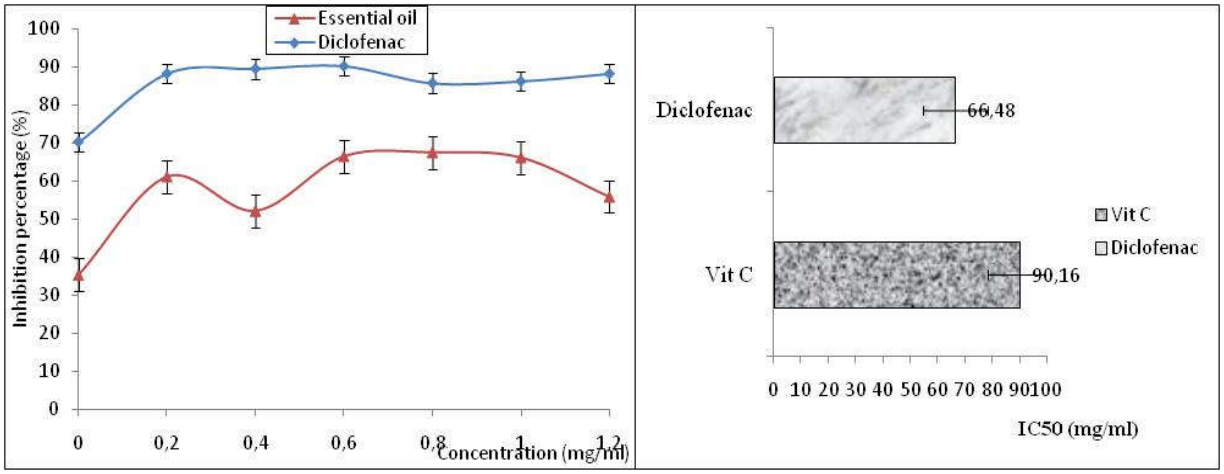

Figure 2. Inhibition percentage of BSA denaturation.

Applying an external stressor, such as heat, an organic solvent, a concentrated inorganic salt, or a chemical like an acid or basic, can cause protein denaturation. Autoantigens may result from this process, which causes proteins to lose their biological activities and tertiary and secondary structures (Pietta, 2000). The percentage of inhibition was used to express it. Figure 2 shows the variation in the percentage of protection against thermal denaturation of BSA according to different concentrations of olive kernel essential oil. This result is compared to that recorded for diclofenac, considered in this test as the reference molecule. According to the results, it appears that there is a proportional relationship between the increase in concentration and the percentage of BSA denaturation by the essential oil and diclofenac.

Our results show that the percentage of inhibition of BSA denaturation by the essential oil is lower than that of diclofenac for all concentrations used. At a concentration of 1 mg/ml, diclofenac showed a maximum percentage inhibition of BSA denaturation of 90.16%, while for the essential oil, the maximum percentage inhibition of BSA denaturation was 66.48%. Changes to the electrostatic, hydrogen, hydrophobic, or disulfide bond may be part of the denaturation pathway (Kar, 2012). Because of this, the bioactive compounds found in olive kernel essential oil can help to protect these various kinds of structural linkages.

Conclusion

This kernel's essential oil is of acceptable grade and conforms with standards, according to the findings of sensory and physicochemical analyses. Thus, this oil's intriguing anti-free radical qualities were demonstrated by the antioxidant activity. When compared to ascorbic acid, the common antioxidant, it showed up as a low IC50 value. There is a good chance that this oil

will be used to create novel anti-inflammatory medications. Given its well-established anti-inflammatory qualities, it might be a useful substitute for existing therapies with fewer adverse effects. For chronic illnesses including arthritis and inflammatory skin disorders, this oil may be especially helpful.

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Conflict of Interest: The authors have no conflict of interest to declare.

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