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Research Article
Comparative Phytochemical Investigations and *in vitro* Pharmacological Activities of Different Brands of Olive Oil

Mawiya Jaifar Talib Al Mawali, Nada Ali Abd El Hamid Nassar, Wegdan Aly Shehata, Nadiya Salim Banioraba and Mohammad Sohail Akhtar

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, Nizwa, Sultanate of Oman

Abstract

Background and Objective: Olive oil is composed of primary triacylglycerol about 99% of the whole components and secondary fatty acids which are mono, diacylglycerols and phosphates. Olive oil is known to contain many active constituents with different pharmacological actions such as antioxidant action. The purpose of this research is thus, to compare phytochemical components of different olive oil brands as well as to determine their cytotoxic and antioxidant properties. **Materials and Methods:** All those four brands of extra virgin olive oil contained all necessary active constituents especially oleic acid, palmitic acid and linoleic acid. **Results:** Due to the presence of healthy constituents, all four brands of olive oil showed equivalent antioxidant activity. No cytotoxic activity was reported on the evaluated concentrations. **Conclusion:** Overall comparison of phytochemicals as well as pharmacological activities of all four brands of extra virgin olive oil was found to be satisfactory.

Key words: Olive, antioxidant, cytotoxic, gas chromatography, oleic acid, palmitic acid, linoleic acid

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Corresponding Author: Mohammad Sohail Akhtar, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, Nizwa, Sultanate of Oman
Tel: +968-25-446-423

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Virgin or pure olive oil is isolated from the fresh olive fruit (*Olea europaea* L.), which is considered a healthy cooking oil. Extraction of the oil from the olive is achieved by specific methods that start from pressing, forming an olive paste, centrifuging and filtration which will not affect its properties[1,2]. Many health benefits are associated with olive oil and it made it of great interest for advanced studies to investigate its biological activities, quality and stability[3]. Many people prefer to use natural constituents in the treatment of various diseases instead of using synthetic drugs which are associated with higher chances of adverse effects[4,5]. Olive oil was known to be extracted and used since ancient times as laxative, sedatives, tonics and emollients[6]. Olive oil contains many components that influence its characteristics. It is known that it contains linoleic acid, tocopherol, polyphenol, squalene, terpene, oleic acid and hydroxytyrosol[7,8]. Olive oil quality is affected by many factors, including varieties availability, maturation, the method used for harvesting, as well as extraction. Other factors that influence the quality of olive oil are grouped as environmental factors, these are light, temperature and exposure to air or oxygen[9-12]. It was reported in the literature that olive oil contains constituents that has an antioxidant activity like polyphenols and vitamin E and which have a role as oil preservatives and have health benefits as the defense mechanism against free radicals[13]. In a study conducted to determine the cytotoxic activity of Spanish olive oil varieties, it was found that olive oil has cytotoxic activity against human cancer especially breast cancer[14]. Because of this, it is important to analyze different varieties of marketed olive oils to verify their quality for human consumption. In addition, it is worthwhile to analyze and investigate the biological properties of olive oils to unravel their health benefits.

The purpose of this research is thus, to compare phytochemical components of different olive oils as well as to determine their cytotoxic and antioxidant properties.

MATERIALS AND METHODS

Study area: The study was carried out at the University of Nizwa, Sultanate of Oman from September, 2019 to June, 2020.

Chemicals: Hexane, Dimethylsulfoxide (DMSO) and 2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma.

Materials:

- Brine shrimp eggs were brought from Taiwan
- Sea salt was brought from Muscat

Instruments:

- UV (Ultra Violet spectroscopy), Thermo Spectronic (Great Britain)
- Yamato Rotary evaporator (Model RE 801) (Japan since 1889)
- GC\MS: GC/MS from Perkin Elmer Model#Clarus 600 connected with the HP-5MS capillary column ((30 m × 0.25 mm i.d. × 0.25 µm) film thickness) were used

Sample collection: Organic olive oil was obtained from the local market. Three different well-known brands of extra virgin olive oil for cooking were brought from the supermarket in Oman.

GC/MS analysis: Organic olive oil from the local market of Oman and three different brands of extra virgin cooking olive oil obtained from the supermarket had been analyzed by the GC/MS instrument. Each one of the compounds was analyzed and identified by comparing the pattern of fragmentation, with what is reported in the literature and also by using the computer to match it with the standard spectra[15].

Antioxidant activity by radical scavenging assay: The antioxidant activity of the isolated and three different brands of extra virgin cooking olive oil was detected and determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) and by modifying the method of Brand Williams. Four concentrations (62.5, 125, 250 and 500 µg mL^{-1}) of each oil were prepared in hexane. From each sample 3 mL was withdrawn and mixed with (1 mL) of hexane of 0.3 mM DPPH. The reaction mixture was strongly shaken and kept in the dark for 30 min at room temperature. The absorbance of each sample was measured at a wavelength of 517 nm. For preparing the control, the same method mentioned was performed and the oil was not added to it[16].

Cytotoxicity assay: The cytotoxicity assay was performed and the brine shrimp (*Artemia salina*) was used, as the indicator organisms[17].

Hatching of shrimp larvae: Artificial seawater was made by adding (37 g) of sea salt to 1 L of distilled water and dissolving

it. Then, it was kept in a plastic trough, separated in two chambers by a perforated polyethylene wall. Shrimp eggs (50 mg) were taken and then sprinkled on one compartment. Aluminium foil was used for covering to prevent light exposure. The second compartment was illuminated to attract the hatched shrimp. These hatched shrimps were used for the bioassay within 48 hrs.

Brine shrimp lethality assay: Solutions corresponding to 62.5, 125, 250 and 500 μg mL^{-1} of each oil in DMSO were prepared in four separate vials. A total of 10 larvae were transferred into each vial and the solution was made up to 5 mL by adding the artificial seawater to dilute it and make up the volume. The vials were then illuminated and maintained at normal room temperature for 24 hrs. The number of survivals was later counted and the percentage of mortalities of the larvae after 24 hrs was calculated. The samples' LC_{50} values were determined by the Finney Probit method for the analysis of the percentage of mortality values[18].

RESULTS

GC/MS analysis: The GC/MS analysis of four different brands of olive oil was conducted and the following results were obtained (Table 1).

Antioxidant activity by using DPPH: The inhibition percentage was calculated and determined for the 4 different brands of olive oil to express the antioxidant activity (Fig. 1).

Cytotoxicity assay

Brine shrimp lethality test: Table 2 shows the mortality percentage of the larvae of brine shrimp exposed to various concentrations of extra virgin olive oil of different brands.

Table 1: Identification of different chemical constituents

Fame names	Retention time (min)	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Sample 4 (%)
Caprylic acid methyl ester	17.666	0.012	0.005	0.005	0.007
Palmitic acid, methyl ester	35.681	12.759	13.087	13.440	15.509
Palmitoleic acid, methyl ester	36.488	1.249	1.485	1.455	1.695
Heptadecanoic acid, methyl ester	37.404	0.124	0.083	0.061	0.234
cis-10-Heptadecenoic acid, methyl ester	38.154	0.270	0.181	0.141	0.360
Oleic acid, methyl ester	40.634	69.106	75.859	71.947	66.747
Linoleic acid, methyl ester	41.545	14.020	7.169	10.884	12.380
Eicosanoic acid, methyl ester	42.6	1.091	0.968	1.060	1.722
Gamma-linolenic acid, methyl ester	42.839	0.070	0.345	0.081	0.091
cis-11-Eicosenoic acid, methyl ester	43.076	0.613	0.471	0.519	0.683
Docosanoic acid, methyl ester	45.653	0.467	0.224	0.326	0.322
Tricosanoic acid, methyl ester	47.147	0.218	0.121	0.082	0.248

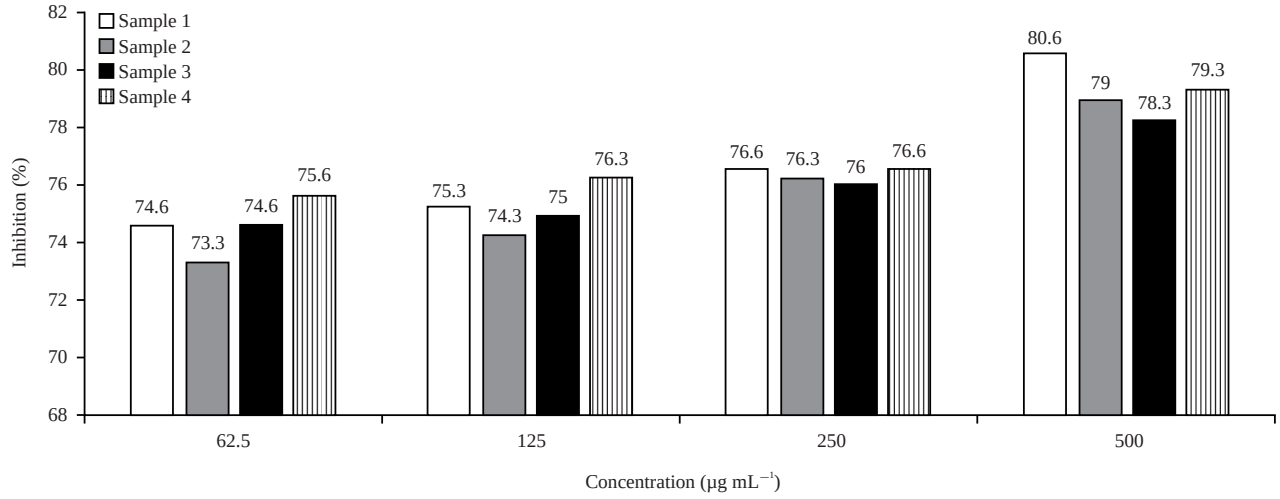

Fig. 1: Comparison of the antioxidant activity of different brands of olive oil

Table 2: Mean mortality of brine shrimp larvae when exposed to various concentrations of extra virgin olive oil of different brands

	Mortality (%)			
Concentration (µg mL^{-1})	Sample 1	Sample 2	Sample 3	Sample 4
62.5	0	0	0	0
125	1	0	0	0
250	1	0	0	0
500	2	0	0	0

n = 10 larvae per treatment

Cytotoxic activity of extra virgin olive oil against brine shrimps larvae: Sample 1 LC_{50} values gathered from the probity analysis resulted in an LC_{50} value of 3508.77. It can be concluded from the results that out of four samples only sample 1 showed little activity against the tested organism.

DISCUSSION

For the GC-MS analysis results of the four brands of olive oil, it was found that sample 1, 2, 3 and 4 contains around 12 main chemical constituents. The major constituent present in all samples 1-4 is oleic acid. The percentage of oleic acid in sample 1 is 69.106%, sample 2 is 75.859%, sample 3 is 71.947% and sample 4 is 66.747%. Other main constituents in samples 1-4 (Table 1) are palmitic acid (12.759-15.509%) and linoleic acid (7.169-14.020%). All four brands of olive oil showed very good inhibition of DPPH at all concentrations. The range of inhibition at 500 µg mL^{-1} for all brands varies from 78.3-80.6% There were no major variations in inhibition of DPPH found among the four different brands. According to the results mentioned in (Table 2), sample 1 is showing very little mortality of brine shrimp against olive oil starting from a concentration of 125 and 500 µg mL^{-1} to and rest of the samples 2, 3 and 4 did not show any mortality. The LC_{50} values were only obtained from sample 1 and it was quite high (3508.77) and the rest of the samples didn't show any activity, so it is concluded that the four samples of the extra virgin olive oil did not show any cytotoxic activity.

As we know that for any pharmacological activity the presence of active constituents in the sample in an adequate amount is necessary. Olive oil contains saturated as well as unsaturated fatty acids[19]. The unsaturated fatty acids are more likely to be oleic acid, palmitoleic acid, a type of monounsaturated fats and polyunsaturated fatty acids like linoleic acid[20]. There are no reports of fluctuations in the percentage of active constituents and most of them are in the range. Our results match the ranges reported in the literature for oleic acid (55-83%), palmitic acid (0.48-17.38%) and linoleic acid (5.30-15.97%)[21]. To conclude, In terms of chemical constituents, all four brands of olive oil have almost the same amount of active constituents.

The antioxidant substance's role is to protect and help the body cells to fight against the negative effects resulting from free radicals formation. Production of free radicals usually results when the body metabolizes constituents of food materials or by exposure to environmental changes like tobacco, smoke and radiation. Damage to the cells can result from the formation of free radicals and as a consequence causes a wide variety of diseases[22,23]. The compound called 2,2-diphenyl-1-picrylhydrazyl (DPPH) is the reagent commonly used to examine the antioxidant activity of a constituent[24]. In this study, we examined the antioxidant activity by DPPH assay of four different brands of extra virgin olive oil brought from the local supermarkets. Our results for the antioxidant activity (73.3-80.6%) were higher than what was reported in a study conducted to determine the antioxidant activity of three different calibration cultivars (18.33-27.37%)[25]. In addition, due to DPPH inhibition that was found, it was clear that extra virgin olive oils are anti-oxidant in nature and can be used as a healthy substitute in place of other cooking oils. There are lots of cooking oil that are hydrogenated and contains trans-fats and are associated with so many negative health effects. Extra virgin cooking olive oil is nutritious and apart from its beneficial fatty acids, it contains considerable amounts of vitamins such as vitamin E and K[26]. Olive oil is loaded with powerful antioxidants and these antioxidants reduce the chances of chronic diseases. Antineoplastic activity of olive oil is assumed to be associated with the fact that they are antioxidants with the ability to act as scavengers for free radicals[27].

As we know that if LC_{50} is high then the cytotoxic activity is less and vice versa. The LC_{50} value was quite high for sample 1. It is assumed that on higher doses like 1000 or 1500 µg mL^{-1} these samples may show better activity. Another factor that may contribute to the lack of cytotoxic activity is the lack of the active constituent responsible for that due to the manufacturing and extracting procedure or due to the types chosen in this study.

As it was determined in this study that olive oil contains active constituents and that it possesses a strong antioxidant activity which has a major role in fighting against many diseases that occur due to oxidative stress. Further studies are needed to investigate more on the pharmacological

constituents of olive oil and their pharmacological activity especially antioxidant activity and how to implement this in the pharmaceutical industry field.

CONCLUSION

All those four brands of extra virgin olive oil contained all necessary active constituents, especially oleic acid, palmitic acid and linoleic acid. Due to the presence of healthy constituents, all four brands of olive oil showed equivalent antioxidant activity. No Cytotoxic activity was reported on the evaluated concentrations. Overall comparison of phytochemicals as well as pharmacological activities of all four brands of extra virgin olive oil was found in this study to be satisfactory.

SIGNIFICANCE STATEMENT

This study discovers the pharmacological constituents, cytotoxic and antioxidant activity of 4 different brands of olive oil that can be beneficial for health and disease-fighting. This study will help the researcher to uncover the critical areas of olive oil pharmacological activity and especially antioxidant activity that many researchers were not able to explore. Thus a new theory on how to implement olive oil extracts in the pharmaceutical industry may be arrived at.

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