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(Original Article)

A Comparative Study on Some Oils as Natural Antioxidants

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Abstract

Over the past few years, several scientific researchers investigated that the rosemary, sweet almond and thyme oils are now recognized as essential components of functional foods. This study evaluates the quantities of flavonoids, total phenolic content (TPC), antioxidant properties, and fatty acid composition of almond, thyme, and rosemary oils. The results of the fatty acid analysis showed that the two oils had different profiles; sweet almond oil had considerable amounts of the important fatty acids; oleic and linoleic acids, while almond oil was rich in monounsaturated fatty acids (MUFA) and low in saturated fatty acids. Moreover, rosemary oil had the highest phenolic content among the other investigated oils; thyme oil had the highest flavonoids and antioxidant activity among the other oils. These results highlight the beneficial nutritional and functional qualities of almond, thyme, and rosemary oils, indicating their potential use as food components or nutritional supplements with positive health effects.

Keywords: *Almond, Thyme, and rosemary, Oils*

Introduction

In recent decades, consumers' attention in healthy eating has turned to the significant health advantages of foods and dietary ingredients. In reality, foods are meant to do more than just sate hunger and supply basic nutritional needs; they're also meant to prevent nutritional diseases, as well as to enhance the physical and mental health of consumers. Consumer demand for foods with better health benefits encouraged the food industry to accelerate the production of functional foods, which today account for a good proportion of new food products. Functional foods are extremely important when studying the relationship between diet, health, and happiness. There are numerous definitions of "functional foods" throughout the world, but none are official or widely acknowledged (Kalra, 2003). According to the European Commission, a food item can only be termed functional if, in addition to having a basic nutritional value, it also positively affects one or more bodily functions, thereby either enhancing general health and well-being or lowering the possibility of diseases (Doyon and Labrecque, 2008). Over the past few years, several researches

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investigated the rosemary, sweet almond and thyme oils are now recognized as an essential component of functional foods.

Almond trees (*Prunus amygdalus dulcis*) are regional tree of Western Asia, including Iran and the nearby countries. Almonds are now grown extensively overseas. The almond oil is the predominant substance of the kernel's weight between 40 and 67% (Roncero *et al*., 2016). This oil is used in a variety of industries, including food, beauty products, and alternative medicine due to its numerous health advantages, such as its anti-inflammatory, antihepatotoxic, natural immune, and inflammation-moderating properties (Lin *et al*., 2017 and Moore *et al*., 2020). Moreover, the almond oil exhibits a rich lipid content (Oliveira *et al*., 2019). Additionally, it provides an excellent nutritional source of antioxidants such as tocopherols, polyphenols, and flavonoids Aires *et al*., 2018 and Kahlaoui *et al*., 2019).

Rosemary (*Rosmarinus officinalis* L., *Lamiaceae*), a woody herbaceous plant native to the Mediterranean zone, is now grown as a decorative and attractive plant around the world (Al-Sereiti *et al*., 1999). The majority of its therapeutic benefits are result of significant antioxidant activity of rosemary's primary chemical components, especially carnosol, carnosic acid, ursolic acid, rosmarinic acid, and caffeic acid (Ngo *et al*., 2011). The pharmacological properties of essential oils and their active components have recently received a lot of attention (Teixeira *et al*., 2013). According to reports, the application of its essential oils in aromatherapy, the perfume industries, and preservation of food (Adel *et al*., 2011). Rosemary oil is able to prolong the storage of foods and keep their quality during preservation because to its antioxidant and antibacterial activities (Bozin *et al*., 2007 and Reynolds *et al*., 2016). Therefore, the food sector currently uses it as a bio preservative (Ojeda-Sana *et al*., 2013).

The genus Thymus, belonging to the Lamiaceae family including numerous subspecies, it is annual aromatic, evergreen or semi-evergreen ornamental plants (Martino *et al*., 2009). Thyme also locally called as "cimbru," is frequently employed in Romanian traditional medicine for its expectorant, antibroncholitic, antispasmodic, anthelmintic, carminative, and diuretic characteristics (Grigore *et al*., 2010). The plant has a good smell and a warming, spicy flavor. This plant's aroma is a result of its essential oil, which also has therapeutic effects and flavoring value for food (Boskabady *et al*., 2006). Therefore, the aim of this research was to identify and compare the chemical composition, total phenolics, total flavonoids, fatty acids and antioxidant activity of rosemary, sweet almond and thyme oils.

Material and Methods

Samples

Rosemary**,** sweet almond and thyme oils were purchased from local market in Assiut Governorate, Egypt. The samples were kept in dry, dark-colored bottles. The bottles were wrapped with carbon sheets to avoid photo oxidation. The average of three repeat samples was used to represent the results.

Peroxide value (PV)

The AOCS cd 8-53(1989) method (acetic acid-chloroform) was employed to determine the peroxide value. In this process, oil samples are added to a saturated potassium iodide solution to react with hydroperoxides. The free (I_2) is titrated using a sodium thiosulfate solution and a starch reagent.

Free fatty acids (FAA)

The FFA content was determined by AOCS cd3a-63(1989) method. This method involves adding isopropyl alcohol and toluene-based neutralized solvent mixture to an oil sample and titrating it using a standard alkali and phenol phthalein reagent.

Total phenolic content (TPC)

Total phenolic contents (TPC) were estimated by Folin-Ciocalteu's methods. The essential oils were diluted to an appropriate concentration for analysis. One milliliter of 20% Na2CO3 (w/v), one milliliter of 1N Folin-Ciocalteu's reagent, and half a milliliter of essential oil were blended. After 2 hours of keeping at room temperature, the blend was centrifuged for 10 min at 8000 rpm. 'The supernatant was detected at 765 nm (Singleton *et al., 1999*).

Total flavonoid content (TFC)

The oil samples were mixed with 0.3 mL of $NaNO_2$ (5%), after 5 min, 0.3 mL of $Alcl_3$ (10%) and 2 mL of NaOH (1 M) was added and added the water to get a final volume of 10 mL. The absorbance was measured at 510 nm. A calibration graph was created using quercetin. The result was expressed as quercetin equivalents (QE; mg QE/g extracts).

Antioxidant Activity

The antioxidant activity was evaluated in terms of its capacity to donate hydrogen or scavenge radicals. tests were conducted according to the method of Gardeli *et al*. (2008) with a minor modification. The reduction of the radical is followed by a decrease in the absorbance at 517 nm. Test tubes were filled with 2 mL of the methanolic stock solution of the essential oils and 2 mL of the 1 mM DPPH solution. After wrapping the tubes with parafilm, they were left in the dark for 1 h. Absorbance at 517 nm was determined by a spectrophotometer.

The formula used to get the DPPH radical's inhibition percentage was percentage inhibition (%) = [(A blank - A sample) / A blank] × 100.

Whereas, a blank is DPPH solution alone, a sample is DPPH solution with tested oil.

Gas chromatography – mass spectrometry (GC – MS) analysis

The oil samples were analyzed by using a gas chromatography (Agilent 8890 GC System), linked to a mass spectrometer (Agilent 5977B GC/MSD) and outfitted with a HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The temperature of the oven was kept at 50 °C at first, then

programmed to rise to 200 °C at a rate of 5 °C/min then to rise to 280 °C at a rate of 10 °C/min. Finally, it was held at 280 °C for 7 min. The carrier gas, helium, was employed at a flow rate of 1.0 mL/min. The oil was diluted in diethyl ether (20 µL oil / mL diethyl ether), and then 1 µL of this mixture was putted in the GC with a divided proportion 1:50. The injection temperature was 230 °C. Mass spectra were acquired at 70 eV in the electron impact mode (EI) scanning a range of 39 to 500 amu in m/z. By comparing the isolated peaks with information from the mass spectra library at Agricultural Research Center in Giza

Statistical Analysis

In order to conduct the statistical analysis, IBM SPSS version 26 was used. Calculated descriptive statistics include means and standard deviation. The Independent-Samples T test was used to determinate differences between the different oils.

Results and Discussion

Fatty Acid Composition

The concern of food authenticity has risen in significance in recent years. Fatty acid composition could be a useful technique for classifying and evaluating oil quality, as well as for detecting adulteration. (Tian *et al*., 2014 and Esteki *et al*., 2020).

In accordance with a recent study by Uchenna *et al*. (2019), and the fact that little is known about the fatty acid profile of oil, it was interesting to determine the fatty acid profile of Thyme oil for the first time in order to comprehend and clarify this oil's antioxidant properties. A total of the six saturated fatty acids found, palmitic acid (C16:0) had the greatest percentage at nearly 58.92%. The major unsaturated fatty acids were linoleic acid (C18:2cis) with 11.70% of total fatty acids oil, followed by linolenic acid (C18:3) with 10.70% of total fatty acids. Therefore, thyme oil had high percentage of saturated fatty acids as 71.29% in comparison to 32.76% of unsaturated fatty acids (Table 1). Since linoleic and linolenic acids are essential fatty acids, this oil may be seen as a unique way to produce useful foods. These fatty acids are also the precursors of other polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA; C22:6 n-3), and eicosapentaenoic acid (C20:5 n-3), which are recognized to have numerous health benefits (Kaur, 2014). This may help to partially explain the oil's antioxidant properties.

Table 1 shows the fatty acid composition of rosemary oil (RO). Fourteen fatty acids were identified and quantified in RO. Fatty acids profile of RO presents four major fatty acids: C16 :0 (Palmitic acid) about 20.51%, C22:0 (Behenic Linolenic acid) about 16.56, and C18:0 (Stearic acid) and C18:1cis (Oleic acid) about 7.82 and 7.10% of total FA, respectively. In contrast, Elbanna *et al*. (2018) identified ten fatty acid in RO with high amounts of linoleic acid C18:2 (41.7%), oleic acid C18:1 (41.2%), palmitic acid C16:0 (8.9%) and stearic acid C18:0 (5.96%). Therefore, it had a high percentage of saturated fatty acids

as 63.84% relative to 33.13% unsaturated fatty acids. It is advised by the Dietary Guidelines for Americans to consume less than 10% of daily calories from saturated fat (USDA, 2010). Even though saturated fat was advised to be avoided for decades, there has been a rise in this belief in recent years. Saturated fat-rich diets may not increase the risk of heart disease, according to several researches (Micha and Mozaffarian, 2010 and Siri-Tarino *et al*., 2010).

In comparison to other nut crops, the almond samples under analysis have very low (<10%) saturated fatty acids, high monounsaturated fatty acid (MUFA), and low polyunsaturated fatty acid (PUPA) (Bakalinsky, 1993). Five fatty acids constitute the majority of almond oil: oleic (18:1), linoleic (18:2), palmitic (16:0), stearic (18:0), and palmitoleic (16:1). Palmitic (16:0) was the predominant saturated fatty acid, accounting for approximately 6.6% of the total oil (Table 1). The oil has the greatest concentration of oleic acid, approximately 70.50 percent (Abdallah *et al*., 1998). Thus, it had a high percentage of unsaturated fatty acids 91.83% in contrast to 8.19% saturated fatty acids. Furthermore, the amount of fatty acids in almond oil is significant for diet. The lipid component of almonds does not promote the formation of cholesterol in humans because it contains a significant amount of unsaturated fatty acids (MUFAs and PUFAs) (Emken, 2013).

Table 1. Fatty Acid Composition

Fatty acids	Relative concentration (%)		
	Thyme	Rosemary	Sweet almond
Myristic acid (C14:0)	2.74	3.42	
Myristoleic acid (14.1)	---	0.57	
Palmitic acid (C16:0)	58.92	20.51	6.60
Palmitoleic acid (C16:1)	---	0.77	0.59
Heptadecanoic acid (C17:0)	3.13	6.28	
Cis-10-Heptadecanoic acid (C17:1)	---	5.17	
Stearic acid (C18:0)	1.59	7.82	1.59
Oleic acid (C18:1cis)	9.12	7.10	70.50
Linoleic acid (C18:2cis)	11.70	9.03	20.74
Linolelaidic acid (C18:2trans)	---	4.67	
Linolenic acid (C18:3)	10.70	4.81	
Arachidic acid (C20:0)	3.57	8.89	
Cis-11- Eicosenoic acid (C20.1)	1.24	1.01	
Behenic Linolenic acid (C22:0)	1.34	16.56	
Total saturated fatty acids	71.29	63.84	8.19
Total unsaturated fatty acids	32.76	33.13	91.83

Total phenol, Flavonoid and Antioxidant activity

Phenolic compounds are natural secondary metabolites that have a wide range of biological activities, where their antioxidant activity is the most essential characteristic mostly for its positive effects on health (Oliveira *et al*., 2016). The total phenolic content (TPC) in the investigated almond oil was 123.17 mg/kg (Table 2). The same result was obtained by Melhaoui *et al*. (2021),

while Rabadan *et al*. (2018) recorded a lower value of phenol content about 18.53 mg/kg. This difference is typically influenced by several variables, including variety, oil extraction technique, and agricultural methods. The total phenolic content in rosemary oil was 147.55 mg/kg (Table 2). When compared to the results of Jordan *et al*. (2013) in the Spain rosemary example, our result indicated a greater TPC. The total phenolic content in thyme was 111.23 mg/kg (Table 2). The obtained results agree with the previous literature reported by Sokmen *et al*. (2004) and Köksal *et al*. (2017). The significant concentration of phenolic compounds indicates that thyme has a high level of antioxidant capacity.

Plants contain large amounts of flavonoids, which are polyphenolic chemicals with a variety of functions. These polyphenolic substances may also act as cell cycle inhibitors, physiological regulators, and chemical transmitters (Galeotti *et al*., 2008 and Viuda Martos *et al*., 2010). The total flavonoids of sweet almond as shown in Table 2 was 55.43 mg/kg as obtained by Alijaniha *et al*. (2023). While, the total flavonoids of rosemary oil were 37.04 mg/kg (Table 2), the same result obtained by El-Gammal. (2016). Also, the total flavonoids of thyme oil were 127.11 mg/kg (Table 2) as reported by Ghandchi and Jamzad. (2015).

The stable free radical DPPH technique was used to assess the antioxidant activity, this method can be done quickly, easily, and sensitively (Ebrahimzadeh *et al*., 2008). As seen in Table 2, obtained results appeared that DPPH radical cation scavenging activity of thyme oil was 94.17 mg/ml. less than the result exhibited by Aljabeili *et al*. (2018). The results showed that the rosemary oil had high radical scavenging action with value of 83.24 mg/ml. According to reports by Viuda Martos *et al*. (2010), phenolic groups in rosemary oils contribute to their antioxidant action. Rašković *et al*. (2014) demonstrated DPPH activity of rosemary oils was 77.6 mg/ml. Furthermore, the DPPH free radical scavenging activities of sweet almond oil were determined, value evaluated for the studied oil was 28.47 mg/ml (table 2), as reported by Csakvari *et al*. (2019).

Table 2. Total phenolic, Total flavonoids, and DPPH

Item	Oil codes		
	Sweet almond**	**Rosemary**.**	**Thyme****
Total phenolic content (mg/kg)	123.17± 1.3	147.55± 1.7	111.23± 1.4
Total flavonoides(mg/kg)	55.43± 1.5	37.04± 1.8	127.11± 1.03
DPPH (Antioxidant activity %)	28.47± 1.85	83.24± 1.44	94.17± 1.92

**High significant difference between values ($p < 0.01$)

Peroxide and acid value

The International Organization for Standardization (ISO) and Codex Alimentarius consider the most important standardization group in the world, when it concerns edible oils and fats, which publish standards including a broad variety of activities. The safety and health of consumers are greatly enhanced by these worldwide standards. Regarding this, Codex Alimentarius advised that acidity levels for oils be less than 5% (Issaoui and Delgado, 2019).

The initial primary parameters that characterize the physicochemical quality of oils are free acidity (AF) and peroxide value displays low values for both measurements, suggesting that oils are of high quality and the lack of acylglycerol enzymatic hydrolysis (Álvarez-Ortí *et al*.,2012). Table 3 shows low values for the two variables peroxide and acid value, suggest that the almond oil was of high quality, similar to the result obtained by Melhaoui *et al*., (2021). As well as peroxide and acid values of rosemary oil were 0.83 and 0.56, respectively. Moreover, peroxide and acid values of thyme oil were 3.07 and 1.4, respectively; these results agreed with Moussa. (2003). All the obtained values are under the Codex Alimentarius recommendation for not exceeding 15 meq O2 kg−1 (Alimentarius, 1999).

Table 3. Acidity and Peroxide values

samples	Acidity mgKOH/g oil**	Peroxide meq/kg oil**
Sweet Almond oil	0.43± 1.02	1.17± 1.35
Rosemary oil	0.56± 1.78	0.83± 1.8
Thyme oil	1.4± 1.09	3.07± 1.45

**High significant difference between values ($p < 0.01$)

Conclusion

A comprehensive investigation of the fatty acid composition, phenolic content, levels of flavonoids, and antioxidant activity offers important new information about the nutritional and practical characteristics of rosemary, thyme, and almond oils. These oils have potential health benefits and food variety, making them promise as nutritional supplements or components in food formulations. Their uses in functional foods and their effects on human health and wellbeing could be investigated further.

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دراسة مقارنة بعض الزيوت كمضادات أكسدة طبيعية

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الملخص العربي

خلال السنوات القليلة الماضية، اهتم العديد من الباحثين بدراسة زيوت إكليل الجبل واللوز الحلو والزعتر والتي أصبحت الآن عنصرًا أساسيًا في الأغذية الوظيفية. تهتم هذه الدراسة بتقدير محتوي الفلافونويدات، والفينولات الكلية والنشاط المضاد للأكسدة، وتركيب الأحماض الدهنية في كلا من زيوت اللوز والزعتر وإكليل الجبل. و قد أظهرت نتائج تحليل الأحماض الدهنية للزيوت الثلاثة أن الزيتين (زيت الزعتر و زيت اللوز) لديهما محتويات مختلفة: حيث كان لزيت اللوز الحلو كميات كبيرة من الأحماض الدهنية المهمة مثل حمض اوليك و حمض اللينوليك، بينما كان زيت اللوز غنيًا بالأحماض الدهنية غير المشبعة ومنخفضًا في الأحماض الدهنية المشبعة.. وقد كان زيت الروزماري أعلى في محتوى الفينولات الكلية مقارنة بالزيوت الاخري. بينما كان زيت الزعتر أعلى في محتوى الفلافونيدات ونشاط مضاد للأكسدة مقارنة بالزيوت الاخري. وتشير هذه النتائج المتحصل عليها الي تميز الخصائص الغذائية والوظيفية المفيدة لزيوت اللوز والزعتر وإكليل الجبل، والتي يمكن استخدامها كمكونات غذائية أو مكملات غذائية ذات اثار صحية إيجابية.