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A COMPARATIVE STUDY BETWEEN THREE ESSENTIAL OILS IN TERMS OF THEIR CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY

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ABSTRACT: The problem of oxidation and free radicals is one of the factors that are very dangerous to human health, which requires searching for safe natural compounds to be included in the diet and work to combat free radicals. The aim of this study was to evaluate the antioxidant effect of essential oils of basil, thyme and lemongrass by two different methods (FRAP and DPPH) as well as to identify the active ingredients of each oil by GCMS. The results of the fractionation on the GCMS showed that the main components of the essential oils were as follows: basil (L-linalool 59.02% and 1,8-cineole 9.52%), thyme (Thymol 48.06%and p-cymene 38.57%), and lemongrass (Geranial 47.59% and neral 36.27%). In the FRAP method, essential oil of basil was the strongest as an antioxidant (2.35± 0.61 mMol Fe^{+2}/g), followed by essential oil of thyme (1.96± 0.42 mMol Fe^{+2}/g), followed by essential oil of lemongrass by a large margin (0.93± 0.21 mMol Fe^{+2}/g). Whereas, when studying the antioxidant activity by the DPPH method, the results showed through comparison with IC_{50} values that, basil essential oil was the strongest antioxidant (IC_{50} =144.78 ± 7.19 µg/ml), followed by lemongrass essential oil (IC_{50} =170.05 ± 6.97 µg/ml) and finally thyme essential oil (IC_{50} =183.45 ± 7.19 µg/ml), with a slight difference. The results clearly indicated that the essential oil of basil was the best as an antioxidant compared to the essential oils of lemongrass and thyme in both methods used for the determination of antioxidant activity in vitro (FRAP and DPPH); this makes it a promising source of natural antioxidants that can be used in diets.

Key words: Essential oil, Antioxidant, Honeybee, Oxidative stress.

INTRODUCTION

Oxidative stress is considered one of dangerous factors for human health, as it plays a major role in the incidence of many diseases (atherosclerosis, cardiovascular diseases and cancer) [Gutteridge, 1993; Hasani-Ranjbar *et al.*, 2009; Rahimi *et al.*, 2010]. The human body deals with free radicals through enzymatic systems and non-enzymatic compounds inside the body, and there are other compounds supplied by the diet.

Despite the great effectiveness of industrially synthesized antioxidants such as BHT and BHA in scavenging free radicals and preserving various processed foods from oxidation well, their long-term use leads to various cancers (Lindenschmidt *et al.*, 1986; Ito and Hirose, 1989). Therefore, it was necessary to search for natural alternatives that are used as antioxidants and have a high degree of safety for human health.

Essential oils are considered one of the most important food sources to obtain the body's needs for natural antioxidants (Emami *et al.*, 2010; Grassmann, 2005; Miguel, 2010). The high antioxidant activity of essential oils is attributed to the fact that they contain different types of terpenes and phenolic compounds, which are known for their high efficiency as free radical scavengers (Edris, 2007).

The essential oil of basil, thyme and lemongrass possesses antioxidant properties due to its major active components especially terpenes. In thyme essential oil, thymol is largely responsible for its antioxidant activity (Sokmen *et al.*, 2004). while L-linalool is answerable for the antioxidant activity of basil (Avetisyan *et al.*, 2017). Lemongrass contains highly active

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monoterpenoid (myrcene, eugenol, β- citral, and α-citral) act as antioxidant, especially eugenol (Lawrence *et al.,* 2015). The aim of this study is to evaluate the essential oils of basil, thyme and lemongrass, which are abundant in the Egyptian environment, to identify the best of them in terms of efficiency as an antioxidant in vitro by using the FRAP and DPPH methods, along with identify potential active substances of each essential oil using by GCMS.

MATERIALS AND METHODS

Essential oils

Lemongrass (*Cymbopogon citratus*), basil (*Ocimum basilicum*) and thyme (*Thymus Vulgaris*) essential oils were obtained from the extraction oil unit in National Research Centre, Dokki, Giza, Egypt.

GC-MS analysis of essential oils

The components of different essential oils were separated and identified using GC-MS apparatus according to the method described by Al-Sayed *et al.,* (2021). A system operating Thermo Scientific Corp. (USA) coupled with Thermo mass spectrometer detector (ISQ Single Quadrupole) was used with the following conditions: TR-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). The identification of volatile metabolites was performed upon comparing the mass spectra as well as the retention index with those of the National Institute of Standards and Technology's (NIST) chemistry webbook library.

Determination of antioxidant activity of tested essential oils

1- Total phenolic content

The level of total phenolic compounds in the tested essential oils was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid. The concentration of the total phenolics was calculated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve (Li *et al*., 2008).

2- Ferric Reducing/Antioxidant Power (FRAP) Assay

FRAP assay was based on the method of (Benzie and Strain, 1996) The results were expressed as mmol Fe (II)/g fresh weight.

3- 2,2-Dipheny l-1-picrylhydrazyl (DPPH) free radical-scavenging capacity assay

Measurement of the DPPH radical scavenging capacity was carried out according to (Karamać *et al.,* 2005). Radical scavenging capacity calculated as follow:

Radical scavenging capacity (%) =

$$(A_{blank} - A_{sample})/Ablank \times 100,$$

Where A_{sample} is the absorbance of DPPH mixed with essential oil and A_{blank} is the absorbance of DPPH in which sample has been replaced with methanol. Vitamin C was used as positive control.

Statistical analysis

The results were expressed as the Mean ± SD using CoStat Software, Version 6.4 (2008).

RESULTS AND DISCUSSION

GC-MS analysis of tested essential oils.

1-Chemical composition of lemongrass essential oil

Data presented in Table (1) showed the GC-MS results of lemongrass essential oil. The data showed that the total identified compounds from lemon grass were 23 represent 97.68 % from total mass. These included monoterpenes, oxygenated monoterpenes and sesquiterpenes. The total amount of monoterpene was (65.87 %), while the total amount of oxygenated monoterpenes was 31.74 % from total mass, Moreover, the total amount of sesquiterpenes was (0.07 %). The major compounds found in lemon grass essential oil, were geranial (47.59%) followed by neral (36.27%), β-Myrcene (7.57%), geraniol (1.5%) and sulcatone (1.1%). The amount of citral compound is the determinant of the quality of lemongrass essential oil (Silva *et al.,* 2008).

Table (1): Chemical constituents and concentration of lemongrass essential oil

No	Compound Name	RT	Area %
1	Geranial	10.21	47.59
2	Neral	9.57	36.27
3	β-Myrcene	4.34	7.57
4	Geraniol	9.78	1.50
5	Sulcatone	4.43	1.10
6	cis-Verbenol	8.27	0.99
7	Geranyl acetate	12.24	0.91
8	L-LINALOOL	6.58	0.75
9	α-Citronellol	9.27	0.53
10	CIS-MYRTANOL	7.67	0.38
11	Trans- α -Bergamotene	13.14	0.21
Monoterpene hydrocarbons			65.87
Oxygenated monoterpenes			31.74
Sesquiterpene hydrocarbons			0.07

Many researchers have estimated the components of lemongrass essential oil in many previous studies, and the results were not far from the results of the current study in terms of the main components of the essential oil (Bhattacharya *et al.,* 1997; Silva *et al.,* 2008; Tyagi and Malik, 2010).

2. Chemical composition of thyme essential oil

Data presented in Table (2) showed the chemical constituents of thyme essential oil. The data showed that the total identified compounds were 10 represent 99.34% from total mass. The major compounds found in thyme essential oil, were thymol (48.06%) followed by p-cymene (38.57%), caryophyllene (8.27%), L-linalool 1.90% and caryophyllene oxide 1.18 %.

Many researchers have estimated the components of the essential oil of thyme in many previous studies (Borugă *et al.,* 2014; Porte and Godoy, 2008; Shabnum and Wagay, 2011) and the results were not far from the results of the current study in terms of the main components of the essential oil, especially thymol which found to be the main component with more than 40% of the total components, followed by cyamine with more than about 25% of the total components of the essential oil.

3. Chemical composition of basil essential oil

Data presented in Table (3) showed the chemical constituents of basil essential oil. The data showed that the total identified compounds were 13 represent 95.69% from total mass. The total amount of monoterpene was (3.28%), while oxygenated monoterpenes was (73.08 %) from total mass; moreover, the total amount of sesquiterpene hydrocarbons was (12.74%) and the oxygenated sesquiterpenes was (6.59%). L-linalool (59.02%), 1,8-cineole (9.52%), α-cadinol (6.59%) and α-bergamotene (5.74%) were the major compounds in thyme essential oil.

In many previous studies (Avetisyan *et al.,* 2017; Cheliku *et al.,* 2015; Daneshian *et al.,* 2009) researchers have estimated the chemical composition of basil essential oil and the results were near similar to that of the current study in terms of the main components of the essential oil, especially since linalool which was the main component with more than 30% of the total components of the essential oil.

Table (2): Chemical constituents and concentration of thyme essential oil

No	compound name	RT	Area %
1	Camphene	4.68	0.02
2	p-Cymene	6.79	38.57
3	γ-Terpinene	7.86	0.46
4	Fenchone	9.05	0.12
5	L-linalool	9.48	1.9
6	Thymol	17.49	48.06
7	α-Copaene	20.36	0.26
8	Caryophyllene	22.17	8.27
9	α-Humulene (CAS)	23.69	0.5
10	Caryophyllene oxide	28.84	1.18
	Monoterpene hydrocarbons		39.05
	Oxygenated monoterpenes		50.08
	Sesquiterpene hydrocarbons		9.03
	Oxygenated Sesquiterpene		1.18
	Total		99.34

Table (3): Chemical constituents and concentration of basil essential oil.

No	Compound name	RT	Area %
1	β-Pinene	5.86	1.23
2	β-Myrcene	6.14	1.24
3	D-Limonene	7.43	0.81
4	1,8-Cineole	7.57	9.52
5	L-Linalool	10.07	59.02
6	Borneol	13.05	0.84
7	α-Terpineol	14.04	1.28
8	Bornyl acetate	17.55	2.42
9	α-Bergamotene	23.51	5.74
10	α-Humulene	24.45	2.03
11	D-Germacrene	25.5	2.5
12	γ-Cadinene	26.84	2.47
13	α-Cadinol	31.95	6.59
	Total		95.69
	Monoterpene hydrocarbons		3.28
	Oxygenated monoterpenes		73.08
	Sesquiterpene hydrocarbons		12.74
	Oxygenated Sesquiterpene		6.59
	Total		95.69

Antioxidant activity of tested essential oils

1- Total phenolic compounds and FRAP assay

The total phenolic compounds of the essential oils used in the experiment were estimated, and the results indicated that basil oil recorded the highest values (74.06 ug µGAE/mg), followed by thyme oil (8.1 ug µGAE/mg), and finally lemongrass oil (5.95 ug µGAE/mg) [Table 4].

The principle of the FRAP assay is based on the antioxidant strength in reducing Fe^{+3}-tripyridyltriazine complex to its Fe^{+3}form. There is a proportional relationship between the intensity of the blue color formed and the ferrous form concentration and the antioxidant capacity of the extract.

If the substance to be tested has an antioxidant effect in the FRAP method, it gives electrons (electron donors) to the free radicals, which eliminate the oxidation chain reaction (Tachakittirungrod *et al.,* 2007).

The results are shown in Table (4) FRAP method results expressed as (mMol Fe^{+2}/g). The results clearly show that basil essential oil recorded the highest values (2.35± 0.61 mMol Fe^{+2}/g), followed by thyme essential oil (1.96± 0.42 mMol Fe^{+2}/g), and finally lemongrass essential oil (0.93± 0.21 mMol Fe^{+2}/g). While the ascorbic acid used for comparison showed the highest values in the experiment (2.75±0.91 mMol Fe^{+2}/g), which were not far from basil essential oil.

The FRAP assay should reflect the one-electron-donating ability of the essential oil components and may correlate with ionization energy. Therefore, the lower the ionization energy, the easier it is to remove the electron, and thus the antioxidant activity of the substance increases (Sharopova *et al.,* 2014). The obtained results indicate that there is a direct relationship between the total phenolic compounds content of essential oils and their antioxidant activity, which was estimated by the FRAP method, where the basil oil was the highest in its content of total phenolic compounds (74.06 ug µGAE/mg) and subsequently recorded the highest antioxidant activity (2.35± 0.61 mMol Fe^{+2}/g). Lemongrass essential oil is the lowest in total phenolic compounds content (5.95 ug µGAE/mg) and then recorded the lowest antioxidant activity as estimated by the FRAP method (0.93± 0.21 mMol Fe^{+2}/g).

When evaluating the antioxidant activity of essential oils, it must be taken into account that the efficiency of essential oil as an antioxidant is affected by many factors such as the method of extraction of the essential oil, the part of the plant from which the oil is extracted, the plant variety in addition to the place of cultivation (Faheem *et al.,* 2022). For example, a study conducted by Hartatie *et al.,* (2019) indicated that the lemongrass essential oil extracted by the method of steam distillation was more efficient as an antioxidant than that extracted by the method of water distillation. As a result of the wide variety of active ingredients in essential oils (phenolic compounds and terpenes of all kinds), it also varies in its mechanisms of action as antioxidants in terms of donating electrons, donating hydrogen ion, scavenging free radicals, or chelating transitional metals, which gives it a strong effect as an antioxidant compared to other compounds (Diniz do Nascimento *et al.,* 2020).

Table (4): Total phenolic compounds and antioxidant activity using ferric reducing antioxidant power (FRAP) method expressed for tested essential oils.

Essntial oil	FRAP (mMol Fe^{+2}/g)	Total phenolic compounds (µGAE/mg)
Basil	2.35 ± 0.61	74.06
Thyme	1.96 ± 0.42	8.1
Lemongrass	0.93 ± 021	5.95
Ascorbic acid	2.75 ± 0.91	

Each sample was three replicates, and result expressed as mean ± SD.

2- Antioxidant activity by DPPH method

The DPPH free radical is a stable free radical, which has been widely used as a tool to estimate the free radical-scavenging activity of antioxidants (Brand-Williams *et al.,* 1995). Antioxidants interact with DPPH radical (by transfer hydrogen atoms or electrons to DPPH), thus neutralizing the free radical character (Archana *et al.,* 2005).

Data in Table (5) shows that the higher the concentration of basil essential oil, the greater the percentage of inhibition (%) in DPPH assay. We find that the higher concentration (1000 µg/ml) gave the highest percentage of inhibition (81.93 %). Similarly, the lowest concentration (125 µg/ml) gave the lowest percentage of inhibition (47.56%); whereas, the IC_{50} was 144.78 ± 7.19 µg/ml. A lower IC_{50} value indicates greater antioxidant activity.

Previous studies of many researchers (Bozin *et al.,* 2006; Hussain *et al.,* 2008; Politeo *et al.,* 2007) indicate the efficiency of basil essential oil as a natural antioxidant compared to synthetic substances such as BHT, as it always showed lower IC_{50}values of basil essential oil than for BHT.

The results shown in Table (6) indicate that as the concentration of thyme essential oil increases, the percentage of inhibition (%) in DPPH method increases. We find that the lowest concentration (125 µg/ml) gave the lowest percentage of inhibition (42.35%). Similarly, the higher concentration (1000 µg/ml) gave the highest percentage of inhibition (78.64 %). whereas, the IC_{50} was 183.45 ± 7.19 µg/ml. A lower IC_{50} value indicates greater antioxidant activity.

Thyme has high antioxidant activity due to its flovanoids content (Lacroix *et al.,* 1997) and terpenoids in their composition (Kadri *et al.,* 2013).

Ruberto and Baratta, (2000) and Tepe *et al.,* (2005), studied the antioxidant activity of Thymus species, and attributed the antioxidant activity to phenolic compounds especially thymol and carvacrol;

The results shown in Table (7) indicate that as the concentration of lemongrass essential oil increases, the percentage of inhibition (%) in DPPH method increases. We find that the lowest concentration (125 µg/ml) gave the lowest percentage of inhibition (43.92%). Similarly, the higher concentration (1000 µg/ml) gave the highest percentage of inhibition (80.65 %) whereas, the IC_{50} was 170.05 ± 6.97 µg/ml. A lower IC_{50} value indicates greater antioxidant activity. The results of this study are similar to the results of another study conducted by a researcher from Egypt (Selim, 2011), in which he studied the antioxidant effect as well as the antimicrobial effect of lemongrass collected from the Ismailia region in Egypt. This study showed the efficiency of lemongrass essential oil as a powerful antioxidant by DPPH method compared with BHT and ascorbic acid. The results of this study are similar to that obtained by (Mansour *et al.,* 2015) which clearly showed that the essential oil of lemongrass collected from Egypt gave higher inhibition values (%) in the DPPH method than those collected from Saudi Arabia.

Table (5): Scavenging activity of the DPPH radical of basil essential oils

Essential oil	Concentration (µg/ml)	Inhibition (%)	IC_{50} values (µg/ml)
Basil	125	47.56 ± 2.02	144.78 ± 7.19
	250	62.98 ± 1.46	
	500	74.15 ± 0.39	
	1000	81.93 ± 0.71	

Each sample was three replicates, and result expressed as mean ± SD.

Table (6): Scavenging activity of the DPPH radical of thyme essential oils

Essential oil	Concentration (µg/ml)	Inhibition (%)	IC50 values (µg/ml)
Thyme	125	42.35 ± 1.73	183.45 ± 7.19
	250	58.71 ± 1.24	
	500	70.39 ± 0.65	
	1000	78.64 ± 0.82	

Each sample was three replicates, and result expressed as mean ± SD.

Table (7): Scavenging activity of the DPPH radical of lemongrass essential oils

Essential oil	Concentration (µg/ml)	Inhibition (%)	IC50 values (µg/ml)
Lemongrass	125	43.92 ± 1.46	170.05 ± 6.97
	250	60.79 ± 1.53	
	500	73.46 ± 0.62	
	1000	80.65 ± 0.41	

Each sample was three replicates, and result expressed as mean ± SD.

In our current study, the three essential oils showed a good effect as antioxidants in both methods (FRAP and DPPH), although there was a difference in the order of their preference as antioxidants between the two methods. In the FRAP method, essential oil of basil was the strongest as an antioxidant (2.35± 0.61 mMol Fe^{+2}/g), followed by essential oil of thyme (1.96± 0.42 mMol Fe^{+2}/g), and finally, essential oil of lemongrass by a large margin (0.93± 0.21 mMol Fe^{+2}/g). Whereas, when studying the antioxidant activity by the DPPH method, the results showed through comparison with IC_{50} values that, basil essential oil is the strongest antioxidant (IC_{50} =144.78 ± 7.19 µg/ml), followed by lemongrass essential oil (IC_{50} =170.05 ± 6.97 µg/ml) and finally thyme essential oil (IC_{50} =183.45 ± 7.19 µg/ml), with a slight difference. The results clearly indicated that the essential oil of basil was the best as an antioxidant compared to the essential oils of lemongrass and thyme in both methods used for the determination of antioxidant activity in vitro (FRAP and DPPH). . The results of various analytical methods of the same samples can differ significantly. The differences may be due to various kinds of antioxidants present in the samples which react differently with the radicals used.

Each method has its own characteristics in terms of the nature of the reagents used, their degree of purity, the sensitivity of the method itself, the time of preparing the samples, as well as the conditions of preparing the samples, etc (Shah and Modi, 2015). The radicals didn't have to be generated before the assay in the DPPH method. On the other hand, there is only disadvantage in the FRAP assay, which is that it takes a long time to prepare the chemicals needed for the method.

Conclusion

The current study indicated that the essential oils used in the study (basil, lemongrass, and thyme) showed that the three essential oils showed a good effect as natural antioxidants, and the best of them was the essential oil of basil in both methods (FRAP and DPPH). As for the essential oil of thyme and lemongrass, they showed conflicting results. Thyme essential oil was better than lemongrass in the FRAP method; while in DPPH method, lemongrass essential oil was better than thyme essential oil. So that, it is

not possible to say that one method is superior to another in assessing the antioxidant activity in vitro. This means that in further work, we need to conduct experiments on experimental animals to evaluate the effect of these essential oils in combating free radicals in vitro.

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دراسة مقارنة بين ثلاثة زيوت أساسية من حيث تركيبها الكيميائي ونشاطها المضاد للأكسدة

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الملخص العربى

يشكل وجود الشقوق الحرة خطورة كبيرة على صحة الإنسان، مما يتطلب البحث عن مركبات طبيعية آمنة لإدراجها في النظام الغذائي والعمل على مكافحة الشقوق الحرة.

الهدف من هذه الدراسة هو تقييم التأثير المضاد للأكسدة للزيوت العطرية للريحان والزعتر وحشيشة الليمون بطريقتين مختلفتين (FRAP و DPPH) وكذلك التعرف على المكونات الفعالة لكل زيت بواسطة جهاز GCMS. أظهرت نتائج التحليل على GCMS أن المكونات الرئيسية للزيوت العطرية كانت كما يلي:

الريحان (L-linalool 59.02% and 1,8-cineole ٩,٥٢٪) أما بالنسبة للزيت العطري للزعتر (Thymol 48.06%and p-cymene ٣٨,٥٧٪)، وأخيرا حشيشة الليمون (Geranial 47.59% and neral ٣٦,٢٧٪) . في طريقة FRAP ، كان زيت الريحان هو الأقوى كمضاد للأكسدة (٢,٣٥ ± ٠.**61** mMol Fe^{+2}/g)، يليه الزعتر (١,٩٦± ٠,٤٢ mMol Fe^{+2}/g)، ثم حشيشه الليمون بفارق كبير (٠,٩٣± ٠,٢١ .mMol Fe^{+2}/g).

أظهرت دراسة النشاط المضادات للأكسدة بطريقة DPPH من خلال المقارنة مع قيم IC_{50} أن الزيت العطري للريحان هو أقوى مضاد للأكسدة (IC_{50} = ١٤٤,٧٨ ± ٧,١٩ ميكروجرام / مل) ، يليه الزيت العطري لحشيشة الليمون (IC_{50} = ١٧٠,٠٥ ± ٦,٩٧ ميكروجرام / مل) وأخيراً الزيت العطري للزعتر (IC_{50} = ١٨٣,٤٥ ± ٧,١٩ ميكروجرام / مل). أشارت النتائج بوضوح إلى أن الزيت العطري للريحان كان الأفضل كمضاد للأكسدة مقارنة بكل من حشيشة الليمون والزعتر في كلتا الطريقتين المستخدمتين في تحديد النشاط المضاد للأكسدة معمليا (FRAP و DPPH). وهذا يجعله مصدرًا واعدًا كمضاد طبيعي للأكسدة التي يمكن استخدامها في الوجبات الغذائية.