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BIOCHEMICAL STUDIES ON THYME AND BASIL ESSENTIAL OILS

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ABSTRACT: The present study was designed to investigate the chemical composition of thyme and basil medicinal plants, studying and evaluating the antioxidant and antimicrobial activity of oils, the oils under study were thyme (*Thymus vulgaris L.)* belonging to the family *Lamiaceae* and basil (*Ocimum basilicum*) belong to family *Lamiaceae* too. The major compounds found in thyme essential oil, were thymol and cymene, while the major compounds found in basil essential oil, were linalool and 1,8-cineole. The antimicrobial activity of the investigated essential oils was tested against eight bacterial (two gram +ve bacteria and six gram -ve bacteria) and four fungal strains. Which were (*S. aureus* and *enterococcus sp.*) for gram positive, (*E. Coli, S. typhi, acinitobacter sp., prouteus vulgaris, prouteus mirabillis* and *klebsiella pneumonaie*) for gram negative, four fungal (*candida albicans, rhizobium sp., fusarium sp*. and *alternaria A.*). The quenching of DPPH free radicals and ferric-reducing antioxidant power assay further highlighted the antioxidant potential of thyme and basil essential oils.

Key words: Thyme – Basil – Antioxidant – Antimicrobial – Composition – Essential oils

INTRODUCTION

Essential oils (EOs) serve a multifaceted role in plant defense in their natural environment. They function as antibacterial, antiviral, and antifungal agents, deterring herbivores by reducing their appetite. Furthermore, EOs play a crucial role in plant-pollinator interactions, attracting beneficial insects for pollination while repelling undesirable ones.

Historically, EOs have been utilized extensively for various health applications. While traditionally employed in perfumery and medicine, their use has expanded significantly in modern times. They are increasingly pervasive in our daily lives, utilized in cosmetics, hygiene products, household fragrances, and aromatherapy. Moreover, EOs are increasingly recognized for their potential in the industrial and agri-food sectors. It is estimated that approximately 3,000 EOs exist, with around 300 possessing significant commercial value, primarily within the perfume and aroma industries. (Lingan, 2018). In Vitro, studies have demonstrated that essential oils (EOs) exhibit potent antibacterial activity, even against strains typically resistant to antibiotics. The antimicrobial properties of EO constituents are primarily attributed to their amphiphilic nature, characterized by both lipophilic hydrocarbon skeletons and hydrophilic functional groups. (Saranraj & Devi, 2018). *Thymus vulgaris* L. (Lamiaceae) is an aromatic perennial plant originating from the Mediterranean region, which has been used by the world population as an aromatic plant, food preservative, and medicinal plant (Jamali *et al*., 2012). The primary constituents of this substance are terpenes, including thymol, carvacrol, p-cymene, γ-terpinene, caryophyllene, linalool, and borneol. These compounds have been demonstrated to exhibit a wide range of biological activities, such as antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, anti-hypertensive, antioxidant, antitumor, pro-apoptotic, anti-proliferative, and anti-nematode properties. (Kohiyama *et al*., 2015). The preservative potential of *Thymus vulgaris* L. EO in food products can be attributed to its multifaceted actions. It acts as an antioxidant, inhibiting lipid oxidation and thereby improving food stability. Additionally, thyme EO possesses potent

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antimicrobial properties, effectively inhibiting the growth of a wide range of microorganisms, such as bacteria, yeasts, and filamentous fungi. (Mahboubi *et al.*, 2017). The chemical composition of basil oil exhibits significant variability, exerting a profound influence on the plant's biological activity. Linalool emerges as a prominent constituent in numerous reports on basil essential oil composition. Moreover, other compounds, such as estragole, 1,8-cineole, and eugenol, are frequently encountered. (Marotti *et al*., 1996). Recent research has highlighted the significant interest in medicinal plants as potential sources of anti-inflammatory and antioxidant compounds. This interest stems from the potential of these compounds to play a crucial role in preventing and treating various diseases. Numerous studies have documented the antioxidant and anti-inflammatory properties exhibited by basil essential oils. (Araújo Couto *et al*., 2019). Treatment with basil essential oil in encapsulated form also demonstrated antioxidant activity from the DPPH sequestering activity assay (Sundararajan *et al*., 2018), Basil essential oil, characterized by its antimicrobial and antioxidant properties, has been shown to possess free radical scavenging activity. This evidence supports its potential for use in the pharmaceutical and cosmetic sectors. (Stanojevic *et al*., 2017).

MATERIALS AND METHODS

Proximate composition

The Association methods of the Official Analytical Chemists AOAC, (1990) were used for proximate analysis. The moisture content of thyme and basil samples (5 g) was determined by oven drying at 105°C to constant weight. Ash content was determined by ashing at 550°C for 3 hours. Protein content was analyzed using the Kjeldahl method. Crude fiber content was determined via the digestion method, and fat content was assessed using the Soxhlet extraction method. All analyses were conducted in triplicate. Carbohydrate content was estimated by the difference method (Pearson, 1976).

%CHO = 100 – (% moisture + % fat. + % ash + % fiber + % protein)

Essential oils

The essential oils of thyme (*Thymus vulgaris*) and basil (*Ocimum basilicum*) were obtained through hydro-distillation, a method for extracting oils from plant material.

Methods

Chemical composition of essential oils

GC-MS analysis of the essential oil samples was performed using a TRACE GC Ultra Gas Chromatograph coupled with a THERMO ISQ Single Quadrupole Mass Spectrometer at the Department of Medicinal and Aromatic Plants Research, National Research Center. The system utilized a TR-5MS column (30 m x 0.25 mm i.e., 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 1.3 mL/min and a split ratio of 1:10. The temperature program involved an initial hold at 60 °C for 1 min, followed by a ramp of 3.0 °C/min to 240 °C and a final hold for 1 min. The injector and detector temperatures were maintained at 240 °C. One microliter of each sample diluted 1:10 in hexane was injected. Electron ionization (EI) at 70 eV was used to obtain mass spectra within an m/z range of 40-450. Compound identification was achieved through mass spectra comparison with authentic chemicals, the Wiley spectral library collection, and the NSIT library Zhang *et al.*, (2010).

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of essential oils and alcoholic extracts was assessed based on their ability to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the recorded by Brand-Williams *et al*., (1995) one milliliter of 0.1 mM DPPH solution was mixed with varying concentrations (25, 50, 75, 100 g/mL) of essential oils and extracts. A comparable blank sample was generated, with ascorbic acid (25-100 µg/mL) utilized as a reference standard. A standard mixture of 1 mL of methanol and 1 mL of DPPH solution served as the control. Triplicate reactions were conducted, and the decrease in absorbance at 517

nm was measured after 30 minutes of incubation in the dark using a UV-Vis spectrophotometer. The percentage inhibition was calculated using a standard formula.

Inhibition % = Ac – As/Ac×100

The Ac is the absorbance of control.

The As is the absorbance of the sample.

Reducing power assay

The reducing power of essential oils and alcoholic extracts was determined according to the reported method by Ebrahimzadeh *et al*., (2008) two and a half milliliters of oil or extract solutions (25-100 µg/ml) were combined with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K3Fe(CN)6]. The reaction mixture was incubated at 50°C for 20 minutes. Subsequently, 2.5 ml of 10% trichloroacetic acid was added to terminate the reaction. Following centrifugation at 3000 rpm for 10 minutes, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride 1 (FeCl3). The absorbance of the resulting solution was measured at 700 nm against a blank. An increase in absorbance of the reaction mixture indicated an increase in reducing power. Vitamin C served as a positive control.

Antimicrobial activity assay

The antimicrobial activity of essential oils was evaluated against a panel of pathogenic microorganisms, including Gram-negative bacteria *(Escherichia coli, Salmonella typhi, Acinetobacter sp., Proteus vulgaris, Proteus mirabilis*, and *Klebsiella pneumoniae*), Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus sp.*), and fungi (*Candida albicans, Rhizobium sp., Fusarium sp*., and *Alternaria sp*.). All bacterial and fungal strains used in this study were obtained from the American Type Culture Collection (ATCC) and were procured from Nozha International Hospital, Cairo, Egypt. The antimicrobial activity was investigated of different essential oils against microorganisms by Kirby-Bauer´s agar disc diffusion modified method Klanenik *et al*., (2010). Standardized microbial suspensions (10^8 CFU/mL) were inoculated onto Mueller-Hinton agar plates for bacterial cultures and Sabouraud agar plates for fungal cultures. Whatman No.3 filter paper discs (6 mm diameter) impregnated with 20 µL of oils at concentrations of 1.25%, 2.5%, 5%, and 10% were then placed on the surface of the solidified media. Plates were subsequently incubated at 37°C for 24 hours for bacterial growth and 28°C for 48 hours for fungal growth. The diameter of the zones of inhibition was measured in millimeters (mm) to assess antimicrobial activity. All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Proximate composition of thyme and basil plants

Table 1 presents the proximate composition of *Thymus vulgaris* (thyme) and *Ocimum basilicum* (basil). Thyme exhibited a moisture content of 4.59%, crude fiber of 21.02%, ash content of 7.86%, crude protein of 9.67%, crude fat of 12.46%, and total carbohydrates of 44.4%. In contrast, basil displayed a moisture content of 4.03%, crude fiber of 18.52%, ash content of 12.24%, crude protein of 6.38%, crude fat of 6.89%, and total carbohydrates of 51.94%. Our data are in line with Badawy *et al*. (2005) who reported that the chemical composition of thyme herb were moisture 7.8 and % protein 9.1 %, also Inas, (2011) reported that, Proximate analysis of basil leaves on a dry weight basis revealed the following composition: 10.58% moisture, 14.12% ash, 17.66% crude protein, 2.53% lipids, 18.09% crude fiber, and 55.11% carbohydrates.

Table 1: Proximate composition of thyme and basil plants (w/w%)

Chemical composition of plant	Moisture	Crude protein	Total lipids	Crude fiber	Total ash	Total carbohydrates
Thyme	4.59	9.67	12.46	21.02	7.86	44.4
Basil	4.03	6.38	6.89	18.52	12.24	51.94

Chemical composition of essential oils

Tables 2 and 3 present the chemical composition of thyme and basil essential oils. A total of 30 compounds were identified across both oils. Thyme oil was characterized by a high abundance of thymol (52.15%), followed by p-cymene (17.68%), γ-terpinene (6.86%), and trans-caryophyllene (4.73%). In contrast, basil oil was dominated by linalool (44.82%), with 1,8-cineole (10.92%), α-farnesene (7.63%), eugenol (6.33%), and germacrene (4.56%) also present in significant amounts. The above data agrees with Rota *et al.,* (2008) who reported that the major part and most active compounds of thyme essential oil are thymol (68.1%) and carvacrol (10%). Also, Marotti *et al.,* (1996) reported that the main constituents for basil essential oil are linalool, and other compounds are also frequent, such as estragole, 1,8-cineole, and eugenol.

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

Compound Name	Area %	Compound Name	Area %	Compound Name	Area %
α Thujene	0.48	β Ocimene	0.03	α Terpinene	1.21
α Pinene	0.53	γ Terpinene	6.86	P-Cymene	17.68
Camphene	0.3	Sabine	0.25	Limonene	0.27
β Pinene	0.15	α Terpinolene	0.26	1,8-Cineole	0.28
1-Octen-3ol	0.76	β Linalool	2.05	Copaene	0.19
3-Octanone	0.04	Camphor	0.25	Trans caryophyllene	4.73
Butanoic 2 methyl ester butyric methyl ethyl ester	0.07	Borneol	1.58	α Murolene	0.12
β Myrcene	0.79	3-Cyclohexen-1-ol	1.19	δ Cadinene	0.88
3-Octanol	0.08	Carvacrol methyl ether	0.86	3-Carene	0.07
Phellandrene	0.14	Thymol	52.15	Thymol acetate	0.1

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

Compound Name	Area %	Compound Name	Area %	Compound Name	Area %
α Thujene	0.04	α Terpinene	0.1	1,8-Cineole	10.92
α Pinene	0.59	γ Cadinene	3.27	β Ocimene	0.65
Camphene	0.12	α Bulnesene	1.87	γ Terpinene	0.11
β Pinene	1.47	Bicyclogermacrene	1.4	α Terpinolene	0.2
Camphor	0.7	Germacrene	4.56	Linalool	44.82
4-Terpineol	0.54	α Humulene	1.02	α Copaene	1
Linalyl propionate	0.88	α Farnesene	7.63	Bicyclosesquiphellandrene	3.19
Estragole	0.81	Trans caryophyllene	0.35	Eugenol	6.33
β Myrcene	0.82	β Elemene	1.63	Endobornyl acetate	1.43
P-Cymene	0.11	Calamenene	0.6	Sabine	0.12

Antioxidant activity

Numerous methodologies exist for evaluating antioxidant capacity, each employing distinct free radical generators and operating via diverse mechanisms. A comprehensive assessment of a product's antioxidant activity necessitates the utilization of multiple methods. While a single method can offer preliminary insights, a combination of assays provides a more comprehensive and accurate characterization of the sample's antioxidant properties.

DPPH radical scavenging activity

The antioxidant potential of thyme and basil essential oils (EOs) was demonstrated by their ability to quench DPPH free radicals. The radical-scavenging activity of the spice EOs was evaluated using the stable free radical DPPH. Table 4 presents the effective concentrations of each EO required to scavenge DPPH radicals, along with the corresponding scavenging values as inhibition percentages. The results revealed varying degrees of radical-scavenging activity among the EOs. Basil EO exhibited the strongest effect of the radical scavenging, reaching 84.3% inhibition at a concentration of 100 µg/ml, which was lower than that of the positive control ascorbic acid (93.6%). Thyme EO followed with a scavenging activity of 81.4%.

Table (4): Antioxidant activity of basil and thyme essential oils measured by DPPH Method.

Essential oils	DPPH % inhibition			
	25 µg	**50** µg	**75** µg	**100** µg
Basil	54.5	67.9	77.5	84.32
Thyme	50	64.1	74.3	81.4
Ascorbic acid	81.6	87.1	91.1	93.6

Reducing power assay

Fe (III) reduction is often used as an electron-donating activity indicator, which concedes an important mechanism in phenolic antioxidant action (Nabavi *et.al.,* 2009). This assay quantifies the reducing capacity of samples, which reflects their antioxidant potential. Reductants within the samples reduce Fe3+ to Fe2+, donating an electron in the process. The resulting Fe2+ ions subsequently form a complex that is measured spectrophotometrically at 700 nm as Prussian blue. An increase in absorbance at 700 nm directly correlates with an increase in reducing ability. Figure 1 depicts the dose-response curves for the reducing power of various plant essential oils. The results demonstrate a concentration-dependent increase in reducing power for all oils tested. At the highest concentration (100 µg/ml), basil essential oil exhibited the highest reducing activity (0.280), followed by thyme essential oil (0.238), compared to the standard, ascorbic acid (0.471).

Studies have shown that the reducing power capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Sofidiya *et al.*, 2006). Thymol in thyme essential oil stands out as the most well-known natural antioxidant, recognized for its role as a classic chain-breaking antioxidant (Pateiro *et al.*, 2018).

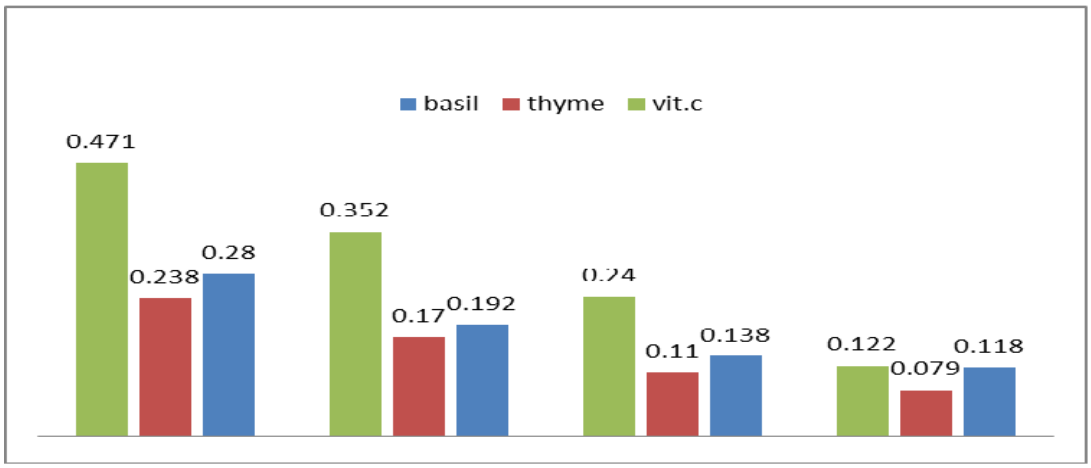

Fig. (1): Reducing power activity of basil and thyme essential oils.

Antimicrobial activity

The antimicrobial activity of the essential oils was evaluated against a panel of microorganisms, including Gram-negative bacteria (E. coli, S. typhi, Acinetobacter sp., Proteus vulgaris, Proteus mirabilis, and Klebsiella pneumoniae), Gram-positive bacteria (S. aureus and Enterococcus sp.), and fungi (Candida albicans, Rhizobium sp., Fusarium sp., and Alternaria sp.) during the screening process. Antimicrobial activity was assessed by measuring the diameter of inhibition zones in millimeters. As presented in Tables 5, 6, and 7, *Thymus vulgaris* demonstrated potent inhibitory effects against *S. aureus* with a 21 mm zone and against *Enterococcus sp.*, *Proteus mirabilis*, and *Proteus vulgaris* with zones of 18, 17, and 15 mm, respectively. This strong activity is likely attributed to the presence of various compounds in thyme oil, notably p-cymene and thymol. In contrast, *Ocimum basilicum* exhibited weaker inhibition against the Gram-negative bacteria *S. typhi* (8 mm) and *E. coli* (11 mm) and showed no significant activity against *Acinetobacter sp.*, *Proteus vulgaris*, *Proteus mirabilis*, and *Klebsiella pneumoniae*. Furthermore, as shown in Table 7, *Thymus vulgaris* displayed significant antifungal activity against *C. albicans* (11 mm) and *Alternaria alternata* (13 mm), while *Ocimum basilicum* demonstrated inhibition zones of 11 mm and 12 mm against these fungi, respectively.

Table (5): The inhibition zones diameter IZD (mm) of the essential oils against gram -ve bacteria.

Microorganisms	Thyme oil %				Basil oil %			
	1.25	2.5	5	10	1.25	2.5	5	10
E. coli	ns	7	9	11	ns	6	8	11
S. typhi	6	7	9	11	ns	ns	6	8
Acinitobacter sp.	ns	ns	6	9	ns	ns	ns	Ns
Prouteus vulgaris	8	10	13	15	ns	ns	ns	Ns
Prouteus mirabillis	9	12	14	17	ns	ns	ns	Ns
Klebsiella pneumonaie	7	10	12	14	ns	ns	ns	Ns

This data is agreed with the published by Kotan *et al.*, (2010); Helmy, (2012), and Küçükbay *et al.*, (2014) which reported that the antibacterial efficacy of thyme oil can be primarily attributed to the presence of phenolic compounds and hydrocarbons. These constituents show antimicrobial properties, acting as bactericides or bacteriostats depending on their concentration.

Table (6): The inhibition zones diameter IZD (mm) of the essential oils against gram +ve bacteria.

Microorganisms	Thyme oil %				Basil oil %			
	1.25	**2.5**	**5**	**10**	**1.25**	**2.5**	**5**	**10**
S. aureus	14	16	19	21	ns	ns	6	8
Enterococcus sp.	10	12	15	18	ns	ns	ns	6

This data agrees with the published by Yousefi *et al.,* (2020) who reported Gram-positive bacteria tend to be more susceptible to EOs than Gram-negative ones, also Smith-palmer *et al.,* (1998), reported that Gram-positive bacteria were more sensitive to inhibition by planting essential oils than the gram-negative bacteria.

Table (7): The inhibition zones diameter IZD (mm) of the essential oils against fungi.

Microorganisms	Thyme oil %				Basil oil %			
	1.25	**2.5**	**5**	**10**	**1.25**	**2.5**	**5**	**10**
Candida albicans	ns	6	8	11	ns	6	9	11
Rhizobium sp.	ns	ns	ns	ns	ns	6	7	9
Fusarium sp.	ns	ns	ns	ns	ns	ns	ns	6
Alternaria A.	6	8	10	13	6	7	9	12

This data agrees with the published by Miao *et al.* (2020), who reported that basil essential oil has traditionally been used to treat bacterial and fungal infections, also Jugreet and Mahomoodally, (2020) and Yap *et al.,* (2014) reported that basil essential oil is composed of several constituents that have antimicrobial activity, so its activity may be due to a synergistic action of its constituents.

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دراسات كيميائية حيوية على زيوت الزعتر والريحان

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قسم الكيمياء الحيوية - كلية الزراعة - جامعة المنوفية - شبين الكوم - مصر

الملخص العربى

تهدف هذة الدراسة إلى دراسة التركيب الكيميائى لكل من نباتات الزعتر والريحان ، دراسة وتقييم النشاط المضاد للأكسدة وكذلك نشاط الزيوت العطرية للزعتر والريحان كمضادات ميكروبية . وأشارت نتائج التحليل الكيميائى للزيوت العطرية إلى ان المركبات الرئيسية الموجودة في زيت الزعتر العطري هي الثيمول والسيمين، بينما المركبات الرئيسية الموجودة في زيت الريحان العطري هي اللينالول و٨،١-سينول .تم اختبار النشاط المضاد للميكروبات للزيوت العطرية المدروسة ضد ثماني سلالات بكتيرية (اثنتان من البكتيريا الموجبة الجرام وستة سلالات من البكتيريا السالبة الجرام) وأربع سلالات فطرية فالسلالات الموجبة لجرام *S. aureus* و *enterococcus sp.* وسالبة الجرام *E.coli* و *S. typhi* و *acinitobacter sp.* و *prouteus vulgaris* و *prouteus mirabillis* و *klebsiella pneumonaie* وأربع سلالات فطرية *candida albicans* و *rhizobium sp.* و *fusarium sp.* و *alternaria A.* .تم دراسة النشاط المضاد للأكسدة لزيوت الزعتر والريحان العطرية من خلال تثبيط الجذور الحرة DPPH واختبار قدرتهم على إختزال الحديديك.

الكلمات المفتاحية: الزعتر – الريحان – مضادات الأكسدة – مضادات الميكروبات – التركيب – الزيوت الأساسية.