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BIOCHEMICAL STUDIES ON ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS ESSENTIAL OILS

Hammam, M.A.[(1)]; El-Sayed, S. M. [(1)]; El-Sayed, A. G. [(2)] and Mohamed, W. R.[(1)]

[(1)] Biochemistry Department, Faculty of Agriculture, Menofia University, Shibin El-Kom, Egypt.

[(2)] Medicinal and Aromatic Plants Research Department National Research Centre, Egypt.

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ABSTRACT: The present study was designed to investigate the chemical composition of four medicinal plants essential oils, studying and evaluation the antioxidant and antimicrobial activity of oils, the oils under study were lemon grass (*Cymbopogon citratus*) belong to family (Poaceae), origanum (*Origanum Majorana*) belong to family (Lamiaceae), thyme (*Thymus Vulgaris*) belong to family (Lamiaceae), and rosemary (*Rosmarinus officinalis*) belong to family (Lamiaceae). The major compounds found in lemon grass, origanum, thyme and rosemary essential oils, were D-Limonene 54.01%, terpinene 4-ol 36.06%, thymol 48.06% and 1,8-Cineole 32.5% respectively. The antimicrobial activity of the investigated essential oils were tested against six bacterial (three gram +ve bacteria and three gram -ve bacteria) and two fungal strains including gram positive (S. aureus, B. subtilis and B. cereus), gram negative (E. Coli , S. typhimurium and Proteus sp) and two fungal (Candida albicans and Aspergillus niger). The antioxidant potential of lemon grass, orignaum, thyme and Rosemary essential oils were further highlighted by the quenching of DPPH free radicals. lemon grass essential oil showed the strongest radical-scavenging effect (69.97%) at 50 g/L.

Key words: EOS, DPPH, TAC, IC50 a.

INTRODUCTION

In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reduction their appetite for such plants. They also may attract some insects to favour the dispersion of pollens and seeds, or repel undesirable others. EOs have been used throughout recorded history for a wide variety of health-related application. Formerly reserved for perfumery and medicine, essential oils are today ubiquitous in our daily lives: in cosmetics, in hygiene products or in home fragrances, in aromatic oils for massage well- be, or even marketed as complexes to purify our polluted air. They are also finding increasing interest in industry and agri-food. It is estimated that around 3,000 essential oils are known and around 300 are commercially valuable, mainly for the perfume and aroma industry (Lingan, 2018).

EOs possesses, in vitro, a potent antibacterial activity, including on the usually antibiotic resistant strains. In the antimicrobial action of EO components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. (Saranraj & Devi, 2018). The medicinal property of lemon grass allegedly because of some phytochemical content in this plant extract. One of the main constituents of the many different species of lemon grass (genus *Cymbopogon*) is citral and terpenes. Infusions of the leaves are used in traditional medicine as antimicrobial, anti-inflammatory, and sedative (Adesegun AS, *et al.*, 2013). Among several essential oils that may be useful as antimicrobial agents, *Origanum majorana* L. (marjoram)essential oil belonging to the family Lamiaceae possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi and therefore, it may have the greatest potential for use in industrial applications (Mohamed,. and Mansour, 2012).

Thymus vulgaris L. (Lamiaceae) is an aromatic perennial plant originates from the Mediterranean region, which has been used by

*Corresponding author: salahmansour79@yahoo.com

the world population as aromatic plant, food preservative and medicinal plant (Jamali *et al*., 2012). The major constituents are terpenes as thymol, carvacrol, pcymene, γ-terpinene, caryophyllene, linalool and borneol. Some of their effects were described such as antibacterial, antiviral, antifungal, anti-inflammatory, anticancer anti-hypertensive, antioxidant, antitumor, pro-apoptotic, anti-proliferative and anti-nematode activities (Kohiyama *et al*., 2015). In addition to acting as an antioxidant agent, the essential oil isolated from rosemary possesses various health benefits and therapeutic effects. (European Medicines Agency 2010). Rosmarinic acid (RA) which is one of the major bioactive compounds of Rosmarinus officinalis has been found to exhibit multiple biological and pharmacological activities, including antioxidant, anti-allergic and anti-inflammatory effects. Recently, our research group reported the synthesis of a new series of amide analogs of rosmarinic acid as effective antioxidants (Ayoob *et al*., 2017).

MATERIALS AND METHODS

Essential oils

The (EOS) essential oils of lemon grass (*Cymbopogon citratus*), origanum (*Origanum Majorana*), Thyme (*Thymus Vulgaris*) and Rosemary (*Rosmarinus officinalis*) were obtained from the extraction oil unit in National Research Centre, Dokki, Giza, Egypt.

Methods:

Chemical composition of essential oils:

The GC-Ms analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min and a split ratio of 1:10 using the following temperature program: 60 ºC for 1 min; rising at 3.0 ºC /min to 240 ºC and held for 1 min. The injector and detector were held at 240 ºC. Diluted samples (1:10 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Antioxidant activity

DPPH radical scavenging activity:

The antioxidant activity of oils was measured on basis of the scavenging activity of the stable 1,1diphenyl-2-picrylhyorazyl (DPPH) free radical according to the recorded by (Brand-Williams *et al*., 1995) with slight modifications 1 ml of 0.1 mM DPPH solution mixed with various concentrations (25, 50, 75, 100)μg/ ml of essential oils corresponding blank sample were repaired and ascorbic acid (25-100µg / ml) was used as refrence . Standard mixture of 1ml methanol and 1ml DPPH solution was used as a control. The reactions carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer The inhibition % was calculated using the following formula

Inhibition % = Ac – As/Ac $\times$100

The Ac is the absorbance of control.

The As is the absorbance of sample.

Antimicrobial activity assay:

The essentials oils were individually tested against Pathogenic strains Gram –ve (Escherichia coli , Salomonella , Proteus) bacilli bacteria, Gram +ve (Staphylococcus aureus , Bacillus subtilis, Bacillus cereus) cocci bacteria, A.niger and Candida albicans . All the strains belonging to the American Type of Culture Collection were obtained from Nozha International Hospital, Cairo. Egypt.

The antimicrobial activity was investigated of different aromatic compounds against microorganisms by well diffusion method (Shalaby *et al.* 2018). One hundred and fifty micro liters of the suspensions were spread over the agar plates using a sterile glass spreader. Each compound was dissolved in 1 mL of DMSO and sterilized by filtration through a 0.22 μm membrane filter (by using Milliopore membrane filter apparatus). 150 μl of each sample were added separately to the appropriate wells in the Petri dishes.

The plates were then incubated at optimum temperature 37 °C for 24 h, followed by the measurement of the diameter of growth inhibition zone expressed in millimeters (mm). All tests were performed in triplicate. The reference bacterial strains were tested by Kirby-Bauer method to evaluate their in vitro sensitivity to Antibiotic: Thiophenicol (100 μg/5 μL/disc).

RESULTS AND DISCUSSION

Chemical composition of essential oils

Data presented in Tables (1, 2, 3 and 4) showed the chemical constituents of lemon grass, origanum, thyme and rosemary essential oils . The data showed that the total identified compounds from lemon grass were 23 represent 99.68 % from total mass. The major compounds found in lemon grass essential oil, were D-Limonene (54.01%) followed by α-Pinene (4.04%), Sabinene (3.53%), β-Pinene (2.93%) as a Monoterpene hydrocarbons while β-Citral (16.56%), E-Citral (9.92%), Eucalyptol (3.61%), β-Myrcene (1.93 %) and L-Linalool (0.51 %) were the major compounds in oxygenated monoterpenes.

The major compounds found in *Origanum Majorana* essential oil, were terpinene 4-ol (36.06%) followed by cis-Sabinene hydrate (7.98%) and α-Terpineol (2.42%) as an oxygenated monoterpenes while p-cymene (17.67%), γ-Terpinene (9.14%), Sabinene 4.3 % and α-Terpinene 3.30% were the major compounds in Monoterpene hydrocarbons. The major compounds found in Thyme (*Thymus Vulgaris*) essential oil, were Thymol (48.06%) followed by p-Cymene (7.98%) and α-Terpineol (2.42%) as an oxygenated monoterpenes while p-cymene (38.57%), Caryophyllene (8.27%), L-Linalool 1.90% and Caryophyllene oxide 1.18%. The major compounds found in Rosemary essential oil, were (1,8-Cineole 32.5%), Menthol (19.14%), Glycerin diacetate (13.96%), β-pinene (8.57%) and D-Limonene (3.72%), D-limonene (3.72%), α-Pinene (1.07%), Sabinene (0.03%) and β-Pinene (8.57%).

Table (1): Chemical constituents of the essential oil from lemon grass.

Compound Name	Area %	Compound Name	Area %
α-Thujene	0.13	cis-Ocimene	0.08
α-Pinene	4.04	L-Linalool	0.51
Camphene	0.78	Limonene oxide, cis-	0.13
Sabinene	3.53	Trans-Limonene Oxide	0.12
β-Pinene	2.93	Isopulegol	0.06
β-Myrcene	1.93	Geranyl vinyl ether	0.05
6-Methyl-5-Hepten-2-One	0.22	cis-Verbenol	0.11
Linalool	0.09	β Fenchyl Alcohol	0.13
D-Limonene	54.01	β-Citral	16.56
Eucalyptol	3.61	E-Citral	9.92
γ-Terpinene	0.44	trans-Caryophyllene	0.07
Dihydromyrcenol	0.23		

Table (2): Chemical constituents essential oil from *Origanum Majorana.*

Compound name	Area %	Compound name	Area %
α-Thujene	1.09	cis-2-P-menthen-1-ol	0.81
α-Pinene	1.21	Terpinene-4-ol	36.06
Sabinene	4.63	α-Terpineol	2.42
β-Pinene	0.74	(e)-P-2-menthen-1-ol	0.2
β-Myrcene	0.24	trans-Sabinene hydrate	0.05
α-Phellandrene	0.19	Linalyl acetate	2.08
α-Terpinene	3.30	Isopulegol acetate	0.65
p-Cymene	17.67	trans-Caryophyllene	1.89
γ-Terpinene	9.14	Aromadendrene	0.06
cis-Sabinene hydrate	5.58	α-Humulene	0.08
α-Terpinolene	1.57	Ledene	0.11
cis-Sabinene hydrate	7.98	(-)-Caryophyllene oxide	0.78
trans-Sabinene hydrate	1.14	Ledene oxide-(ii)	0.06

Table (3). Chemical constituents essential oil from *Thymus Vulgaris*

Compound name	Area %	Compound name	Area %
Camphene	0.02	Thymol	48.06
p-Cymene	38.57	α-Copaene	0.26
γ-Terpinene	0.46	Caryophyllene	8.27
Fenchone	0.12	α-Humulene (CAS)	0.5
L-linalool	1.9	Caryophyllene oxide	1.18

Table (4): Chemical constituents essential oil from Rosemary *Rosmarinus Officinalis*

Compound name	Area %	Compound name	Area %
α-PINENE	1.07	Isoborneol	0.34
Camphene	0.16	endo-Borneol	0.46
Sabinene	0.03	Menthol	19.14
β-PINENE	8.57	p-Menth-1-en-8-ol	1.12
D-Limonene	3.72	Isobornyl acetate	1.1
1,8-Cineole	32.51	Glycerin diacetate	13.96
Isopinocarveol	0.14	Benzene, ethyl	6.61
(+)-2-Bornanone	10.6		

Antioxidant activity

There are many different methods for determining antioxidant function that rely on different generators of free radicals, acting by different mechanisms. It is very difficult to assess the antioxidant activity of a product on the basis of a single method. A single method will provide basic information about antioxidant properties, but a combination of methods describes the antioxidant properties of the sample in more detail.

The antioxidant potential of lemon grass, origanum, thyme and rosemary essential oils were further highlighted by the quenching of DPPH free radicals. The radical-scavenging capacity of the spice EOs were tested using the “stable” free radical DPPH. Table (5) show the effective concentrations of each EO required to scavenge DPPH radical and the scavenging values as inhibition percentage. It can be seen that EOs exhibited various degrees of scavenging ability. Lemon grass EO showed the strongest radical-scavenging effect (69.97%) at 50 g/L, which is lower than those observed for the positive controls, ascorbic acid (92.68 %). This activity was followed by the thymus EO (69.85%). Marjoram and rosemary EOs showed the lowest scavenging activities. The values of IC_{50} were in the order ascorbic acid > lemon grass > thyme > origanum > rosmary. The antioxidant activity measured by this method is slightly lower than that reported in the scientific literature for thyme, oregano, and rosemary EOs (Viuda-Martos *et al.*, 2010).

Antimicrobial activity

From screening process, the antimicrobial activity of the investigated essential oils was tested against six bacterial (three gram +ve bacteria and three gram -ve bacteria) and two fungal. Antimicrobial activity had been defined as mean diameter of inhibition zone (mm). The results shown in Table (6) revealed that *thymus Vulgaris* recorded 32 mm for *S. aureus* and 23 and 21 mm for *B. cereus* and *B. subtilis* respectively so thyme oil exhibited a strong inhibitory effect due to structure of this oil which characterized by presence of different compounds included P-cymene and thymol . Meanwhile, *rosmarinus Officinalis* exhibited weak inhibitory effect against three gram-positive with inhibition zone ranged from 10-12 mm and no significant activity showed against *S. aureus*.

Overall other essential oils were exhibited moderate antibacterial activity against tested bacterial owing to structure and active component of these oils. meanwhile *Thymus Vulgaris* recorded 29 mm for *E. coli* and 35 and 20 mm for *S. typhimurium* and *Proteus sp* respectively, meanwhile *Proteus sp* showed resistance to *Cymbopogon citratus* , *Origanum Majorana* and *Rosmarinus Officinalis* while *E. Coli* showed resistance to *Origanum Majorana.* The results showed in table (6) revealed that *Cymbopogon citratus* and *Thymus Vulgaris* had a significant antifungal activity against *C. albicans* with inhibition zone 44 and, 38 mm respectively meanwhile *Thymus Vulgaris* and *Origanum Majorana* recorded inhibition zone 20 and 16 respectively for *A. niger*. Overall other essential oils were exhibited moderate antifungal activity against tested fungal strains owing to structure and active component of these oils.

Table (5): Antioxidant activity of lemon grass, origanum, thyme and rosemary essential oils measured by DPPH Method

Essential oils	DPPH % inhibition				IC50 [a]
	5 g/L	10 g/L	20 g/L	50 g/L	
Cymbopogon citratus	25.77	39.84	54.92	69.97	19.17
Origanum Majorana	13.71	24.62	37.83	56.50	35.54
Thymus Vulgaris	20.75	32.38	53.91	69.85	22.43
Rosmarinus Officinalis	15.58	21.75	28.50	49.32	54.68
ascorbic acid	55.20	56.64	73.44	92.68	4.35

Table (6): The inhibition zones diameter IZD (mm) of the essential oil against gram +ve , gram –ve bacteria and fungi.

EO (10 µL/disc)	Gram +ve bacteria			Gram -ve bacteria			Fungi	
	S. aureus	***B. subtilis***	***B. cereus***	***E. Coli***	***S. typhimurium***	***Proteus sp***	***C. albicans***	***A. niger***
Lemon grass	10±0.7	9.9±0.2	15±0.7	13±0.1	20±0.7	NA	44±0.4	9±0.1
Origanum	11±0.7	13±0.3	14±0.1	NA	15±0.3	NA	21±0.3	16±0.2
Thyme	32±0.8	21±0.6	23±0.2	29±0.5	35±0.8	20±0.4	38±0.5	20±0.2
Rosmary	NA	10±0.4	12±0.4	14±0.7	13±0.4	NA	19±0.4	9±0.1
Thiophenicol	30±0.1	27±0.2	31±0.2	30±0.1	27±0.2	31±0.2	29±0.4	14±0.3

REFERENCES

Adesegun, A.S.; Samuel, F.O.; Olawale, R.G.; Funmilola, S.A. (2013). Antioxidant activity of the volatile oil of Cymbopogon citratus and its inhibition of the partially purified and characterized extracellular protease of Shigella sonnei. American Journal of Research Communication. 1(1): 31–45.

Ayoob I., et al. (2017). “New semi-synthetic rosmarinic acid-based amide derivatives as effective antioxidants”. Chemistry Select 2.31: 10153-10156.

Brand-Williams, W.; Cuvelier, M.E.; Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 28: 25-30.

European Medicines Agency: Community herbal monograph on Rosmarinus officinalis L., aetheroleum. 2010.

Jamali, C. A.; El Bouzidi, L.; Bekkouche, K.; Lahcen, H.; Markouk, M. and Wohlmuth, H. (2012). Chemical composition and antioxidant and anticandidal activities of essential oils from different wild Moroccan Thymus species. Chemistry & Biodiversity. 9: 1188–1197.

Kohiyama, C. Y.; Yamamoto Ribeiro, M. M.; Mossini, S. A.; Bando, E.; da Silva Bomfim, N. and Nerilo, S. B. (2015). Antifungal properties and inhibitory effects upon aflatoxin production of Thymus vulgaris L. by Aspergillus flavus Link. Food Chemistry, 173: 1006–1010.

Lingan, K. A. (2018). Review on Major Constituents of Various Essential Oils and its Application. Translational Medicine, 08(01), 1–5. https://doi.org/10.4172/2161-1025.1000201

Mohamed, M.H. and Mansour, H.A. (2012). Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. LWA-Food Science and Technology, 45: 79-87.

Saranraj, P. and Devi, D. (2018). Life Science Archives (LSA) REVIEW, https://doi.org/10.22192/lsa.2017.3.2.6.

Shalaby Al.G.; Ragab I.M. Tamer, Ahmed Belal M.Mehany, Mohamed M. I. Helal and Wafaa Abbas Helmy. (2018). Antitumor and prebiotic activities of novel sulfated acidic polysaccharide from ginseng Biocatalysis and agriculture Biotechnology, 402-409

Viuda-Martos, M.; Ruiz Navajas, Y.; Sánchez Zapata, E.; Fernández-López, J.; Pérez-Álvarez, J.A. (2010). Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour Frag J, 25: 13–19.

دراسات كيميائية على بعض الزيوت العطرية كمضات ميكروبية ومضادات اكسدة

مصطفى عبد الله همام(١)، صلاح منصور عبد الجواد(١)، عبد الناصر جابر السيد الجندى(٢)، وليد رفعت محمد احمد الصعيدى(١)

(١) قسم الكيمياء الحيويه- كليه الزراعة - جامعه المنوفية

(٢) قسم النباتات الطبيه والعطريه- المركز القومى للبحوث

الملخص العربى

تهدف هذة الدراسة إلى دراسة التركيب الكيميائى لأربعة من الزيوت العطرية ودراسة نشاطهم كمضادات أكسدة ومضادات ميكروبية، والزيوت تحت الدراسة هى زيت حشيشة الليمون – زيت الاورجانو – زيت الزعتر ووزيت الحصالبان ، والمركبات الفعالة بالزيوت هى D-Limonene 54.01 % بزيت حشيشة الليمون ، terpinene 4-ol 36.06 % بزيت الاورجانو، thymol 48.06% بزيت الزعتر و 1,8-Cineole 32.5 % بزيت الحصالبان ، تم تقييم نشاط الزيوت العطرية تحت الدراسة كمضادات ميكروبية ضد ست سلالات بكتيرية ثلاث سلالات موجبة لجرام (S. aureus, B. subtilis, B. cereus) وثلاث سلالات سالبة لجرام (E. Coli , S. typhimurium , Proteus sp) وسلالاتين فطر (Candida albicans, Aspergillus niger) كما تم دراسة خواص الزيوت العطرية كمضادات أكسدة مستخدما DPPH كشق حر وسجل زيت حشيشة الليمون أقوى نشاط مضاد للأكسدة حيث سجل نسبة (٦٩,٩٧٪) لكل ٥٠ جرام / اللتر .