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In Vitro Antimicrobial and Antioxidant Activities of Monoterpenes against some Food-Borne Pathogens

Gehan I. Kh. Marei[1]; Entsar I. Rabea[1] and M. E. I. Badawy[2]
[1] **Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour 22516, Egypt**
[2] **Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545 El-Shatby, Alexandria University, Alexandria, Egypt**

ABSTRACT

The present work describes the antimicrobial activity of twenty-five monoterpenes against Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* and antifungal activity against *Aspergillus flavus.* The antibacterial activity was evaluated by broth microdilution technique as a minimum inhibitory concentration (MIC) and the antifungal activity was estimated by mycelia radial growth technique as (EC_{50}). The results showed that thymol and α-terpineol were the most potent against *E. coli* with MIC of 45 and 55 mg/L, 135 and 225 mg/L against *S. aureus*, respectively. The results also showed that thymol exhibited the highest antifungal activity against *A. flavus* with EC_{50} 20 mg/L. Furthermore, the antioxidant properties were explored using *N,N*-dimethyl-1,4-phenylenediamine (DMPD) and the results showed that geraniol were the most potent compound (IC_{50} = 19 mg/L).

Keywords: Monoterpenes; *Escherichia coli*; *Staphylococcus aureus; Aspergillus flavus*; Antimicrobial activity; Antioxidant activity.

INTRODUCTION

There is main attention for the food producer, food integrity and consumers authorities to Foodborne diseases. Lately, there is a major attempt to detection the natural antimicrobials that can inhibit fungal and bacterial development in foods in order to improve quality and shelf life. Also, synthetic preservatives caused worried about the safeness food used by food consumers. As a result, natural products have become used increasing as alternative food preservatives (Gyawali and Ibrahim 2014; Smid and Gorris 1999; Tajkarimi *et al*. 2010). Due to that, there are continued in research about the antimicrobials derived from a variety of natural sources. Natural antimicrobials have obtained from diverse sources like animals, plants, algae, fungi, and bacteria. plant antimicrobials showed high efficiency in food applications like food safety and preservation (Gyawali and Ibrahim 2012; Myszka *et al.* 2019; Rafiq *et al*. 2016; Tajkarimi *et al.* 2010). Polyphenolic compounds, from plant-derived compounds, have change and structural diversities in chemical composition, and thus they have antibacterial effectiveness (Stojković *et al*. 2013). The antimicrobial efficacy of plant extracts may be due to the presence of phenolic compounds or another hydrophobic component in the essential oils (Dorman and Deans 2000; Oyedeji-Amusa and Ashafa 2019). The monoterpenes secondary metabolites of plants first isolated by extraction and distillation procedures (Correa *et al.* 2019; Croteau *et al.* 2000). They are naturally formed from the condensation of two isoprene units. They have shown in a broad extent of pests such as bacteria, fungi, and insects, which make these compounds useful as potential alternatives to harmful synthetic pesticides (Garcia *et al*. 2008; Abdelgaleil *et al.* 2009; Badawy *et al.* 2010; Abdel Rasoul *et al.* 2012; Rabea and Badawy 2014; Herrera *et al.* 2015; Marchese *et al.* 2017;Ieri *et al.* 2019; Saad *et al.* 2019).

Pathogenic fungi cause diversity diseases in humans or plant organisms. *Aspergillus flavus* is responsible for aflatoxin contamination of crops before to harvest or during storage (Presterl *et al*. 2019; Yu *et al.* 2004). A. flavus causes disease in different ways like the production of mycotoxins , induction of allergenic responses and through systemic infections (Machida and Gomi 2010).

Escherichia coli is a notable pathogen that causes food borne illness (Karch *et al*. 2005; Mayton *et al*. 2019). illness Hemorrhagic colitis due to Infection to E. coli. Most illness has been connected with eating undercooked contaminated ground beef and drinking unpasteurized milke (Cody *et al*. 1999; Thompson and Darwish 2019) or drinking contaminated water (Sharma and Dean-Nystrom 2003). E. coli can occure the infection in various ways like asymptomatic fecal shedding of the organism. Staphylococcus aureus is a cause of hospital- and community-acquired infections. It is a Gram-positive round-shaped bacterium and It is a facultative anaerobe that can grow without the need for oxygen (Buchan *et al.* 2019; Masalha *et al*. 2001).

the purpose of this research was to study the comparative toxicities of different classes of monoterpenes as the major components of plant essential oils against Gram-negative (Escherichia Coli ATCC 8739), and Gram-positive (Staphylococcus aureus ATCC 6538) bacteria and Aspergillus flavus fungus. All compounds were evaluated by the antibacterial, antifungal, and antioxidant activities *in vitro*.

MATERIALS AND METHODS

1. Chemical used

β-cymene (99%), Camphene (95%), 3-carane (90%), (R)-(+)-limonene (97%), myrcene (95%), α-pinene (98%), camphor (98%), (±)-carvone (98%), (1R)-(-)-fenchone (98%), menthone (90%), (R)-(+)-pulegone (97%), citral (96%), (1*R*)-(−)-myrtenal (98%), citronellol (95%), geraniol (98%), linalool (97%), (-)-menthol (99%), α-terpineol (96%), thymol (98%), cinnamyl acetate (98%), citronellyl acetate (95%), eugenyl acetate (98%), geranyl acetate (97%), linalyl acetate (97%), α-terpinyl acetate (95%) were obtained from Sigma-Aldrich Co. (USA). Triphenyltetrazolium chloride (TTC), *N,N*-dimethyl-1,4-phenylenediamine (DMPD), α-tocopherol and ascorbic acid were purchased from Sigma Aldrich Co. (≥ 95% purity). The chemical structures of the tested monoterpenes are present in Figure 1. Potato Dextrose Agar (PDA) was purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK). Ceftriaxone was purchased from Pharco Co. Carbendazim was purchased from Kafr Elzyat company, Egypt. All of the other reagents used were of high purity grade.

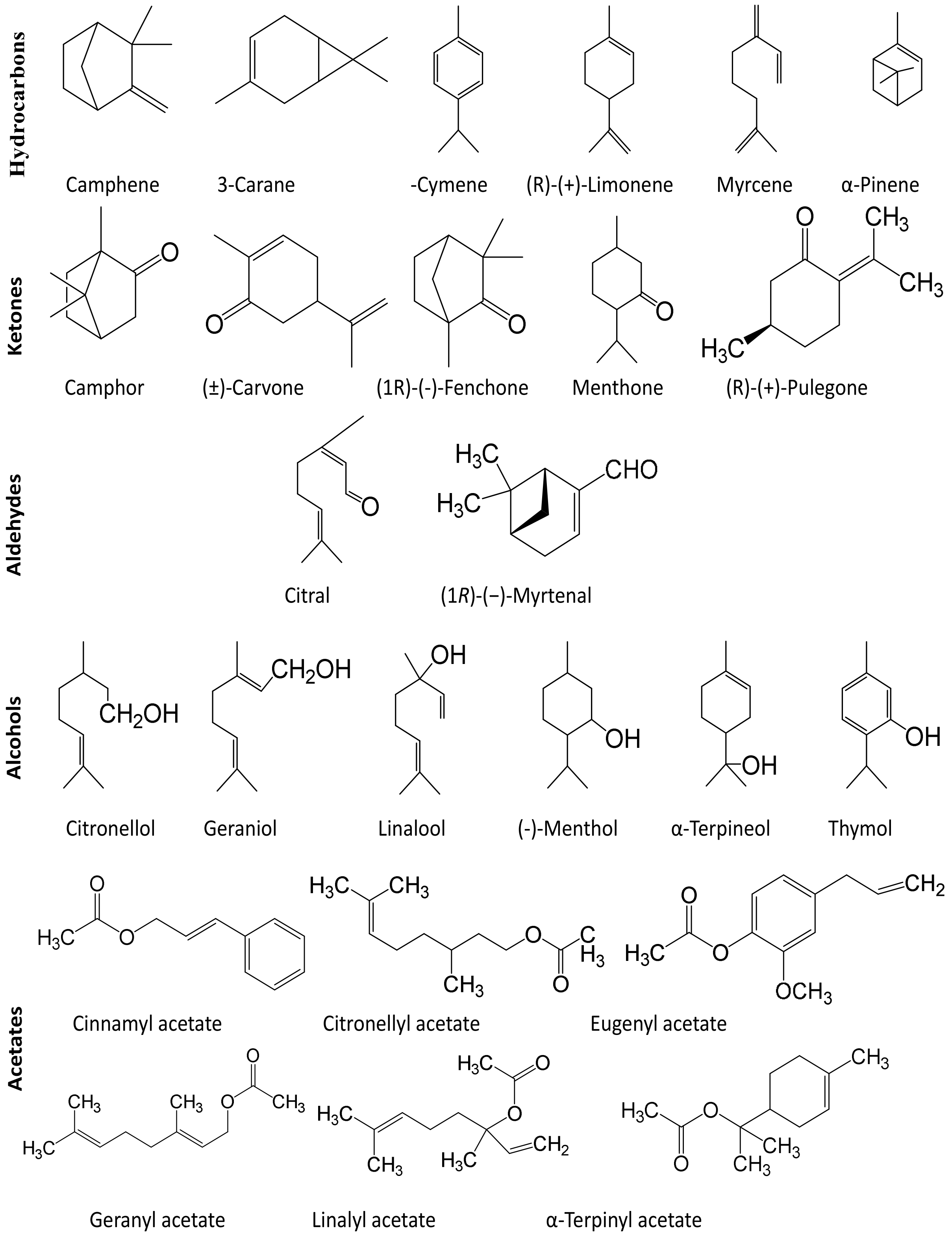

Figure 1. Chemical structure of monoterpenes.

3. *In vitro* assay of antibacterial activity

(Escherichia Coli ATCC 8739) Gram-negative and (Staphylococcus aureus ATCC 6538) Gram-positive bacteria were obtained from the Faculty of Agriculture, Alexandria University, Department of Dairy Science, Microbiology Laboratory. The cultures were maintained on (NA: Peptone 5 g, Beef extract 3 g, NaCl 8 g) nutrient agar medium at 37ºC. The antibacterial activities of the monoterpenes were evaluated out in 96-well microtiter plates according (Aruna Kumari *et al*. 2017; El-Kilany *et al*. 2015). It has been diluted with (NB) sterile Nutrient broth medium to a concentration of 5×105 CFU/mL calculated as a number of colonies× dilution factor/volume of culture plate using hemocytometer. The compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain the main stock solution (5000 µg/mL).

The solutions contain the compounds put in the wells, followed by the addition of NB medium and then 20 μL of bacterial suspension. The final volume in each well was 200 μL and the concentrations of 0.0, 37.5, 62.5, 75, 150, 250, 300, 500, 600, and 1000 µg/mL were tested for each compound. Negative and positive controls wells were performed without monoterpenes. The contents of each well were mixed on a microplate shaker at 200 rpm for 1 min prior to incubation for 24 h at 37°C. To indicate respiratory activity the presence of color was determined after adding 25 μL/well of triphenyltetrazolium chloride (TTC, Sigma) dissolved in water (0.25%, w/v) as a chromogenic marker and incubated under appropriate cultivation conditions for 30 min in the dark (Badawy *et al.* 2016). The absorbance was measured at 492 nm in an Ultra Microplate Reader (Robonik, PVT, LTD). Ceftriaxone as a standard drug was also tested for comparison at the concentrations of 0.0, 3.75, 6.25, 7.5, 15, 25, 30, 50, 60, and 100 µg/mL. The (MIC) minimum inhibition concentration values have been calculated.

3. *In vitro* assay of antifungal activity

It determined the antifungal activity of the tested monoterpenes by (Bajpai *et al.* 2007). It was added suitable volumes of the stock solutions of monoterpenes in DMSO to the PDA medium immediately. The monoterpenes were examined at concentrations of 0.0, 1.0, 10, 50, 100, 150, 200, 250, 350, 500, 1000, and 2000 mg/L. A carbendazim as a standard fungicide was evaluated at concentrations of 1, 10, 25, 50, 100 and 200 mg/L. Each concentration was tested in triplicate. Growth inhibition was calculated as the following equation (Pandey *et al.* 1982):

$$\text{Mycelial growth inhibition } (\%) = \frac{DC - DT}{DC} \times 100$$

DC and DT are the diameters of growth at the control and treatment. The linear regression method was used to determine the concentration of monoterpenes that inhibits the colony growth of fungi by 50% (EC50).

4. *In vitro* assay of antioxidant activity

The free radical scavenging activity as antioxidant of the monoterpenes was evaluated using the stable radical *N,N*-dimethyl-1,4-phenylenediamine (DMPD) with concentrated 100 mM DMPD solution (Asghar *et al.* 2007; Badawy *et al.* 2016; Fogliano *et al.* 1999). Standard solution of ascorbic acid (50-1000 mM) was prepared in deionized water. 100 µL of diluted samples were introduced into the sample and % inhibition of the radical cation was calculated for the standards and sample solutions as follows:

$$\text{Inhibition } (\%) = \left(\frac{\text{Absorbance in the control} - \text{Absorbance in the sample}}{\text{Absorbance in the control}}\right) \times 100$$

The concentration at which there is 50% fall in absorbance of DMPD radical solution was determined from the graph (IC_{50}). The IC_{50} value in µM was calculated from the above obtained concentration value. Ceftriaxone was used as antioxidant reference.

5. Statistical Analysis

Statistical analysis was did using the SPSS 25.0. The log dose-response curves to determine the IC50 and EC50 values for the antioxidant and antifungal bioassay, respectively depended on the probit analysis.

RESULTS AND DISCUSSION

1. *In vitro* antibacterial activity

The hydrocarbon and oxygenated monoterpenes were assayed for *in vitro* antibacterial activity against (E. coli) and (S. aureus) in comparison with ceftriaxone as a standard antibacterial agent. The MIC values of the tested compounds are summarized in Table 1 and expressed as mg/L. The control showed no effect in the experiments. The results revealed that all the tested compounds exhibited remarkable *in vitro* antibacterial activity against tested bacterial strains. However, the activity was lower than the standard drug (MIC of ceftriaxone = 0.72 and 13.1 mg/L against *E. coli* and *S. aureus*, respectively). The groups of compounds divided to five groups, hydrocarbons, ketones, aldehydes, alcohols and acetates as seen in Table 1. Among hydrocarbons group, myrcene and 3-carane were the most potent with MIC of 65 and 140 mg/L, against *E. coli* and with MIC of 260 and 275 mg/L against *S. aureus*, respectively. However, camphene and α-pinene were less active compounds with MIC of 540 and 550 mg/L against *E. coli* and with MIC of 610 and 600 mg/L against *S. aureus*, respectively. From ketones group, (R)-(+)-pulegone and menthone were most effective compounds with MIC of 60 and 70 mg/L, against *E. coli* and with MIC of 385 and 400 mg/L against *S. aureus*, respectively. There was no significance deference between (±)-carvone and (1R)-(-)-fenchone against the two tested bacteria. Nevertheless, camphor exhibited the lowest activity with MIC of 560 and 800 mg/L against *E. coli* and *S. aureus*, respectively. In aldehydes group, citral displayed the highest inhibition with MIC of 180 and 290 mg/L against *E. coli* and *S. aureus*, respectively, while (1*R*)-(−)-myrtenal was the lowest active (MIC = 275 and 550 mg/L against *E. coli* and *S. aureus*, respectively). Among alcohols group, thymol and α-terpineol were the most persuasive with MIC of 45 and 55 mg/L, against *E. coli* and with MIC of 135 and 225 mg/L against *S. aureus*, respectively. However, geraniol and (-)-menthol were the lowest active compounds with MIC of 250 and 275 mg/L against *E. coli* and with MIC of 300 and 400 mg/L against *S. aureus*, respectively. There was no significance deference between linalool and citronellol against the two tested bacteria. For acetates group, citronellyl acetate and geranyl acetate showed the greatest activity with MIC of 390 and 400 mg/L against *E. coli* and with MIC of 450 and 600 mg/L against *S. aureus*, respectively. There was no significance deference between eugenyl acetate, cinnamyl acetate and α-terpinyl acetate against the two tested bacteria. Conversely, linalyl acetate displayed the bottommost effective with MIC of 600 and 850 mg/L against *E. coli* and *S. aureus*, respectively. It can be concluded that alcoholic monoterpenes were the most antibacterial agents against the two tested bacteria compared with the other groups. When we consider the susceptibility of the microorganisms, another point deserves awareness; *E. coli* was more sensitive to the tested compounds than *S. aureus*.

Thymol, eugenol and carvacrol were highly inhibition against 16 Gram-negative bacteria and nine Gram-positive bacteria (Dorman and Deans 2000). The categories of this compounds have bactericidal or bacteriostatic agents, depending upon the concentration used. EOs containing phenols or aldehydes, such as citral, thymol, cinnamaldehyde, carvacrol, eugenol or carvacrol as the major components have the highest antibacterial activity, followed by EOs containing terpene alcohols. Other EOs, containing esters or ketones, such as α-thujone, geranyl acetate or β-myrcene had much weaker antibacterial activity. While volatile oils including terpene hydrocarbons were usually inactive (de Barros *et al.* 2009; Tajkarimi *et al.* 2010; Ait-Ouazzou *et al.* 2011; Rao *et al.* 2019).

High antimicrobial activity of Thymus and Origanum species has been attributed to their phenolic components such as thymol and carvacrol (Hazzit *et al.* 2009; Nabet *et al.* 2019; Soković *et al.* 2009). The presence of an oxygen function in the framework increases the antimicrobial properties of terpenoids (Naigre *et al.* 1996).

Table 1. Antibacterial activity of monoterpenes and ceftriaxone as a standard drug against *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538)

Class	Compound	MIC (mg/L)	
		E. Coli	***S. aureus***
Hydrocarbons	Camphene	540	610
	3-Carane	140	275
	β-Cymene	275	300
	(R)-(+)-Limonene	400	550
	Myrcene	65	260
	α-Pinene	550	600
Ketone	Camphor	560	800
	(±)-Carvone	200	420
	(1R)-(-)-Fenchone	275	500
	Menthone	70	400
	(R)-(+)-Pulegone	60	385
Aldehyde	Citral	180	290
	(1*R*)-(−)-Myrtenal	275	550
Alcohol	Citronellol	135	250
	Geraniol	250	300
	Linalool	130	265
	(-)-Menthol	275	400
	α-Terpineol	55	225
	Thymol	45	135
Acetate	Cinnamyl acetate	530	675
	Citronellyl acetate	390	450
	Eugenyl acetate	500	650
	Geranyl acetate	400	600
	Linalyl acetate	600	850
	α-Terpinyl acetate	550	790
	Ceftriaxone	0.727	13.10

MIC: Mininmum inhibitory concentration.

2. *In vitro* antifungal activity

The hydrocarbon and oxygenated monoterpenes compounds were assayed for *in vitro* antifungal activity against the aflatoxin-producing fungus *Aspergillus flavus* in comparison with carbendazim as a standard fungicide. The EC_{50} values of the tested compounds are summarized in Table 2 and expressed as mg/L. DMSO was taken as a control, which showed no effect in the experiments. The results revealed that all the tested essential oils exhibited remarkable *in vitro* antifungal activity against tested fungus strain. However, the activity was lower than the standard fungicide (EC_{50} of carbendazim = 19.0 mg/L against *A. flavus*). The groups of compounds divided to five groups, hydrocarbons, ketones, aldehydes, alcohols and acetates. Among hydrocarbons group, (R)-(+)-limonene, 3-carane and myrcene were the strongest antifungal agents with EC_{50} of 238, 259 and 288 mg/L, respectively. Nonetheless, β-cymene was the lowest antifungal activity compound with EC_{50} of 1051 mg/L. From ketone group, (R)-(+)-pulegone was most effective compound with EC_{50} of 255 mg/L. There was no significance deference between camphor and menthone against the tested fungus. On the other hand, (±)-carvone exhibited the lowest activity with EC_{50} of 550 mg/L. In aldehydes group, citral revealed effective action with EC_{50} of 212 mg/L while (1*R*)-(−)-myrtenal was the lowest antifungal activity one in this group with EC_{50} of 501 mg/L. Among alcohols group, thymol was the most persuasive against *A. flavus* with EC_{50} of 20 mg/L followed in descending order by citronellol (EC_{50} = 87 mg/L). However, α-terpineol was the lowest active compound with EC_{50} of 407 mg/L. There was no significance deference between linalool and geraniol against the tested fungus. For acetates group, geranyl acetate presented the greatest activity with EC_{50} of 348 mg/L. There was no significance deference between eugenyl acetate and citronellyl acetate. Conversely, linalyl acetate and α-terpinyl acetate displayed the bottommost effective with EC_{50} of 636 and 755 mg/L, respectively. It can be concluded that alcoholic monoterpenes were the most antifungal agents against the tested fungus compared with the other groups.

Aspergillus genus, which presents species infesting living plants (e.g. *A. flavus*) and stored food products, is responsible for food contamination all over the world (Kohiyama *et al.* 2015). The growth of in foodstuffs is toxicologically significant since some species are known to produce mycotoxins when exposed to suitable conditions (Moreira *et al.* 2010).

Several research studies reported that the monoterpenes exhibited antifungal activity against a wide range of microorganisms (Marchese *et al.* 2016; Santos *et al.* 2018; Teixeira *et al.* 2018; Zhou *et al*. 2019). Thymol, Trans-anethole, menthol, and zingiberene are the major element of essential oils of thyme, fennel, mint, and ginger, respectively. The effective concentrations for ginger, thyme, mint and fennel were 80, 50, 50 and 50% (oil/DMSO; v/v), respectively. Thymol, Trans-anethole, menthol, and zingiberene showed antifungal effect and the thyme essential oil highlighted in the inhibition of mycelial growth and sporulation of *A. flavus* (Silva *et al.* 2012). Nguefack *et al.* (2004) reported that the thyme essential oil at 200 mg/L reduced 81% of the radial growth of *A. flavus*. At 1000 mg thyme/L reduce 100% of the radial growth of *A. flavus*. Antifungal activity of thyme was assessed in culture medium and tomato paste against *A. flavus*. Results showed that 350 ppm of the thyme oil has strongest inhibition of *A. flavus* growth (Omidbeygi *et al.* 2007).

Carvacrol and thymol were tested *in vitro* against seven kinds of plant pathogenic fungi and the results showed both compounds exhibited broad spectrum of activity and strong antifungal activity has been attributed to their monoterpene and phenolic hydroxyl, and the position of phenolic hydroxyl showed less effect on antifungal activity. Ester derivatives of carvacrol and thymol were more antifungal activity than carvacrol and thymol (Wang *et al*. 2018).

Table 2. Antifungal activity of monoterpenes and carbendazim as a standard fungicide

Class	Compound	EC_{50} (mg/L)	95% Confidence limits		Slope ± SE	Intercept ±SE	χ^2
			Lower	Upper			
Hydrocarbons	Camphene	318	194	429	1.14±0.20	-2.85±0.57	3.71
	3-Carane	259	203	309	2.47±0.30	-5.96±0.80	3.17
	β-Cymene	1051	881	1297	1.76±0.21	-5.32±0.61	0.16
	(R)-(+)-Limonene	238	143	323	1.34±0.22	-3.19±0.61	3.54
	Myrcene	288	203	365	1.60±0.22	-3.93±0.62	3.15
	α-Pinene	433	293	568	1.11±0.20	-2.92±0.56	2.38
Ketone	Camphor	373	165	562	3.04±0.30	-7.82±0.81	4.82
	(±)-Carvone	550	244	1082	3.36±0.28	-9.20±0.79	8.80
	(1R)-(-)-Fenchone	412	90	715	1.91±0.22	-5.00±0.61	4.51
	Menthone	369	311	425	2.53±0.26	-6.50±0.71	3.5
	(R)-(+)-Pulegone	255	224	286	2.62±0.35	-6.31±0.86	0.40
Aldehyde	Citral	212	141	275	1.79±0.25	-4.17±0.67	0.86
	(1*R*)-(−)-Myrtenal	501	305	741	3.26±0.29	-8.80±0.79	4.61
Alcohol	Citronellol	87	66	121	1.07±0.15	-2.08±0.27	2.02
	Geraniol	287	163	396	1.10±0.20	-2.71±0.57	1.19
	Linalool	201	114	280	1.39±0.23	-3.20±0.63	3.66
	(-)-Menthol	304	183	412	1.15±0.20	-2.85±0.57	0.58
	α-Terpineol	407	335	478	2.08±0.23	-5.42±0.64	1.89
	Thymol	20	14	27	1.19±0.15	-1.57±0.25	2.98
Acetate	Cinnamyl acetate	514	265	810	2.44±0.24	-6.63±0.66	4.28
	Citronellyl acetate	493	430	559	2.65±0.25	-7.13±0.69	3.77
	Eugenyl acetate	401	49	759	2.53±0.25	-6.59±0.69	7.80
	Geranyl acetate	348	217	466	1.11±0.20	-2.83±0.57	1.17
	Linalyl acetate	636	562	718	2.72±0.24	-7.62±0.69	2.94
	α-Terpinyl acetate	755	671	850	2.82±0.25	-8.12±0.71	2.38
	Carbendazim	19	9.0	34	1.08±0.09	-1.40±0.14	11.27

EC_{50}: Half maximal effective concentration.
χ^2: Chi-squared.

3. *In vitro* antioxidant activity

The hydrocarbon and oxygenated monoterpenes compounds were assayed for *in vitro* antioxidant activity in comparison with α-tocopherol as a standard antioxidant agent. The IC_{50} values of the tested compounds are summarized in Table 3 and expressed as mg/L. DMSO was taken as a control, which showed no effect in the experiments. The results revealed that all the tested compounds exhibited remarkable *in vitro* antioxidant activity. Among hydrocarbons group, myrcene was the strongest antioxidant agent with IC_{50} of 22.13 mg/L followed by (R)-(+)-limonene and 3-carane (IC_{50} = 291.8 and 297.7 mg/L, respectively). Nevertheless, camphene, α-pinene and β-cymene were the lowest active compounds with IC_{50} of 868, 880 and 916 mg/L, respectively. For ketones, (R)-(+)-pulegone was the most effective compound with IC_{50} of 218 mg/L. There was no significance deference between (±)-carvone and (1R)-(-)-fenchone. On the other hand, camphor and menthone exhibited the lowest activity with IC_{50} of 1101 and 1217 mg/L, respectively. In aldehydes group, (1*R*)-(−)-myrtenal revealed effective action with IC_{50} of 285 mg/L, while citral was the lowest active one with IC_{50} of 1052 mg/L. Among alcoholic monoterpenes, geraniol was the most persuasive antioxidant with IC_{50} of 19 mg/L followed in descending order by thymol (IC_{50} = 31 mg/L). However, (-)-menthol was the lowermost active compound with IC_{50} of 1047 mg/L. For acetates group, geranyl acetate presented the greatest activity with IC_{50} of 534 mg/L followed in descending order by eugenyl acetate and α-terpinyl acetate (IC_{50} = 606 and 646 mg/L, respectively). In contrast, cinnamyl acetate displayed less action with IC_{50} of 1067 mg/L. It is clear from the results that the antioxidant potential of compounds is associated with the chemical structure and its efficiency against pests where alcoholic monoterpenes were the most antioxidant agents compared with the other groups.

Terpenes, one of the most extensive and diverse structural compounds happening in nature, exhibition a inclusive range of biological activity and antioxidant properties (Gonzalez-Burgos and Gomez-Serranillos 2012). Due to their antioxidant behavior terpenes have been shown to provide appropriate protection under oxidative stress conditions in different diseases. The main classes of terpenes, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, benzene derivatives, and non-isoprenoid components including alcohols, aldehydes, ketones have been tested for their antioxidant efficiency in comparison with α-tocopherol as a reference compound (Ruberto and Baratta 2000).

phenols have the highest antioxidant activity. In specific, some monoterpene hydrocarbons, namely, terpinolene, α- and γ-terpinene showed a significant protective action. Sesquiterpene hydrocarbons and non-isoprenoid components showed a low or no antioxidant effect. The antioxidant activities of eucalyptol, Linalool, α-terpineol, and α-pinene with both the ferric reducing ability of plasma (FRAP) and 1,1'-diphenyl-2-picrylhydrazyl (DPPH) methods were determined (Zengin and Baysal 2014).

Table 3. Antioxidant activity of monoterpenes and α-tocopherol as a standard antioxidant

Class	Compound	IC_{50} (mg/L)	95% Confidence limits		Slope ± SE	Intercept ±SE	χ^2
			Lower	Upper			
Hydrocarbons	Camphene	868.40	738.69	1056.41	1.63±0.23	-4.79±0.66	3.40
	3-Carane	291.80	222.55	351.88	2.02±0.25	-4.97±0.69	0.97
	β-Cymene	916.89	762.66	1166.16	1.43±0.23	-4.24±0.64	4.46
	(R)-(+)-Limonene	297.74	233.15	354.21	2.17±0.25	-5.36±0.7	0.95
	Myrcene	22.136	13.512	32.243	0.91±0.08	-1.22±0.18	2.95
	α-Pinene	880.74	598.39	1743.27	1.82±0.22	-5.35±0.67	7.32
Ketone	Camphor	1101.33	932.24	1385.43	1.75±0.24	-5.31±0.69	5.17
	(±)-Carvone	644.18	547.16	752.89	1.71±0.23	-4.80±0.64	4.11
	(1R)-(-)-Fenchone	877.64	558.92	2223.41	1.41±0.22	-4.15±0.64	5.97
	Menthone	1217.47	816.08	4762.26	1.99±0.25	-6.14±0.73	9.34
	(R)-(+)-Pulegone	218.38	163.18	266.58	2.43±0.28	-5.82±0.76	0.87
Aldehyde	Citral	1052.36	698.45	3360.74	1.82±0.24	-5.51±0.69	8.75
	(1*R*)-(−)-Myrtenal	285.39	105.19	416.53	1.93±0.25	-4.74±0.68	5.57
Alcohol	Citronellol	289.67	217.73	351.8	1.94±0.24	-4.77±0.68	2.77
	Geraniol	19.153	12.038	27.406	1.01±0.09	-1.30±0.19	2.94
	Linalool	530.68	422.44	637.06	1.43±0.22	-3.89±0.63	4.5
	(-)-Menthol	1047.71	879.20	1334.16	1.60±0.23	-4.83±0.67	4.26
	α-Terpineol	480.56	383.03	571.7	1.55±0.22	-4.16±0.63	4.85
	Thymol	31.426	19.152	45.738	0.80±0.08	-1.20±0.18	5.62
Acetate	Cinnamyl acetate	1067.29	699.56	3853.62	1.64±0.23	-4.95±0.67	7.65
	Citronellyl acetate	685.04	586.38	800.62	1.75±0.23	-4.95±0.65	4.86
	Eugenyl acetate	606.66	513.99	705.79	1.74±0.23	-4.85±0.65	2.72
	Geranyl acetate	534.83	471.32	596.72	2.57±0.25	-7.13±0.70	0.37
	Linalyl acetate	721.779	449.174	1245.916	1.79±0.23	-5.13±0.65	7.77
	α-Terpinyl acetate	647.08	551.15	754.72	1.74±0.23	-4.88±0.65	3.87
	α-Tocopherol	5.02	1.88	8.71	1.86±0.14	-1.30±0.14	28.21

IC_{50}: Half maximal inhibition concentration.
χ^2: Chi-squared.

CONCLUSION

The pure hydrocarbon and oxygenated monoterpenes exhibited *in vitro* antimicrobial and antioxidant effects against selected food-borne pathogens. The results showed that thymol and α-terpineol were the most potent against *E. coli* and *S. aureus*. The results also showed that thymol exhibited the highest antifungal activity against *A. flavus*. Furthermore, Geraniol showed it the most effective compound as the antioxidant. These monoterpenes can use as natural alternatives for application in food preservation to inhibit or retard the bacterial, fungal growth, and safety the shelf life of the food products.

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الأنشطة المضادة للأكسدة و الميكروبات من التربينات الاحادية ضد بعض مسببات الأمراض التي تنتقل عن طريق الأغذية

جيهان ابراهيم خليل مرعى[1] ، انتصار ابراهيم ربيع[1] و محمد الطاهر ابراهيم بدوى[2]

[1]قسم وقاية النبات – كلية الزراعة- جامعة دمنهور

[2]قسم الكيمياء و تقنية المبيدات- كلية الزراعة بالشاطبى- جامعة الاسكندرية

تم دراسة النشاط المضاد للميكروبات من خمسة وعشرين مركب من التربينات الاحادية ضد بكتريا ايريشريشيا كولاى السالبة لصبغة جرام و الايستافيلوكوكس ايوريوس الموجب لصبغة جرام والنشاط المضاد للفطريات ضد فطر الاسبرجلس فلافس(التى تصيب الاغذية). وتم تقييم النشاط المضاد للبكتريا بتقنية (broth microdilution technique) من خلال حساب أدنى تركيز مثبط (MIC) وايضا تم تقييم النشاط المضاد للفطريات عن طريق (mycelia radial growth technique) من خلال(EC_{50}) حساب التركيز الفعال الذى يسبب تثبيط 50٪ من النمو الهيفى .أظهرت النتائج أن الثيمول و α-terpineol كانا الأكثر فاعلية ضد *(E. coli*) بكتريا ايريشريشيا كولاى بقيمة MIC =45 و 55 ملجم / لتر، و 135 و 225 ملجم / لتر ضد الايستافيلوكوكس ايوريوس (*S. aureus*) ، على التوالي. أظهرت النتائج أيضا أن الثيمول أعلى نشاط ابادى لفطر الاسبرجلس فلافس EC_{50} = 20 ملجم / لتر. وعلاوة على ذلك ، تم اكتشاف الخصائص المضادة للأكسدة لهذه المركبات باستخدام N,N-dimethyl-1 ،4- phenylenediamine (DMPD) وأظهرت النتائج أن geraniol كان المركب الأكثر فاعلية كمضادات اكسدة بقيمة IC_{50} =19 ملجم / لتر.