



Research Journal of **Microbiology**

ISSN 1816-4935



Academic
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Research Article

Antimicrobial Activities of Essential Oil of Eight Plant Species from Different Families Against some Pathogenic Microorganisms

¹Hanan G. Mohamed, ¹Ahmed M. Gaafar and ²Amira Sh. Soliman

¹Departement of Special Food and Nutrition, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

²Department of Natural Resources, Institute of African Research and Studies, Cairo University, Giza, Egypt

Abstract

The beneficial effect of many types of plant extract used as seasoning agents in foods have been claimed for centuries. The purpose of this study was conducted to investigate the antibacterial activity of essential oils from i.e., ginger, thyme, coriander, marjoram, mustard, chamomile, licorquorice and nigilla against some bacterial strains of food borne pathogen (*Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium* and *E. coli*). Two different methods named the optical density assay and the well diffusion method were applied in the present study. The antibacterial effect of the essential oils coriander, thyme and ginger gave the highest antibacterial effect on all strains with inhibition percentages ranged from 90-99% and their inhibition zone ranged between 80-90 mm in diameters for both method used. While, marjoram, mustard and chamomile were moderate effect with inhibition percentages values ranged from 60-89% with inhibition zone between 50-70 mm. The lowest inhibitory effects were recorded for licorquorice and nigilla with inhibition percentages ranged from 33-65 and their inhibition zone of 8-45 mm. *Bacillus cereus* and *Staphylococcus aureus* were more resistant than *Salmonella typhimurium* and *E. coli* with all the examined essential oils. The results of this study revealed that these essential oils possesses some antibacterial properties as antibiotics, therefore, they can be used as a potential source of active ingredients for food preservatives.

Key words: Essential oils, antibacterial activity, well diffusion method, optical density assay, food borne pathogen

Received: August 26, 2015

Accepted: October 22, 2015

Published: December 15, 2015

Citation: Hanan G. Mohamed, Ahmed M. Gaafar and Amira Sh. Soliman, 2016. Antimicrobial Activities of Essential Oil of Eight Plant Species from Different Families Against some Pathogenic Microorganisms. Res. J. Microbiol., 11: 28-34.

Corresponding Author: Hanan G. Mohamed, Department of Special Food and Nutrition, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food safety is an increasingly important public health issue. Antimicrobial drugs are subjected to the microbial resistance and this has become a growing problem in recent years. Therefore, sufficient research to discover potent natural antibiotics is desirable and compulsory. Since many essential oils have been reported to possess strong antimicrobial effects (Orhan *et al.*, 2012). Essential oils are aromatic oily liquids obtained from plant material which have been long known for exhibiting antibacterial, antifungal, antiviral activities and they are regularly used in medicine and in the food industry (Bassole and Juliani, 2012). It is now acceptable by public and green food consumers to use essential oils with low taste concentrations in food to keep it safe and extend its shelf-life (Burt, 2004).

Essential oils or their components have been shown to exhibit insecticidal antiviral, antitoxigenic, antiphlastic and antimycotic properties. Those characteristic are possibly related to the function of those compounds in plants (Mahmoud and Croteau, 2002). The essential oils or their compounds extracted from various types of plants i.e., mustard, thyme, coriander, ginger, chamomile, nigella, liquorice and marjoram have been used for preserving foods and drinks have an inhibitory effect on the growth of microorganisms, which used as spices or aromatic herbs to give special aromas or flavors to foods are also known to have antimicrobial properties (Burt, 2004). Essential oils showed antimicrobial activity against a wide range of bacteria including antibiotic resistant species and fungal species. They can affect both Gram positive and Gram negative bacteria in addition to yeasts and filamentous fungi (Soni and Soni, 2014).

Elgayyar *et al.* (2001) evaluated the inhibition of essential oils from some herbs and mentioned that organo, coriander and basil showed the greatest inhibition effect while anis has no particularly inhibitory effect to bacteria. Donaldson *et al.* (2005) tested the activity of different essential oils and essential oil blends against *Staphylococcus aureus* and *Candida albicans* and found that all essential oils were active against both microorganisms.

The antibacterial activity of essential oils of thyme, marjoram, chamomile was investigated against *Bacillus cereus* and *Bacillus subtilis* by Gurgulova *et al.* (2006) and Abd El Mageid *et al.* (2009) and they reported that the highest antibacterial activity recorded for the essential oils of thyme and wild marjoram for all strains and the oil of chamomile had low antibacterial activity against the same strains. Also, Witkowska *et al.* (2013) indicate that crude extracts of some

herbs and spices possess *in vitro* activity against food spoilage and pathogenic bacteria, displayed bactericidal or bacteriostatic activities with consequent damage to bacterial cell membranes of both Gram positive and Gram negative bacteria. The reduction of the microbial population depends on the concentration of the essential oil where high concentrations of essential oil gave high antibacterial effect and may completely inhibit the growth of microorganisms (Kalemba and Kunicka, 2003). There are many methods that quantify microbiological activity of essential oils includes assays such as optical density, agar well diffusion, disk diffusion and hole plate diffusion (Burt, 2004).

The present study was aimed to evaluate the potentiality of essential oil of some commonly used spices and herbs against of different pathogenic bacteria by the optical density assay and by the well diffusion method.

MATERIALS AND METHODS

Type of essential oils: The volatile oils of thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), coriander (*Coriandrum sativum*), mustard (*Brassica alba*), chamemil (*Matricaria chamomilla*), marjorum (*Majorame hortensis*), nigella (*Nigella sutiva*) and liquorica (*Glycyrrhizza glabra*) were obtained from Kato Aromatic Company, Egypt and they were isolated by hydro distillation.

Bacterial strain: Four bacterial strains of significant importance were used to test the antibacterial properties of the essential oils. Two of them were Gram positive (*Bacillus cereus* ATCC6538 and *Staphylococcus aureus* ATCC25923) and the others were Gram negative (*E. coli* ATCC25922 and *Salmonella typhimurium* ATCC9027). The cultures of strains used in this study were obtained from Microbiological Resources Centre (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Bacterial strains were inoculated into Mueller Hinton broth (Difco) and incubated at 37°C for 24 h. The cultures were subjected to three successive 24 h. transfers before use. All cultures were adjusted to 10⁶ CFU mL⁻¹ prior to use.

Media: Muller Hinton liquid and solid media (Difco) were used in this study. The liquid medium was sterilized by autoclaving at 121°C for 20 min and then used for subculture and optical density assay. Solid media was used for agar-well diffusion assay.

Well diffusion assay: Twenty milliliter of Muller Hinton agar was placed into 10 mL petri dishes and 0.1 mL of the active

cultures was spread over the plate using a sterile glass spreader in order to get a uniform microbial growth for all plates (Donaldson *et al.*, 2005). A well was done using a 6 mm diameter cork borer in the agar plate. The wells were filled with 10, 20 or 30 μL of the essential oil. All plates were sealed by parafilm with sterile laboratory conditions to avoid evaporation of the agar plates. The plates were left for 30 min at room temperature to allow the diffusion of oil and then plates were incubated at 37°C for 24 h. The inhibition zone was measured with a caliper. Values were performed in triplicate and the mean value was recorded.

Optical density assay: The tested essential oils were emulsified into 5% propylene glycol aqueous solution and then the essential oils were added to sterile test tube contained Muller Hilton Broth (MHB) to obtain final concentrations of 2, 4, 6, 8 and 10 $\mu\text{L mL}^{-1}$. All tubes were inoculated with an active 24 h bacterial culture (500 μL per tube). Then all tubes were incubated in shaking incubator at 37°C for 24 h then microbial growth was measured, by taking the optical density at 600 nm and the results was expressed as growth inhibition percentage in order to compare the inhibitory effect of essential oil on the culture growth (Kalemba and Kunicka, 2003). Standard curves for microbial growth against optical density were executed.

Statistical analysis: The data analysis of this experiment was carried out by using the statistical analysis system by Robert (1990) and SAS (1996). Measured data was analyses by ANOVA. Least significance difference test was used to determine differences between means. Significance was as summed at $p \leq 0.05$.

RESULTS

Table 1-4 show the effect of different essential oils on the growth of bacterial strains using the turbidity method and the effect was expressed as inhibition percentage. All essential oils showed an inhibitory effect against the tested bacterial strains (*Salmonella typhimurium*, *E. coli*, *Bacillus cereus* and *S. aureus*) and the inhibition increased with increasing oil concentration. Ginger, thyme and coriander revealed the highest inhibitory activity (90-99%), while marjoram, mustered and chamomile showed moderate effect against the bacterial strains (60-90%), the lowest inhibition was found with licquorice for all strains (33-57%). The same effect was noticed for nigella, marjoram and mustered on *Bacillus cereus* (51-63%). With, regard to *S. aureus* this effect was obtained with the same essential oils and chamomile. Also, nigilla and chamomile exhibited the lowest effect on *Salmonella*, *E. coli* and *Bacillus*, respectively (Table 1 and 2).

Table 1: Inhibition percentage of different essential oils against *Salmonella typhimurium* by optical density assay

Essential oil types	Essential oil concentration ($\mu\text{L mL}^{-1}$)				
	2	4	6	8	10
Ginger	49 ^{Eb}	68 ^{Da}	78 ^{Cb}	86 ^{Bb}	97 ^{Aa}
Thyme	59 ^{Ca}	70 ^{Ba}	99 ^{Aa}	99 ^{Aa}	99 ^{Aa}
Coriander	45 ^{Db}	61 ^{Cb}	75 ^{Bb}	98 ^{Aa}	99 ^{Aa}
Marjoram	40 ^{Ec}	46 ^{Dc}	67 ^{Cc}	73 ^{Bc}	83 ^{Ac}
Musterd	29 ^{Ed}	33 ^{Dd}	51 ^{Cd}	75 ^{Bc}	89 ^{Ab}
Chamomile	34 ^{Ec}	36 ^{Dd}	58 ^{Cd}	69 ^{Bd}	90 ^{Ab}
Licquorica	19 ^{Ed}	23 ^{De}	34 ^{Ce}	42 ^{Be}	57 ^{Ad}
Nigilla	18 ^{Ed}	25 ^{De}	30 ^{Ce}	39 ^{Be}	43 ^{Ae}

Data in the column followed by different letters are significantly different at $p < 0.01$ according to ANOVA test

Table 2: Inhibition percentage of different essential oils against *Escherichia coli* by optical density assay

Essential oil types	Essential oil concentration ($\mu\text{L mL}^{-1}$)				
	2	4	6	8	10
Ginger	60 ^{Ea}	72 ^{Da}	80 ^{Ca}	92 ^{Ba}	99 ^{Aa}
Thyme	58 ^{Ea}	64 ^{Pb}	78 ^{Ca}	86 ^{Bb}	96 ^{Aa}
Coriander	57 ^{Ea}	63 ^{Pb}	81 ^{Ca}	89 ^{Ba}	99 ^{Aa}
Marjoram	17 ^{Ec}	34 ^{Pc}	49 ^{Cbc}	51 ^{Be}	67 ^{Ab}
Musterd	14 ^{Ecd}	31 ^{Pc}	45 ^{Cc}	63 ^{Bc}	70 ^{Ab}
Chamomile	22 ^{Eb}	36 ^{Pc}	54 ^{Cb}	63 ^{Bc}	72 ^{Ab}
Licquorica	10 ^{Ed}	14 ^{De}	23 ^{Cd}	28 ^{Be}	33 ^{Ad}
Nigilla	15 ^{Ec}	29 ^{Pd}	42 ^{Cc}	48 ^{Bd}	57 ^{Ac}

Data in the column followed by different letters are significantly different at $p < 0.01$ according to ANOVA test

Table 3: Inhibition percentage of different essential oils against *Bacillus ceruse* by optical density assay

Essential oil types	Essential oil concentration ($\mu\text{L mL}^{-1}$)				
	2	4	6	8	10
Ginger	53 ^{Db}	55 ^{Db}	74 ^{Cb}	83 ^{Bb}	92 ^{Aa}
Thyme	58 ^{Ea}	67 ^{Da}	77 ^{Ca}	89 ^{Ba}	96 ^{Aa}
Coriander	60 ^{Fa}	71 ^{Da}	82 ^{Ca}	91 ^{Ba}	97 ^{Aa}
Marjoram	29 ^{Ec}	39 ^{Dc}	47 ^{Cc}	52 ^{Bc}	60 ^{Ab}
Musterd	32 ^{Ec}	44 ^{Dc}	50 ^{Cc}	58 ^{Bc}	63 ^{Ab}
Chamomile	18 ^{Ed}	22 ^{Dd}	31 ^{Cd}	38 ^{Bd}	42 ^{Ad}
Licquorica	17 ^{Ed}	20 ^{Dd}	28 ^{Ce}	34 ^{Be}	40 ^{Ad}
Nigilla	23 ^{Dd}	25 ^{Dd}	37 ^{Cd}	43 ^{Bd}	51 ^{Ac}

Data in the column followed by different letters are significantly different at $p < 0.01$ according to ANOVA test

Table 4: Inhibition percentage of different essential oils against *Staphylococcus aureus* by optical density assay

Essential oil types	Essential oil concentration ($\mu\text{L mL}^{-1}$)				
	2	4	6	8	10
Ginger	51 ^{Eb}	60 ^{Da}	74 ^{Ca}	82 ^{Ba}	90 ^{Aa}
Thyme	58 ^{Ea}	64 ^{Da}	78 ^{Ca}	86 ^{Ba}	96 ^{Aa}
Coriander	55 ^{Ea}	62 ^{Da}	75 ^{Ca}	83 ^{Ba}	94 ^{Aa}
Marjoram	17 ^{Ed}	34 ^{Dc}	49 ^{Cb}	50 ^{Bbc}	67 ^{Ab}
Musterd	23 ^{Ec}	41 ^{Db}	53 ^{Cb}	56 ^{Bb}	70 ^{Ab}
Chamomile	19 ^{Ecd}	32 ^{Dc}	45 ^{Cc}	54 ^{Bb}	68 ^{Ab}
Licquorica	13 ^{Fe}	29 ^{Dd}	38 ^{Cd}	45 ^{Bc}	52 ^{Ac}
Nigilla	21 ^{Ec}	36 ^{Dbc}	42 ^{Cc}	53 ^{Bb}	65 ^{Ab}

Data in the column followed by different letters are significantly different at $p < 0.01$ according to ANOVA test

Table 5: Inhibition zone of different essential oils against some pathogenic bacteria by well diffusion method

Microorganisms	Concentration ($\mu\text{L well}^{-1}$)	Essential oil							
		Ginger	Thyme	Coriander	Marjoram	Mustered	Chamomile	Licoquorica	Nigella
<i>Salmonella typhimurium</i>	10	15 ^{Cc}	75 ^{Ba}	16 ^{Cc}	15 ^{Cc}	50 ^{Bb}	8 ^{Cd}	8 ^{Cd}	17 ^{Cc}
	20	33 ^{Bc}	(-) ^{Aa}	55 ^{Bb}	20 ^{Bd}	(-) ^{Aa}	15 ^{Be}	13 ^{Be}	28 ^{Bc}
	30	(-) ^{Aa}	(-) ^{Aa}	(-) ^{Aa}	85 ^{Aa}	(-) ^{Aa}	25 ^{Ac}	19 ^{Ac}	38 ^{Ab}
<i>Escherichia coli</i>	10	18 ^{Cd}	32 ^{Bb}	17 ^{Cd}	20 ^{Cc}	50 ^{Ca}	15 ^{Cd}	7 ^{Ce}	25 ^{Cc}
	20	38 ^{Bc}	(-) ^{Aa}	24 ^{Bd}	38 ^{Bc}	80 ^{Bb}	17 ^{Be}	13 ^{Be}	39 ^{Bc}
	30	(-) ^{Aa}	(-) ^{Aa}	(-) ^{Aa}	81 ^{Ab}	(-) ^{Aa}	40 ^{Ac}	18 ^{Ad}	45 ^{Ac}
<i>Bacillus ceruse</i>	10	35 ^{Ca}	35 ^{Ca}	20 ^{Cb}	17 ^{Cb}	15 ^{Cc}	22 ^{Cb}	7 ^{Cd}	15 ^{Cc}
	20	50 ^{Bb}	65 ^{Ba}	30 ^{Bc}	25 ^{Bd}	35 ^{Bc}	36 ^{Bc}	10 ^{Be}	22 ^{Bd}
	30	80 ^{Ab}	(-) ^{Aa}	85 ^{Aa}	46 ^{Ac}	60 ^{Ac}	48 ^{Ac}	12 ^{Ad}	46 ^{Ac}
<i>Staphylococcus aureus</i>	10	25 ^{Cb}	34 ^{Ca}	22 ^{Cb}	19 ^{Cb}	25 ^{Cb}	18 ^{Cc}	8 ^{Cd}	17 ^{Cc}
	20	40 ^{Bc}	60 ^{Ba}	40 ^{Bc}	27 ^{Bd}	48 ^{Bb}	35 ^{Bc}	11 ^{Be}	25 ^{Bd}
	30	(-) ^{Aa}	(-) ^{Aa}	(-) ^{Aa}	36 ^{Ad}	70 ^{Ab}	60 ^{Ac}	13 ^{Ae}	35 ^{Ad}

:- No growth or complete inhibition, Data in the column followed by different letters are significantly different at $p < 0.01$ according to ANOVA test

In particular, *Bacillus cereus* was more sensitive for coriander and thyme which exhibit the highest percentage of 97 and 96% and it was more resistant for licquorica. While, *S. aureus* was more sensitive for thyme and coriander and it was also more resistant for liquorica (Table 3 and 4). Thyme was significantly the most effective essential oil used against *Salmonella typhimurium* as it resulted inhibition percentage of 99% when it was used with concentration of 6 $\mu\text{L mL}^{-1}$, while coriander gave the same result at concentration of 10 $\mu\text{L mL}^{-1}$. These mean that *Salmonella typhimurium* is the most sensitive strain this strain was more resistant for treatment of nigilla (Table 1). The highest effect of essential

oils on *E. coli* was obtained when it was treated with ginger and coriander at concentration of 10 $\mu\text{L mL}^{-1}$ as it exhibited inhibition percentage of 99% (Table 2). Also *E. coli* was more sensitive for liquorices.

Data presented in Table 5 and illustrated in Fig. 1 summarize the effect of each essential oil against some tested bacterial strains using the well-diffusion assay and data presented as diameter of zone inhibition (mm) of Table 5 and inhibition percentage of Fig. 1. As noticed from the data, ginger and coriander showed similar trend as they possess complete inhibition of *Salmonella typhimurium*, *E. coli* and *S. aureus* when it used in concentration of 30 μL per well,

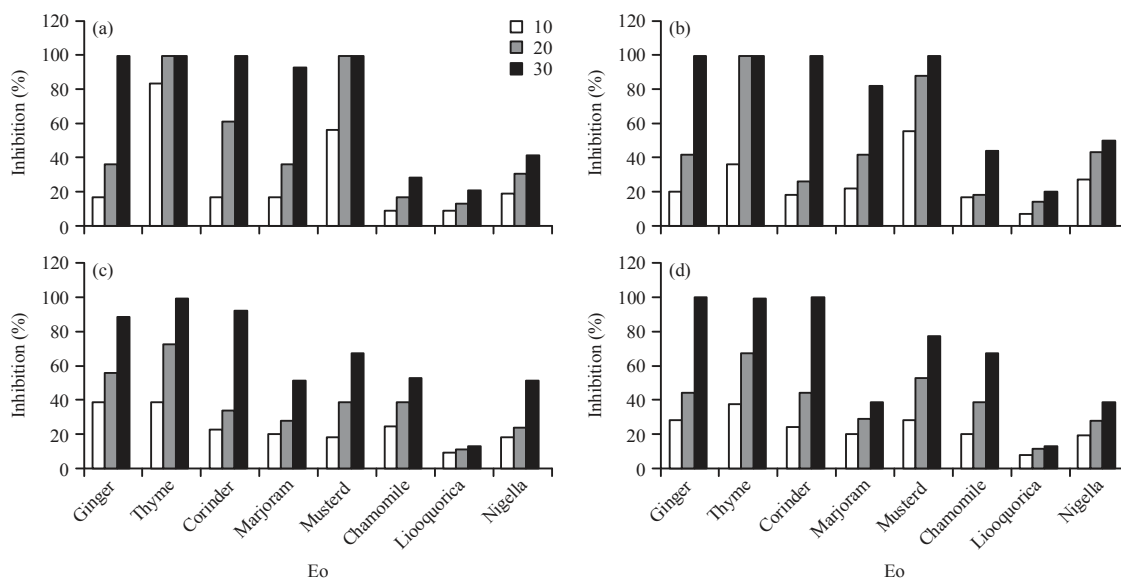


Fig. 1(a-d): Inhibition percentage of different essential oils against some pathogenic bacteria by well diffusion method, (a) *Salmonella typhimurium*, (b) *E. coli*, (c) *Bacillus cereus* and (d) *Staphylococcus aureus*

this concentration was the most effective concentration of ginger and coriander against *Bacillus cereus*.

Thyme is significantly the most effective essential oil against all tested strains where it showed a complete inhibition against *Salmonella typhimurium* and *E. coli* at a concentration of 20 μL per well. The same result was obtained for all tested strains when thyme was added in concentration of 30 μL per well. *Salmonella typhimurium* was significantly more sensitive for mustered than *E. coli* as it exhibit complete inhibition when it was treated with mustered in concentrations of 20 or 30 μL per well, while *E. coli* showed complete growth reduction only at a concentration of 30 μL per well.

Regarding to *Bacillus cereus* and *S. aureus* mustered did not show complete inhibition it gave inhibition zone diameter of 60 and 70 mm. with inhibition percentage of 67 and 78%, respectively. Marjoram showed high effect on *Salmonella typhimurium* and *E. coli* when concentration of 30 μL per well was used, but *Bacillus cereus* and *S. aureus* were more sensitive for marjoram as they showed inhibition zone diameter of 46 and 36 mm. With inhibition percentage of 51 and 39%, while *Salmonella typhimurium* and *E. coli* gave inhibition zone of 85 and 81 mm with inhibition percentage of 93 and 83%.

Both of *Bacillus cereus* and *S. aureus* were more sensitive For chamomile oil while *Salmonella typhimurium* and *E. coli* were more resistant. Nigella showed lower effect on growth of all strains if it compared with the offer mention essential oils.

The growth of *E. coli* and *Bacillus cereus* were affected with nigella more than *Salmonella typhimurium* and *S. aureus*. Finally, liquorice oil has the lowest inhibition effect on growth of all strains ranged from 10-19 mm. inhibition zone diameters with inhibition percentage of 13 and 21%, respectively. Where *Bacillus cereus* and *S. aureus* were slightly more resistant than *Salmonella typhimurium* and *E. coli*.

DISCUSSION

Generally, all of tested essential oils reduced the growth of the bacterial strains used in this experiment in different rates depending on the type and the concentration of essential oil and the type of tested organisms. These results are in agreement with Paster *et al.* (1990) and Mardafkan *et al.* (2015), who concluded that Gram positive and Gram negative organisms were both susceptible to the essential oils. As shown from data of optical density assay experiment, the essential oils of coriander, thyme and ginger gave the highest antibacterial effect on all strains while marjoram, mustered and chamomile are of moderate effect. The lowest effect was recorded for liquorice and nigella. In this respect Singh *et al.* (2007) found that coriander posse excellent antibacterial activity against some Gram positive and Gram negative bacterial strain tested and Ghaly (2006) reported that thyme oil gave the maximum inhibitory action against all the bacteria examined in their study followed by marjoram oil. Thyme and coriander exhibit the highest inhibition effect on both

S. aureus and *Bacillus cereus*. The obtained result is agreement with those reported by Dorman and Deans (2000) and Donaldson *et al.* (2005).

The antibacterial activity of thyme towards *Bacillus cereus* can be explained by the presence of a compound in oil fraction of thyme called carvacol regarding to all concentrations (Ultee *et al.*, 2000). Thyme showed the highest inhibition effect on *Salmonella typhimurium* when it was used with a concentration of 6 $\mu\text{L mL}^{-1}$, these data is a good agreement with the previously reported by Donaldson *et al.* (2005). Present data of the present study showed that, ginger and coriander were the most effective essential oils on growth of *E. coli* as it exhibit the highest inhibition percentage at a concentration of 10 $\mu\text{L mL}^{-1}$. In this regard, Elgayyar *et al.* (2001) found that the oil of coriander completely inhibited the growth of *S. aureus* and it was less effective in inhibiting *E. coli* although it still strongly has inhibitory effect. Also, Chaisawadi *et al.* (2005) agreed with the obtained data for ginger oil in this study as they found that ginger oil showed higher antibacterial properties against *Bacillus cereus*, *Salmonella typhimurium* and *S. aureus*.

The antibacterial effect of essential oils remains unchanged with changing the method used in determination of the antibacterial effect, but the only difference lies with oil concentration used. Where, similar trends were obtained when the same essential oils tested either by well diffusion assay or optical density assay. Complete inhibition of growth of all tested strains was obtained when treated with thyme at concentrations of 20 and 30 μL per well and 6-10 $\mu\text{L mL}^{-1}$, respectively. Also, ginger and coriander gave the same results with *Salmonella typhimurium*, *E. coli* and *S. aureus*. While, mustard showed the same trend with both *Salmonella typhimurium* and *E. coli*. Generally, inhibition growth of bacterial strains increased by increasing the concentration of essential oils. These data agreement with Ghaly (2006), who reported that thyme oil gave the maximum inhibitory action against all bacteria tested including, *E. coli* and *S. aureus* using well diffusion method and it was followed by marjoram oil. Additionally, this study indicated that optical density assay is a good method for determination of strength of antibacterial properties perhaps this is due to the direct attachment between the microorganism and the antibacterial. While, well diffusion assay is useful in screening for antibacterial activity of essential oils (Donaldson *et al.*, 2005). The high efficacy of optical density assay in determining the antibacterial effect of essential oils may be referred to the direct contact of essential oil molecules with the microbial cells rather than in diffusion through agar media.

It could be concluded that all tested essential oils exhibit high significant inhibition effect on both Gram positive and Gram negative strains used in this study, also there was a high significant differences between the concentrations of essential oils examined. Regardless, the oil concentration the same trend of growth inhibition of the examined essential oils was recorded using both methods. Therefore, they can be used as a potential source of active ingredients for food preservatives.

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