Egypt. J. Microbiol. **Vol. 56**, pp. 61-74 (2021)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Impact of Lemon Grass and Tea Tree Oils on Foodborne Pathogens and their Produced Spoilage Enzymes

Sofia M. Asem[(1)], Ali M. Saeed[(2)], Fawzy G. Khedre[(1)], Adel A. El Mahalawy[(2)], Samar S. Mohamed[(2)#]

[(1)]Department of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt; [(2)]Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt.

ESSENTIAL oils (EOs) are one of antimicrobial agents that naturally found in many plant's parts and have long been recognized for their medicinal properties. The efficacy of ten essential oils (Tea tree, Lavender, Eucalyptus, Rosemary, Lemon grass, Basil, Olbanum, Cumin, Onion and Cress) against food pathogens isolates and their enzymes have been evaluated. Lemon grass exhibited a potent inhibitory activity against all isolates and *Pseudomonas aeruginosa* was the most susceptible with MIC and MBC values of 3.12µL/mL and 6.25µL/mL, respectively. Tea tree oil was most effective against fungal isolates, *Aspergillus flavus* was the most susceptible with MIC value 1.56µL/mL and MFC value 3.12µL/mL. Transmission electron micrographs also confirmed that MIC concentrations of both oils caused many alternations in cell structure of both sensitive bacterial and fungal isolates. The most active antimicrobial fractions of Lemongrass and Tea tree oil were separated using TLC and analyzed by GC-MS as Citral and Terpinen-4-ol, respectively. These compounds exhibited a pronounced inhibitory effect on food spoilage enzymes (amylases, proteases, lactase, and lipases) with inhibition percentages ranged between 80 and 90%.

Keywords: Citral, Enzymatic activity, Essential oils, Foodborne pathogens, Terpinen-4-ol.

Introduction

Foodborne diseases are considered the most significant global public health troubles. Bacteria considered the main causes of food borne illness; *Escherichia. coli* infection, *Clostridium perfringen* gastroenteritis, Botulism, Salmonellosis and staphylococcal food poisoning are the major food illness caused by bacteria (Addis & Sisay, 2015). Yet, a great share of food-borne infections might be due to fungi or their byproducts, some molds contaminate or spoil food are known pathogens, such as *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, and mucormycetes (Tomsikova, 2002; Brenier-Pinchart et al., 2006; Pitt & Hocking, 2009) that can cause serious diseases specially in immunocompromised individuals .

Food spoilage, food poisoning and other food-related diseases have become a vital subject of worry among various food industries (Sokmen et al., 2004; Wu et al., 2009). Preservation approaches are useful to extend the shelf life, improve the hygienic quality, and confirm the safety of food. In food commerce, bacteriocins or other natural preservatives such as herbal extracts and essential oils are used as alternative to prevent the growth of both pathogenic and spoiling microorganisms (Martínez et al., 2019; Nazari et al., 2019).

Essential oils (EOs) comprise many nontoxic bioactive volatile compounds that have been revealed to be that are safe as food additives used in food industries (Bhavaniramya et al., 2019). Essential oils (EOs) are also against numerous microorganisms associated with several food products (Burt, 2004; Selim, 2010). In addition to their antiseptic, antibacterial, antiviral,

#Corresponding author email: samar_samer78@yahoo.com
Received 27/3/2021; Accepted 27/4/2021
DOI: 10.21608/ejm.2021.68718.1193

antioxidant, anti-parasitic, antifungal, and insecticidal activities (Kaloustian et al., 2008).

Different EOs have variable antimicrobial activity, this may be related to variation in their chemical compositions that change according to seasons, geographical location of plants and the methodology used in EOs extraction (Kokkini et al., 1997; García-Díez et al., 2016). The antimicrobial activity of essential oils may be attributed to their ability to penetrate bacterial membranes and inhibit functional, lipophilic properties of the cell (Burt, 2004; Trombetta et al., 2005; Calo et al., 2015) and destroy many bacterial enzymes such as ATPase, amylase, histidine carboxylase, and proteases (Thoroski 1989; Devi et al., 2010). They also have capability to penetrate through a permeabilization process and disturb the fungal cell wall and cytoplasmic membranes, which leads to the disintegration of mitochondrial membranes due to alterations in the flow of electrons inside the electron transport system (ETS) pathway. This also damages the lipids, proteins, and nucleic acid contents of cells infected by the fungal pathogens (Arnal-Schnebelen, 2004).

The aim of this study is to determine the antimicrobial activities of some essential oil and active compounds on food borne pathogens and their enzymatic activity in attempt to evaluate their efficiency as food preservative compounds.

Materials and Method

Sample collection

Seventy food samples were aseptically collected from different markets of health departments in Sharkia governorate, Egypt in the period from March 2016 to January 2017. The samples were kept cool in an icebox after collection and were transferred to the Microbiology Laboratory. Food samples are listed in Table 1.

Essential oils

Essential oils were purchased from sekem national company. They include *Eucalyptus globulus* (Eucalyptus), *Melaleuca alternifolia* (Tea tree), *Lavandula officinalis* (Lavender), *Cymbopogon citratus* (Lemongrass), *Allium cepa* L. (Onion), *Rosmarinus officinalis* (Rosemary), *Ocimum basilicum* L. (Basil), *Boswellia sacra* (Olbanum), *Cuminum cyminum* L. (Cumin) and *Lepidium sativum* L. (Cress) oils.

Isolation of foodborne microorganisms

Tenfold dilution for each food sample was carried out in sterile saline solution and peptone water, then 100µL from each dilution was sub-cultured on different media (nutrient agar, MacConkey, Brilliant green lactose Bile Broth 2% and Eosin Methylene Blue agar) and incubated at 37°C for 24-48hrs for isolation of total bacteria, total coliforms and faecal coliforms, respectively. While fungi were isolated on potato dextrose agar and incubated at 28°C for 4-5 days.

Identification of foodborne microorganisms

Bacterial isolates were identified according to Bergey's manual of systematic bacteriology (Krieg & Holt, 1984), this including growth on different selective media and biochemical tests. Fungal isolates were identified according to their macromorphological characteristics on different media and micromorphological criteria according to Samson (1981), Suhaib et al. (2011) and Afzal et al. (2013).

Antimicrobial activity of essential oils

Antimicrobial activity of collected essential oils against food boring microorganisms were examined by the disk diffusion assay according to Clinical and Laboratory Standards Institute (CLSI, 2012). A bacterial suspension of 0.5McFarland density standard at 530nm, resulting in a concentration of 1-5x10^6 CFU/mL (CLSI, 2012) and fungal cultures suspensions were prepared from fresh, mature (3 to 5 day old) cultures grown on potato dextrose agar slants. The colonies were covered with 5mL of sterile distilled water and, suspensions were obtained by comprehensive scraping of the surface with a sterile loop. The inoculum size was determined by spectrophotometer reading at a wavelength of 530nm to a transmittance level of 80 to 82%, as described in the NCCLS M38–A (NCCLS, 1997). Microbial suspensions were spread on the surface of Muller Hinton agar plate using sterilized cotton swabs. A hole with a 6-8mm diameter was then punched aseptically with a sterile cork borer and inoculated with a 200µL volume of each essential oil. The plates were then incubated at 37°C for 24hrs for bacteria and 48-72hrs for fungi. Ceftazidime and Amphotericin B at 10µg/mL were as standard positive control for bacteria and fungi, respectively (Fontenelle et al., 2007).

TABLE 1. Sources of collected food samples

Food		Source	No. of samples
Meat	Luncheon	Zagazig Health Administration	3
	Dumpling meat	Zagazig Health Administration	3
	Meat of cow	Belbeis Health Administration	4
	Heart meat	Belbeis Health Administration	1
	Sheep meat	Belbeis Health Administration	1
	Beef	Zagazig Health Administration	3
	Liver of cow	Belbeis Health Administration	1
	Cooked meat	Zagazig Health Aadministration	1
Poultry	- Chicken	Zagazig Health Administration	3
	- Cooked chiken	Kafr Saqr Health Administration	1
	- Liver of chiken	Kafr Saqr Health Administration	1
	- Hips of chicken	Zagazig Health Administration	1
	-Egg	Zagazig Health Administration	1
Dairy product	-Milk	Alqnayat Health Administration	3
	Yogurt	Tenth of Ramadan Health Administration	3
	Cheese	Tenth of Ramadan Health Administration	3
	-Butter	Alqnayat Health Administration	1
	- Vita cheese	Tenth of Ramadan Health Administration	1
	- Unpacked milk	Alqnayat Health Administration	1
Syrup	- Mango juice	Tenth of Ramadan Health Administration	1
	- Guava juice	Tenth of Ramadan Health Administration	2
Vegetables & fruits	-Watermelon	Zagazig Health Administration	1
	-Guava	Hehia Health Administration	1
	-Tomato	Zagazig Health Administration	2
	-Cucumber	Hehia Health Administration	2
	- Prepared eggplant	El-Salehia Health Administration	2
	- Okra	El-Salehia Health Administration	1
Grains	-Wheat	Alqnayat health administration	2
	- Rice	El-Salehia Health Administration	1
	-Cooked rice	Zagazig Health Administration	1
	-Packed beans	Zagazig Health Administration	2
Fish	- Fillet-fish	Zagazig Health Administration	2
	- Shrimp	Zagazig Health Administration	2
	- Tuna	Alqnayat Health Administration	1
	- Fish	Zagazig Health Administration	3
	- Salamon fish	Zagazig Health Administration	2
Bakery	-Bread	Zagazig Health Administration	3
	- Pancake	El-Salehia Health Administration	1
Total			70

Determination of MIC, MBC and MFC

Broth microdilution assay in Mueller-Hinton broth medium was used to determine the minimum inhibitory concentration (MIC) of the most potent essential oils against the most sensitive microorganisms (CLSI, 2012; NCCLS, 1997). Briefly, tubes containing 0.9mL of each concentrations used for each of the essential oil (100-1.25) µg/mL aqueous solution and those of standard antibacterial (125-7.8) µg/mL and antifungal drugs (25-1.56) µg/mL were prepared by serial dilution in Mueller-Hinton broth. The tubes were then inoculated with 100*µ*L of each of the bacterial or fungal suspensions and incubated as previous. Blank Mueller-Hinton broth was used as negative control. The MIC was defined as the lowest essential oil concentration, which prevented visible growth (Takahata et al., 1999). In order to determine The Minimum bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC), the tubes with no growth after 24hrs for bacteria and 4 days for fungi were sub-cultured on freshly prepared nutrient agar and sabouraud dextrose agar, respectively by the streaking method and incubated at proper temperature appropriately and then observed for growth. Petri dishes with not any growing bacterial or fungal cultures represent MBC and MFC, respectively (Cheesbrough, 2006).

Transmission electron microscopy

Transmission electron microscope (TEM) was used to examine the cells of *Pseudomonas aeuroginosa* and *Aspergillus terrues* (most sensitive species, after the treatment with 3.12µL/mL lemon grass oil and 1.56µL/mL of tea tree oil, respectively. TEM was carried out in the laboratory of electron microscope in National Research Centre (NRC), Dokki, Giza, Egypt using Joel-Jem-100 CX electron microscope.

Fractionation of active compounds of lemon grass and tea tree oils by thin layer chromatography

The TLC plates were prepared according to Stahl (1969). The Lemongrass oil and tea tree oil (most active essential oils) were loaded on TLC plate using hexane: ethylacetate (90:10, v:v) and n-hexane:acetone (90:10 v/v) as a developing system, respectively. The dried chromatograms were visualized by UV illuminations using 254nm and 366 nm for Lemongrass oil and tea tree oil, respectively. The plate was also stained by spraying anisaldehyde reagents (Savira et al., 2018; Tabanca et al., 2020). Retention factor (Rf) was calculated by the following equation:

Rf= Distance of active component/ distance of running solvent

Bands spots on TLC plate were scraped and eluted by shaking incubation with methanol for 12hrs, then the solvent was centrifuged at 10,000rpm for 5min. The supernatant was concentrated in a hot air oven at 45◦C for 48hrs (Sasidharan et al., 2011; Dhivya, 2017). The extracted fractions were dissolved in Dimethyl Sulfoxide (DMSO) and bio-assayed against both sensitive pathogens by well diffusion method (Fontenelle et al., 2007).

GC-MS analysis

Active fractions of both essential oils were analyzed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30m x 0.25mm x 0.25µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/min to 230°C hold for 2min. increased to the final temperature 290°C by 30°C/min and hold for 2min. The injector and MS transfer line temperatures were kept at 250, 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1mL/min. The solvent delay was 3min and diluted samples of 1µL were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Effect of tea tree, lemon grass oils and active compounds on enzymatic activity of most sensitive pathogens

Inhibitory effects of tea tree, lemongrass oils and active compounds on protease, lipase, and lactase produced by *Pseudomonas aeuroginosa* and *Aspergillus flavus* were evaluated. The amylatic activity was determined using Dinitrosalycalic acid method (DNS) (Miller, 1959). The proteolytic activity was assayed according to Tremacold & Carmonda (2005). Lactase enzyme activity was measured spectro-photometrically at 510nm (Bargmayer, 1985)

while lipase activity was evaluated using p-nitrophenol (pNP) method as described by Massadeh et al. (2011).

Statistical analysis

The results with three replicates were expressed as mean (±) standard deviation. Data was statistically analyzed using analysis of variance (ANOVA) procedure using MSTAT-C Statistical Software Package (Michigan State University, 1983).

Results

Isolated foodborne pathogens

The most common isolated foodborne pathogenic bacteria were *Escherichia coli, Salmonella* spp*., Pseudomonas aeruginosa* and *Staphylococcus aureus*. *E. coli* found to be the most frequent pathogen representing 30% of collected isolates followed by *Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa* and *Bacillus* sp. with percentages 22.8, 15.7, 12.8 and 10%, respectively. On the other hand*, Klebsiella* sp*.,* and *Neisseria* sp. were the less frequent pathogen within collected isolates (4.2%). Meat and poultry products were the most contaminated food source with foodborne pathogenic bacteria followed by dairy products, vegetables, fruit products, flour and bakery products. Whereas the least contaminated food source were canned products.

Concerning foodborne pathogenic fungi, the most common isolated fungi were *Aspergillus flavus* (32.8%)*, Aspergillus terreus* (20%), *Aspergillus niger* (17%) and *Aspergillus fumigatus* (10%). Vegetable and fruit products were the most contaminated food source with fungi followed by bakery products, dairy products, meat, and poultry products.

Antimicrobial activity of essential oils

Results of well diffusion assay revealed that most of tested essential oils possess antimicrobial activity against all isolated foodborne pathogenic bacteria and fungi except Olbanum and Cress which exhibit a weak inhibitory effect. Lemongrass exhibited a potent inhibitory activity against all isolates and *Pseudomonas aeruginosa* was the most susceptible (48mm) followed by *Bacillus* sp. (35mm). *Staphylococcus aureus* and *Salmonella* sp. were the most sensitive to tea tree oil with 27mm and 22mm inhibition zone diameter, respectively. Tea tree and lavender oils were very effective against *Aspergillus flavus, A. terreus* and *A. fumigatus* with inhibition zone diameters ranging from 60-40mm if compared with amphotericin B. It is apparent that lemon grass was the most potent inhibitory essential oil to bacterial isolates and tea tree oil was the most effective one against fungal isolates as shown in Table 2.

Determination of Minimum inhibitory concentration of the antimicrobial agent is a very important measure in evaluating its effectiveness. Lemongrass exhibited a great inhibitory effect against bacterial pathogens in approximately low concentrations especially against *Pseudomonas aeroginosa* (MIC 3.12 µL/mL and MBC 6.25µL/mL). Similarly, Tea tree oil exposed its potency against fungal pathogens in extremely low concentrations especially against *Aspergillus flavus* with MIC value 1.56µL/mL and MFC 3.12µL/mL (Table 3).

Transmission electron microscope

The mechanism of action of essential oils was analyzed by recording the changes in cell morphology and cell organelles of both tested pathogens using transmission electron microscope. Results revealed that, lemon grass oil affected greatly *P. aeruginosa* cell morphology, they seem to be irregular with abnormal distribution of the cytoplasm and the cell wall became denser, moreover, part of the cytoplasmic material released through formation of cracks comparing with control rods cells that appeared normal, showing a multilayered cell surface consisting of an outer membrane, a peptidoglycan layer in the periplasmic space and a cytoplasmic membrane and cytoplasm was relatively uniform and that the plasma membrane was continuous close contact with the cell wall. (Fig. 1). As for *Aspergillus flavus*, cell wall and plasma membrane of the non-treated cells were smooth, uniform, surrounded by intact fibrillar layer and all organelles are suspended in highly dense cytoplasm having normal appearance while after tea tree oil treatment, cells showed remarkable accumulation of osmophil bodies closely under the plasma membrane which became rough and irregular with continuous folding in the cytoplasm and detachment of fibrillar layer. The hyphal tips were also collapsed and disintegrated (Fig. 2).

TABLE 2. Inhibition zone diameter of ten tested essential oils on foodborne microbial isolates using well diffusion method

Microbial isolates	Inhibition zone diameter (mm)											
	Tea tree	Lavender	Eucalyptus	Rosemary	Lemon-grass	Basil	Onion	Olbanum	Cumin	Cress	Ceftazidime (30µg)	Amphotericin (20µg)
Bacillus sp.	14.0^{jk}±2.0	10.0 $^{m-o}$±1.0	10.0 $^{m-o}$±1.0	20.0^{fg}±1.0	35.0 ^{b}±4.0	15.0^{ij}±1.0	12.0^{km}±2.0	10.0^{mo}±1.0	8.0^{o}±2.0	10.0^{mo}±1	29.0^{a}±5	
E. coli	0.0^{p}±0.0	17.0^{hi}±2.0	12.0 $^{k-m}$±0.0	13.0 $^{j-l}$±1.0	13.0 $^{j-l}$±1.0	9.0^{no}±0.0	12.6 ^{jl}±1.0	0.0^{p}±0.0	10.0^{mo}±1.0	0.0^{p}±0.0	37.0^{de}±2	
Klebsiella sp.	0.0^{p}±0.0	12.0 $^{k-m}$±3.0	0.00^{p}±0.0	15.0 ^{ij}±3.0	18.0 ^{gh}±3.0	11.0^{l-n}±0.0	15.0^{ij}±2.0	0.0^{p}±0.0	10.0^{mo}±1.0	0.0^{p}±0.0	25.0^{e}±4	
Neisseria sp.	0.0^{p}±0.0	14.0^{jk}±1.0	10.0 $^{m-o}$±0.0	15.0^{ij}±1.0	22.0 ^{ef}±2.0	10.0^{mo}±2.0	24.0^{e}±3.0	0.0^{p}±0.0	8.0 ^{o}±1.0	0.0^{p}±0.0	20.0^{cd}±3	
Pseudomonas aeruginosa	11.0 $^{l-n}$±1.0	10.0 ^{mo}±1.0	14.0 ^{jk}±1.0	18.0^{gh}±1.0	48.0 ^{a}±4.0	11.0^{l-n}±0.0	2.0^{km}±1.0	0.0^{p}±0.0	0.0 ^{p}±0.0	0.0^{p}±0.0	40.0^{a}±2	
Staphylococcus aureus	27.0^{d}±2.0	10.0 $^{m-o}$±2.0	12.0 $^{k-m}$±1.0	12.0 ^{km}±0.0	14.0 ^{jk}±2.0	30.0^{c}±3.0	13.0 ^{jl}±0.0	0.0^{p}±0.0	8.0^{o}±2.0	0.0^{p}±0.0	27.0^{b}±1	
Salmonella enterica.	22.0^{ef}±1.0	10.0 $^{m-o}$±2.0	20.0^{fg}±3.0	14.0 ^{jk}±2.0	18.0 ^{gh}±3.0	12.0^{km}±3.0	10.0^{mo}±1.0	0.0 ^{p}±0.0	0.0 ^{p}±0.0	0.0^{p}±0.0	20.0^{bc}±2	
LSD at 0.05					**2.56**						5.25	
Aspergillus flavus	60.0 ^{b}±5.0	35.0 ^{d}±5.0	12.0 $^{l-n}$±0.0	20.0 ^{gh}±2.0	18.0 $^{h-j}$±2.0	15.0 $^{j-l}$±1.0	13.0 ^{kn}±1.0	12.0^{l-n}±2.0	0.0 ^{o}±0.0	0.0 ^{o}±0.0	63.0 ^{b}±3	
Aspergillus terrus	50.0^{a}±2.0	40.0 ^{c}±2.0	30.0^{ef}±4.0	35.0 ^{d}±1.0	20.0 ^{gh}±1.0	15.0 $^{j-l}$±2.0	15.0^{j-l}±1.0	0.0 ^{o}±0.0	0.0 ^{o}±0.0	0.0 ^{o}±0.0	70.0^{a}±5	
Aspergillus fumigatus	40.0^{c}±4.0	40.0 ^{c}±3.0	32.0 ^{de}±3.0	15.0 $^{j-l}$±2.0	20.0 ^{gh}±2.0	28.0^{f}±4.0	12.0^{l-n}±2.0	10.0^{n}±0.0	10.0^{n}±2.0	0.0^{o}±0.0	50.0^{c}±2	
Aspergillus niger	20.0 ^{gh}±0.0	20.0 ^{gh}±5.0	12.0^{l-n}±2.0	14.0 ^{km}±0.0	10.0^{n}±0.0	13.0 ^{kn}±0.0	12.0^{l-n}±3.0	10.0 ^{n}±0.0	10.0^{n}±3.0	0.0^{o}±0.0	31.0^{e}±3	
Rhizopus sp.	19.0 ^{hi}±2.0	10.0 ^{n}±0.0	15.0^{j-l}±1.0	16.0 $^{j-l}$±1.0	12.0 $^{l-n}$±3.0	12.0 $^{l-n}$±2.0	30.0 ^{ef}±6.0	0.0^{o}±0.0	10.0^{n}±1.0	0.0 ^{o}±0.0	45.0^{c}±5	
Fusarium sp.	16.0 $^{i-k}$±1.0	19.0 ^{hi}±2.0	10.0^{n}±2.0	12.0 $^{l-n}$±0.0	16.0 $^{i-k}$±1.0	12.0^{l-n}±1.0	1.0^{mn}±0.0	10.0^{n}±2.0	10.0 ^{n}±2.0	0.0^{o}±0.0	27.0^{e}±2	
Penicillium sp.	23.0^{g}±3.0	18.0 $^{h-j}$±1.0	15.0 $^{j-l}$±2.0	13.0 $^{k-n}$±3.0	21.0 ^{gh}±3.0	13.0^{kn}±2.0	20.0^{gh}±4.0	12.0^{l-n}±3.0	10.0^{n}±3.0	0.0^{o} ±0.0	38.0^{d}±4	
LSD at 0.05					**3.64**						**6.34**	

- Means having the same letter (s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.
- Values are means (±SD) of three replicates.

TABLE 3. MIC, MBC and MFC values of tested essential oils against foodborne pathogens

Isolates	Oil	MIC (µL/mL)	Positive control (µg\µL)	MBC/MFC (µL/mL)	Positive control (µg\µL)
Bacillus sp.	Lemon grass	6.25^{c}±0.25	31.25	12.5^{c}±1.50	62.5
Escherichia coli		50.0^{a}٦,٠±	31.25	100.0^{a}±2.0	62.5
Klebsiella sp.		25.0^{b}±3.0	31.25	50.0^{b}±2.0	31.25
Nesseria sp.		50.0^{a}١,٥±	31.25	100.0^{a}±3.0	62.5
Pseudomonas aeruginosa		3.12^{c}±0.12	31.25	6.25^{d}±1.25	31.25
Salmonella entrica		25.0^{b}±2.5	62.5	50.0^{b}±3.0	62.5
Staphylococcus areus		50.0^{a}±2.50	31.25	100.0a±6.0	62.5
LSD at 0.05 level		**4.15**		**5.55**	
Aspergillus flavus	Tea tree	1.56^{e}±0.06	6.5	3.12^{e}±0.12	12.5
Aspergillus fumigatus		12.50^{c}±0.50	6.5	25.00^{c}±3.0	12.5
Aspergillus terreus		6.25^{d}±0.25	6.5	12.50^{d}±0.50	12.5
Aspergillus niger		25.00^{b}±2.0	12.5	50.00 ^{b}±5.0	25
Penicillum sp.		12.50^{c}±0.50	6.5	25.00^{c}±1.0	12.5
LSD at 0.05 level		**3.01**		**6.08**	

- Means having the same letter (s) within the same column are not significantly different according to Duncan's multiple range test at 5% level of probability.
- Values are means (±SD) of three replicates.

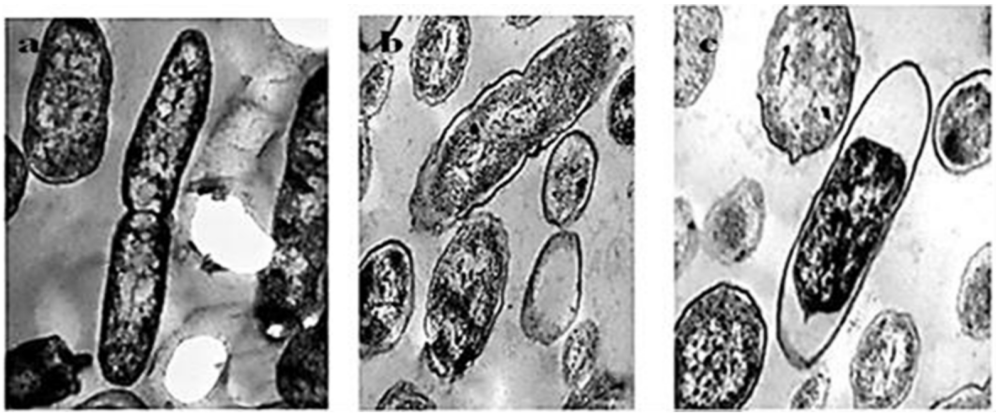

Fig. 1. TEM micrographs of *P. aeruginosa*: a, without oil treatment control and b & c, after sample treatment with 3.12µL/mL Lemongrass oil for 24hrs

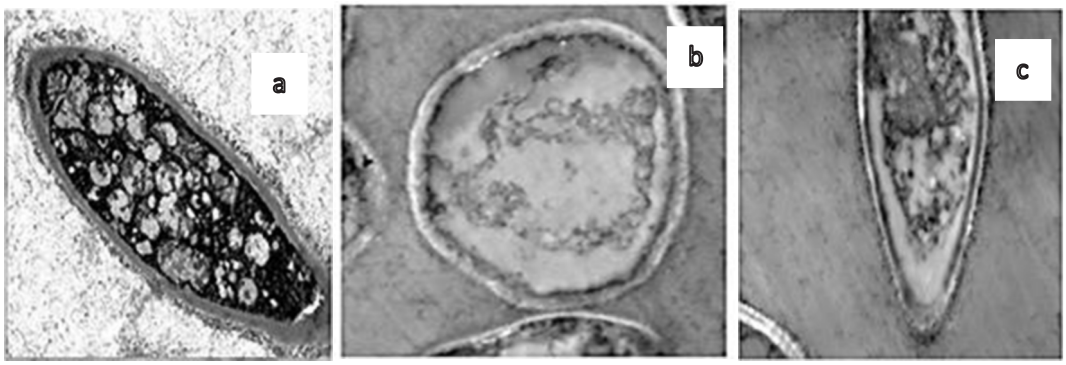

Fig. 2. TEM micrographs of *A. flavus*: a, without oil treatment control and b & c, after sample treatment with 1.56µL/mL of tea tree oil for 7 days

TLC and antimicrobial activity of fractions

Lemongrass and tea tree oils were purified and fractioned by TLC technique (Fig. 3). The active antimicrobial fractions of lemongrass and tea tree oils were determined. The highest antimicrobial activity of lemongrass oil against *Pseudomonas aeruginosa* was obtained with active fraction of Rf value 0.45 with inhibition zone 46mm. Whereas, tea tree oil exhibited the highest activity against *Aspergillus flavus* at Rf value 0.72 with 39mm inhibition zone diameter (Table 4).

GC–MASS analysis

The active fraction of lemongrass and tea tree oils were analyzed by GC-MS. The GC-MS chromatogram of lemon grass oil showed two high peaks at retention time of 12.9 and 13.8min which was corresponding to 22 and 34% relative intensity. It was identified according to its molecular mass to be neral and geranial, respectively.

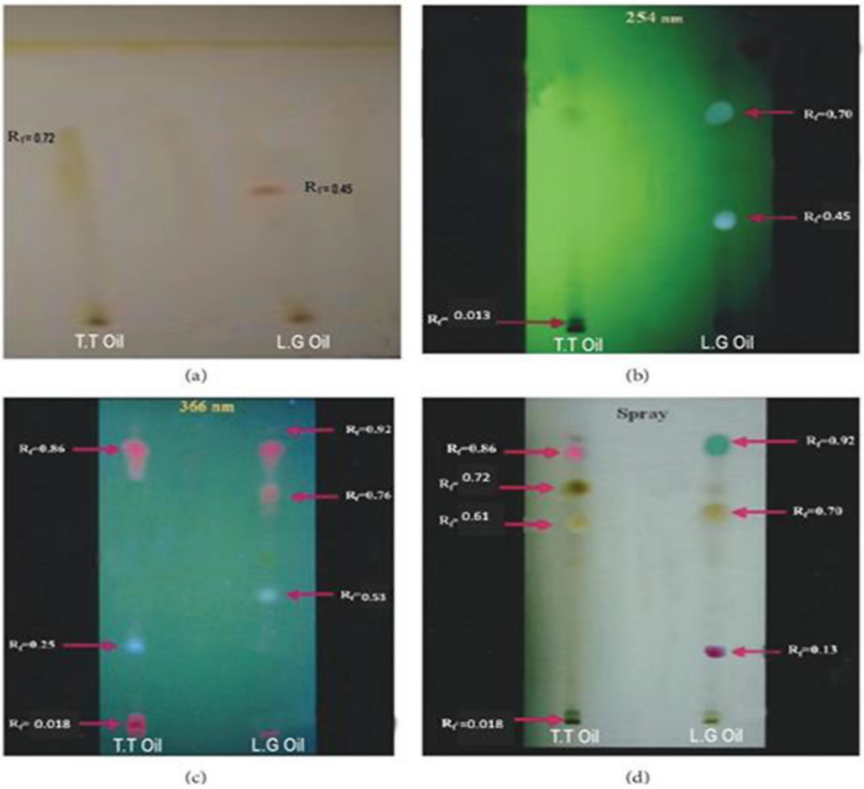

Fig. 3. TLC plate for lemongrass and tea tree oils, giving (a) dark yellow color spots for L.G oil and faint yellow color spots for T.T oil under visible light. (b) Dried plates chromatograms were visualized by UV illuminations at short wave length (254nm, CAMAG). (c) Dried plates chromatograms were visualized by UV illuminations at long wavelength (366nm, CAMAG). (d) colored bands appeared after spraying

TABLE 4. Antimicrobial activity of extracted fractions of lemongrass and tea tree oils against *Pseudomonas aeruginosa* and *Aspergillus flavus*, respectively

Bands	Lemongrass oil against *Pseudomonas aeruginosa*		Tea tree oil against *Aspergillus flavus*	
	R_f	Inhibtion zone diameter (mm)	R_f	Inhibtion zone diameter (mm)
Band 1	0.45	46	0.018	8.5
Band 2	0.13	10	0.25	13
Band 3	0.53	18	0.61	18
Band 4	0.70	5	0.72	39
Band 5	0.76	7.7	0.86	7.7
Band 6	0.92	9	-	-

Citral ($C_{11}H_{16}O$), 3,7-dimethyl-2,6-octadienal, is a monoterpene aldehyde mixture of geranial, cis-isomers, and neral, trans-isomers, with 152.23 g/mol molecular mass. While tea tree oil active fraction showed high peaks at retention time of 7.25min which was corresponding to 15.21% relative intensity. It was identified according to its molecular mass 154.2493 to be Terpinen-4-ol ($C_{10}H_{18}O$), 4-methyl-1-propan-2-ylcyclohex-3-en-1-ol. (Fig. 4).

Effect of essential oils and active compounds on enzymatic activity of Pseudomonas aeruginosa and Aspergillus flavus

The inhibitory effect of (lemongrass oil and citral) & (tea tree oil and Terpinen 4-ol) on production of some food degrading enzymes by *Pseudomonas aeruginosa* and *Aspergillus flavus,* respectively were assessed. It was found as shown in Fig. 5 that lemongrass and tea tree oils possess powerful inhibitory effect on production of proteinases, amylase, lipase and lactase enzymes with inhibition percentages among 80 and 90%. This inhibitory effect was noticeably increased after treatment with active compounds separately.

Discussion

Plentiful cases of foodborne illnesses and chronic complications are reported every year in many countries (Heredia & García, 2018). Most of the foodborne pathogens are abundant in nature with high opportunity of cross contamination between one or several products during processing (Linscott, 2011). Meat and poultry products were the most contaminated food source with foodborne pathogenic bacteria this is because meat have high amounts of proteins, essential amino acids, B complex vitamins and minerals that considered advantageous environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, processing, storage, and distribution at slaughterhouses (Gill, 1998). *E.coli* was found to be the most frequent pathogen followed by *Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa* and *Bacillus sp.* High coliform and *E. coli* counts are usually accompanying with significant levels of enteric pathogens and confirm hygiene-related problems and contamination of fecal origin (Jay, 2005).

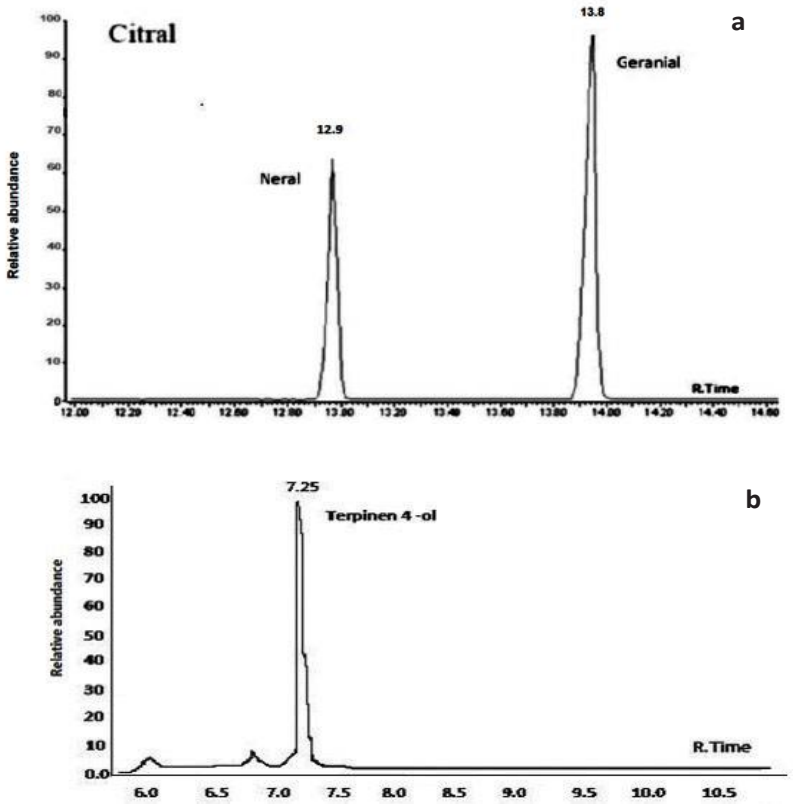

Fig. 4. The GC-MS chromatogram of the antimicrobial active fraction of lemongrass oil (a) and tea tree oil (b)

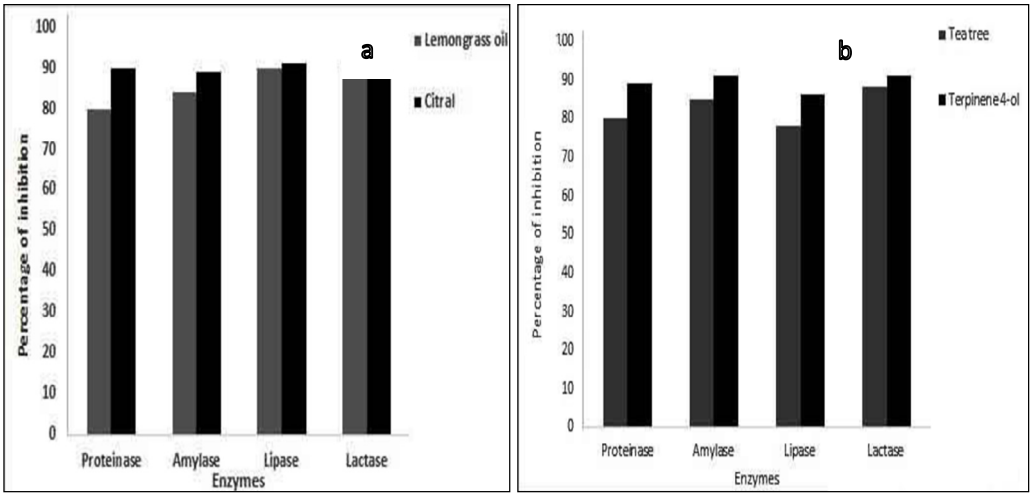

Fig. 5. Inhibitory effect of lemongrass and citral on enzymatic activity of *Pseudomonas aeruginosa* (a), inhibitory effect of tea tree oil and Terpinen 4-ol on enzymatic activity of *Aspergillus flavus* (b)

Vegetable and fruit products were the most contaminated food with fungal pathogens, this may be due to the low pH, especially of fruits that gives fungi a competitive advantage over the majority of bacteria, these molds produce mycotoxin which are the main cause of spoilage, especially in products that are refrigerated in open boxes (Giampieri et al., 2012; Mailafia et al., 2017). These metabolities are heat stable recommends that they remain in the food after heat processing and continue to cause toxicity. *Aspergillus flavus* was the most frequent food borne fungal pathogen. *Aspergillus nig*er was the most common species in Brazil from 117 samples investigating toxigenic fungi on dried fruits (Iamanaka et al., 2005). Also, in Lebanon a high level of contamination of grapes with various strains of *Aspergillus* was observed by El Khoury et al. (2006).

Food preservatives added to the food to increase the shelf life of food products by preventing fungal and bacterial contamination. Because, some of these additives have been associated to human health problems (Vally et al., 2009; Weitzberg & Lundberg, 2013), many researchers are focused recently on the exploration and development of new natural or 'green' preservative substances for making food safe. One of such possibility is the use of essential oils as antimicrobial additives (Gassara et al., 2016). In this study, lemongrass and tea tree oils were very effective against isolated food borne pathogens specially against *streptococcus aureus* and *Aspergillus flavus*, respectively. This is attributed to hydrophobicity of their chemical constituents which enables them to partition with the lipids present in the cell membrane of microbial cell and mitochondria, rendering them more permeable by disturbing the cell structures (Devi et al., 2010). According to results of transmission electron microscope, the antimicrobial activity of the lemongrass and tea tree oils against *pseudomonas aeruginosa* and *Aspergillus flavus*, respectively was found to provoke morphological changes this was explained by several reports which observed that the bioactive components present in EOs might attach to the surface of the cell, and subsequently penetrate to the phospholipid bilayer of the cell membrane and accumulate and this leading to disturbance of the structural integrity of cell membrane, disturb the cellular membrane and influence the cell metabolism by reacting with active sites of enzyme or act as an H+ carrier depleting ATP pool and hence causing cell death (Bajpai et al., 2013; Lv et al., 2011; Utlee et al., 2002).

Fractionation of lemon grass and tea tree oils by TLC and GC mass identification of active fractions revealed that Citral, composed of geranial and neral, and Terpinen-4-ol were the active fractions of the two oils, respectively. These active compounds belonging to Phenylpropenes terpenins that are known by their powerful antimicrobial activity (Silva et al., 2015; Aleksic & Knezevic, 2014; Semeniuc & Rotar, 2017).

Microbial enzymes are one of the most important targets in control approaches of food

borne pathogens, some of these enzymes are heat stable and thus they can escape from food preservation procedures and remain in food causing their degradation and spoilage. In this study, Citral and Terpinen 4-ol exhibited a great inhibitory effect on amylases, proteases, lipases and lactase activities of both sensitive bacterial and fungal pathogens suggesting that these compounds exerts their inhibitory activities through not only their effect on microbial cell constituents but also enzymatic activities of these pathogens. These suggestions agree with Marei & Abdelgalei (2018) who found that *trans*-cinnamaldehyde, *p*-cymene and eugenol caused potent inhibition of pectin methyl esterase and cellulase produced by eight plant pathogenic fungal species. Similarly Zahani & Khaledi (2018) concluded that a part of the inhibitory effect of essential oil of *Syzygium aromaticum* against *Penicillium digitatum* is associated to the indirect influence on their infection process by affecting the activity of cell wall degrading enzymes which are produced by the fungal pathogens and associated with virulence such as cellulase and pectinase, in addition to the direct effect on the pathogens growth.

Conclusion

Lemongrass and tea tree oils and their active compounds, citral and terpenin-4-ol, poses a great inhibitory effect on microbial food borne pathogens in low concentrations. They also have massive adverse effects on microbial cell growth and production of food degrading enzymes by these microbes in addition they are safe as food additives, so these active compounds are considered a good choice for preservation of food and food products.

References

Addis, M., Sisay, D. (2015) A review on major food borne bacterial illnesses. *Journal of Tropical Diseases,* **3**(4), 1000176. DOI: 10.4176/2329-891X.1000176.

Afzal, Hina, Shahzad, S., Qamar, Syeda, Nisa, Un (2013) Morphological identification of *Aspergillus* species from the soil of larkana district (Sindh, Pakistan). *Asian Journal of Agriculture and Biology*, **1**(3), 105-117.

Aleksic, V., Knezevic, P. (2014) Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. *Microbiological Research,* **169**(4), 240–254.

Arnal-Schnebelen, Hadji-Minaglou F., Peroteau, J.F., Ribeyre, F., Billerbeck, V.G.D. (2004) Essential oils in infectious gynaecological disease; a statistical study of 658 cases. I*nternational Journal of Aromatherapy*, **14**(4), 192–197.

Bajpai, V.K., Sharma, A., Baek, K.H. (2013) Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food borne pathogens. *Food Control*, **32**, 582–590.

Bargmayer H.U. (1985) "*Method of Enzymatic Analysis*", 3rd ed., Vol. 2 (Verlag Chemie, 1985, pp. 208-216, 227-230.

Bhavaniramya, S., Vishnupriya, S., Saleh Al-Aboody, M., Vijayakumar, R., Baskaran, D. (2019) Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain & Oil Science and Technology*, **2**, 49–55.

Brenier-Pinchart, M.P., Faure, O., Garban, F., Fricker-Hidalgo, H., Mallaret, M.R., Trens, A., Lebeau, B., Pelloux, H., Grillot, R. (2006) Ten-year surveillance of fungal contamination of food within a protected haematological unit. *Mycoses*, **49**, 421–425.

Burt, S. (2004) Essential oils and their antibacterial properties and potential applications in foods - A review. *International Journal of Food Microbiology,* **94**, 223-253.

Calo, J., Crandal, J.R., O'Bryan, C.A., Ricke, S.C. (2015) Essential oils as antimicrobials in food systems- A review. *Food Control*. **54**, 111-119.

Cheesbrough, M. (2006) "*District Laboratory Practice in Tropical Countries*"; 1st edn., Cambridge University Press, Cambridge, UK., ISBN-10: 0521665450, 434p.

CLSI (2012) "*Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard*", 7th ed., CLSI document M02-A11; Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA.

Devi, K.P., Nisha, S.A., Sakthivel, R., Pandian, S.K.

(2010) Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. *Journal of Ethnopharmacology,* **130**(1), 107–115.

Dhivya, K.K.S. (2017) Screening of phytoconstituents, UV-VIS spectrum and FTIR analysis of *Micrococca mercurialis* (L.) benth. *International Journal of Herbal Medicine*, **5**, 40–44.

El Khoury, A., Rizk, T., Lteif, R., Azouri, H., Delia, M.L., Lebrihi, A. (2006) Occurrence of ochratoxin A and aflatoxin B1-producing fungi in Lebanese grapes and ochratoxin A content in musts and finished wines during 2004. *Journal of Agricultural and Food Chemistry,* **54**(23), 8977-8982.

Fontenelle, R.O.S., Morais, S.M., Brito, E.H.S., Kerntopf, M.R., Brilhante, R.S.N., Cordeiro, R.A., Tomé, A.R., Queiroz, M.G.R. (2007) Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. *Journal of Antimicrobial Chemotherapy*, **59**, 934–940.

García-Díez, J., Alheiro, J., Falco, V., Fraqueza, M.J., Patarata, L. (2016) Chemical characterization and antimicrobial properties of herbs and spices essential oils against pathogens and spoilage bacteria associated to dry-cured meat products. *Journal of Essential Oil Research*, **29**, 117-125.

Gassara, F., Kouassi, A.P., Brar, S.K., Belkacemi, K. (2016) Green alternatives to nitrates and nitrites in meat-based products– A review. *Critical Reviews in Food Science and Nutrition*, **56**, 2133–2148.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Quiles, J.L., Mezzetti, B., Battino, M. (2012) The strawberry, composition, nutritional quality, and impact on human health. *Nutrition*, **28**(1), 9–19.

Gill, C.O. (1998) Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: "*The Microbiology of Meat and Poultry*", Davies, A., Board, R. (Eds.), pp. 118-157. London: Blackie Academic and Professional, 1998.

Heredia, N., García, S. (2018) Animals as sources of food-borne pathogens: A review. *Animal Nutrition,* **4**, 250–255.

Jay, J.M., Loessner, M.J., Golden, D.A. (2005) Indicators of food microbial quality and safety. In: "*Modern Food Microbiology*", Berkely: Springer (Eds.), pp. 387-409.

Kaloustian, J., Chevalier, J., Mikail, C., Martino, M., Abou, L., Vergnes, M.F. (2008) Etude de six huiles essentielles: Composition chimique et activité antibactérienne. *Phytothérapie*, **6**, 160–164.

Kokkini, S., Karausou, R., Dardioti, A., Keigas, N., Lanaras, T. (1997) Autumn essential oils of Greek oregano. *Phytochemistry*, **44**, 883-886.

Krieg, N.R., Holt, J.G. (1984) "*Bergey's Manual of Systematic Bacteriology*", Vol. 1, Williams & Wilkins Co., Baltimore, pp. 161- 172.

Iamanaka, B.T., Taniwaki, M.H., Menezes, H.C., Vicente, E., Fungaro, M.H. (2005) Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. *Food Additives and Contaminants,* **22**(12), 1258-1263.

Linscott, A.J. (2011) Foodborne illnesses. *Clinical Microbiology Newsletter*, **33**(6), 41-46.

Lv, F., Liang, H., Yuan, Q., Li, C. (2011) *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food related microorganisms. *Food Research International*, **44**, 3057–3064.

Mailafia, S., Okoh, G.R., Olabode, H.O.K., Osanupin, R. (2017) Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Veterinary World,* **10**(4), 393–397.

Marei, G.I.Kh., Abdelgalei, S.A.M. (2018) Antifungal potential and biochemical effects of monoterpenes and phenylpropenes on plant pathogenic fungi. *Plant Protection Science*, **54**, 9–16.

Martínez, B., García, P., Rodríguez, A. (2019) Swapping the roles of bacteriocins and bacteriophages in food biotechnology. *Current Opinion in Biotechnology,* **56**, 1–6.

Massadeh, M.I., Sabra, F.M. (2011) Production and characterization of lipase from *Bacillus stearothermophilus*. *African Journal of Biotechnology,* **10**(61), 13139-13146, 10 October, 2011.

Michigan State University (1983) MSTAT-C Micro

Computer Statistical Program, Version 2. Michigan State University, East Lansing.

Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry,* **31**(3), 426–428.

National Committee for Clinical Laboratory Standards (NCCLS) (1997) Reference method for broth dilution antifungal susceptibility testing for yeasts; approved standard Document M27-A. Villanova, National Committee for Clinical Laboratory Standards; v. 17, pp. 1-29.

Nazari, M., Ghanbarzadeh, B., Kafil, H.S., Zeinali, M., Hamishehkar, H. (2019) Garlic essential oil nanophytosomes as a natural food preservative; its application in yogurt as food model. *Colloid and Interface Science Communications*, **30**, 100176. doi: 10.1016/j.colcom.2019.100176.

Pitt J.I. and Hocking A.D. (2009) "*Fungi and Food Spoilage*"; 3. New York; Springer.

Tabanca, N., Niogret, J., Kendra, P.E., Epsky, Nancy D. (2020) TLC-based bioassay to isolate Kairomones from Tea tree essential oil that attract male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann). *Biomolecules*, **10**, 683. doi:10.3390/biom10050683.

Samson, R., Hoekstra, E., Oorschot, C. (1981) Chapter1, "*Introduction to Food-Born Fungi*, Delft-Holland; pp.1- 200.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M., Yoga Latha, L. (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, **8**(1), pp. 1–10.

Savira, A., Syahrani, A., Rudyanto, M. (2018) A Simple Method for Isolation of Citral using Column Chromatography. *Proceedings of BROMO Conference*, pp. 12-20; DOI: 10.5220/0008356900120020

Selim, S. (2010) Antimicrobial activity of essential oils against Vancomycin-Resistant Enterococci (VRE) and *Escherichia coli* O157:H7 in Feta soft cheese and minced beef meat. *Brazilian Journal of Microbiology*, **42**, 187-196.

Semeniuc, C.A., Pop, C.R., Rotar, A.M. (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *Journal of Food and Drug Analysis*, **25**, 403-408.

Silva-Angulo, A.B., Zanini, S.F., Rosenthal, A., Rodrigo, D., Klein, G., Martínez, A. (2015) Comparative study of the effects of citral on the growth and injury of *Listeria innocua* and *Listeria monocytogenes* cells. *PLoS ONE,* **10**, 0114026.

Sokmen, A., Gulluce, M., Akpulat, H.A., Daferera, D., Tepe, B., Polissiou, M., Sokmen, M., Sahin, F. (2004) The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius. Food Control,* **15**, 627–634.

Stahl, E. (1969) TLC in thin layer chromatography. "*A Laboratory Handbook*", 2nd ed.; Springer-Verlag, New York.

Suhaib, A., Kamili, Azra N., Bashir, A.G. (2011) Identification of some Penicillium species by traditional approach of morphological observation and culture. *African Journal of Micro- Biology Research,* **5**(21), 3493- 3496.

Takahata, M., Mitsuyama, J., Yamashiro, Y. (1999) "*In vitro* and *in vivo* antimicrobial activities of T-3811ME, a novel des-F (6)-quinolone. *Antimicrobial Agents and Chemotherapy*, **43**(5), 1077–1084.

Tomsikova, A. (2002) Risk of fungal infection from foods, particularly in immunocompromised patients. *Epidemiologie, Mikrobiologie, Imunologie*, **51**, 78–81.

Thoroski, J. (1989) Eugenol induced inhibition of extracellular enzyme production by *Bacillus cereus*. *Journal of Food Protection*, **52**, 399–403.

Tremacoldi, C.R., Carmona, E.C. (2005) Production of extracellular alkaline proteases by *Aspergillus clavatus*. *World Journal of Microbiology & Biotechnology,* **21**, pp. 169–172.

Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., Bisignano, G. (2005) Mechanisms of antibacterial action of three monoterpenes. *Antimicrobial Agents and Chemotherapy*, **49**, 2474-2478.

Ultee, A., Bennik, M.H., Moezelaar, R. (2002) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology,* **68**, 1561–1568.

Vally, H., Misso, N.L., Madan, V. (2009) Clinical effects of sulphite additives. *Clinical and Experimental Allergy,* **39**, 1643–1651.

Weitzberg, E., Lundberg, J.O. (2013) Novel aspects of dietary nitrate and human health. *Annual Review of Nutrition*, **33**, 129–159.

Wu, V.C.H., Qiu, X., Delos-Reyes, B.G., Lin, C.S., Pan, Y. (2009) Application of cranberry concentrate (Vaccinium macrocarpon) to control *Escherichia coli* O157:H7 in ground beef and its antimicrobial mechanism related to the down regulated slp, hdeA and cfa. *Food Microbiology,* **26**, 32–38.

Zahani, F.H., Khaledi, N. (2018) Biological effects of various essential oils on citrus decay pathogens. *International Journal of New Technology and Research (IJNTR)*, **4**(4), 129-139.

تأثير عشب الليمون وزيوت شجرة الشاي على مسببات الأمراض المنقولة بالغذاء وإنزيمات التلف الناتجة عنها

صوفيا مصطفي عاصم البسيوني[1]، علي محمدعلي سعيد[2]، فوزي جمال خضر[1] ،عادل أحمد رمضان المحلاوى[2]، سمر سمير محمد[2]

[1]: قسم النبات- كلية العلوم- جامعة الزقازيق- الزقازيق - مصر، [2]قسم الميكروبيولوجى- كلية العلوم- جامعة عين شمس- القاهرة– مصر.

الزيوت الأساسية هي أحد العوامل المضادة للميكروبات التي توجد بشكل طبيعي في العديد من أجزاء النبات وقد تم التعرف عليها منذ فترة طويلة لخصائصها الطبية. تم تقييم فعالية عشرة زيوت أساسية (شجرة الشاي، اللافندر، الكافور، إكليل الجبل، عشب الليمون، الريحان، البلبانوم، الكمون، البصل، حب الرشاد) ضد مسببات الأمراض الغذائية المعزولة وإنزيماتها. أظهر عشب الليمون نشاطًا مثبطًا قويًا ضد جميع العزلات وكانت البسودوموناس ايروجينوزا هي البكتريا الأكثر حساسية بقيم MIC و MBC 3.12 ميكرولتر/ مل و 6.25 ميكرولتر/ مل على التوالي. اظهر زيت شجرة الشاي أكثر فاعلية ضد العزلات الفطرية، وكان اسبرجلس فلافس الفطر الأكثر حساسية بقيمةMIC 1.56ميكرولتر/ مل وقيمة MFC. 3.12ميكرولتر/ مل. أكدت الصور المجهرية لميكروسكوب الإلكتروني الناقل أيضًا أن تركيزات MIC لكلا الزيتين تسببت في العديد من التغييرات في بنية الخلية لكل من العزلات البكتيرية والفطرية الحساسة. تم فصل الأجزاء المضادة للميكروبات الأكثر نشاطًا في زيت عشب الليمون وزيت شجرة الشاي باستخدام TLC وتحليلها بواسطة GC-MS وتم تعريفها إلى Citral و Terpinen-4-ol، على التوالي. أظهرت هذه المركبات تأثيرًا مثبطًا واضحًا على إنزيمات تلف الطعام (الأميليز، البروتياز، اللاكتاز، والليباز) مع نسب تثبيط تراوحت بين 80 و 90%.