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Screening for antibacterial activity of some essential oils and evaluation of their synergistic effect

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Abstract

Biological properties associated to many medicinal and aromatic plants have recently gained a great scientific interest. In this study, seven essential oils of famous plants reputed with their therapeutic vertus were screened in order to underline their limit antibacterial spectrum separately then establishing the correlated effect in their combination (antagonism, synergy). The antibacterial potential of seven essential oils was screened against ten Gram negative bacteria; *E. coli* (ATCCC 25922), *Ps. aeruginosa* (ATCC 27853), *Acinetobacter sp, Klebsiella pneumonaie* ESBL, *Klebsiella oxytoca*, *Enterobacter sp*, *E. coli* ESBL, *Proteus sp*, *Morganella morganii*, *Pseudomonas aeruginosa* MBL, *Serratia sp* and three strains of Gram-positive bacteria; *S. aureus* (ATCC 25923), *Streptococcus sp*, *Staphylococcus aureus* MRSA using disk diffusion method on agar medium (Mueller-Hinton agar), the strains were inoculated by swabbing technique and a volume of 25µl of each oil was tested. Synergy and antagonist effects were studied to evaluate single and binary combined antibacterial activities against reference strains (*S. aureus, E.coli and Ps. aeruginosa*).Results revealed a varing antibacterial activities against the examined pathogens according to the oil where ginger oil presented the highest activity and its larger inhibition zone (28.43 mm) toward the multidrug-resistant pathogen E. coli ESBL, while juniper, lavender and tarragon oils were the less effective ones. Maximum activity of the tested essential oils was obtained from the combination of Ginger and rosemary essential oils against *Staphylococcus aureus* (36,36 mm) and *E coli* (34, 53 mm).In conclusion, the tested essential oils exhibited a very intense antibacterial potential toward the Gram-positive and the Gram negative bacteria even the drug-resistant ones.

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Introduction

Essential oils (Eos) are mixtures of compounds obtained from spices, aromatic herbs, fruits, flowers and characterized by their aroma. The antimicrobial properties of essential oils have been known for a long time, and various researches have been conducted into their antimicrobial activities using various bacteria and fungi. Considering the increased pathogen resistance, investigations into the antimicrobial activities, mode of action and potential uses of essential oils and their components have gained a new impulse (Mazumder *et al.*, 2014). Wide spread of antibiotic resistance remains a serious clinical problem, which stimulates studies for search of new methods for coping with drug resistance or renews interest in traditionally used and forgotten methods, such as treatment with antibacterial plant extracts and essential oils. Combined therapy is traditionally used to increase antimicrobial activity and reduce toxic effects of agents (Kateryna and Mahendra,2012). Plant secondary metabolites and essential oils can be used as an alternative remedy for the treatment of many infectious diseases (Hemali *et al.*, 2015), their application in controlling pathogens could reduce the risk of food borne outbreaks and assure consumers safe food products. Some plants and extracts used as flavoring agents are known to possess antimicrobial activity offering a potential alternative to synthetic preservatives (György, 2010).

Many studies have been carried out to extract various natural products for screening antimicrobial activity but attention has not been focused intensively on studying the combinations of these products for their antimicrobial activity (Raho Ghalem and Benali, 2008).

The aim of this study was to investigate the antibacterial activity of seven essential oils alone and in combination with ginger essential oil against some pathogenic Gram positive and Gram-negative bacteria. Furthermore, the assessment of binary combinations of the EOs against tested microorganisms was performed to detect synergic, antagonist or additive effects.

Materials and methods

Essential oils

Seven essential oils (EOs) obtained from Arko essential (commercial producers of plant essential oils and aromatic substances- France) were used in this study (Table 1). The selection of these oils was based on literature survey and their therapeutic proprieties in traditional medicine.

Bacterial strains

For the antibacterial activity of each EO, a total of thirteen bacteria was tested where ten were Gram-negative ones; *E. coli* (ATCCC25922), *Ps. aeruginosa* (ATCC 27853), *Acinetobacter sp*, *Klebsiella pneumonaie* ESBL, *Klebsiella oxytoca*, *Enterobacter sp*, *E. coli* ESBL, *Proteus sp*, *Morganella morganii*, *Pseudomonas aeruginosa* MBL, *Serratia sp* and three Gram-positive strains; *S. aureus* (ATCC 25923), *Streptococcus sp*, and *Staphylococcus aureus* MRSA. These pathogens were isolated from clinical specimens and have been identified as multidrug resistant bacteria. They are provided from the Microbiology laboratory, Anti-Cancer Center, Batna, Algeria.

For the synergy activity of Eos mixture, we tested; *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922).

Antibacterial activity

In order to evaluate the antibacterial efficiency of the selected essential oils, we use the disk diffusion method (Dobre, *et al.,* 2011). Bacterial suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab to get a uniform microbial growth on both control and test plates. Under aseptic conditions, empty sterilized discs (Whatman no. 5, 6 mm diameter) were impregnated with 25 µl of essential oils. The Petri dishes were left for 30 min at room temperature (20-22°C) for better oil diffusion and then incubated at 37 °C. After incubation for 24 hours, the inhibition zone diameters were measured and documented. (Mahboubi and Farzin,2009).

Testing synergy of essential oils and ginger oil

For this purpose, we prepared blends of EOs in sterile Eppendorf tubes by mixing 100µl of ginger oil with 100µl of correspondent second oil. Paper disks were then impregnated with 25µl of Eos mixture and the same protocol above was applied. After incubation, the results are read and formulated in accordance the size of the inhibition zone.

Statistical analysis

All the experimental results were performed in triplicate and the results were expressed as means ± SD. Comparison of groups was performed by analysis of the ANOVA variance; using Graph Pad Prism 5.03. The differences detected were considered significant when $p<0.05$.

Results

Antibacterial activity

The seven aromatic medicinal plants investigated in this work (*Juniperus communis, Eucalyptus globulus, Artemisia dracunculus L, Citrus limon, Lavandula angustifolia, Rosmarinus officinalis L* and *Zingiber officinale*) have wide use in traditional medicine, for treatment of infections, respiratory and gastric diseases, and other health problems (Table 1).

Table 1. The selected essential oils and their properties.

Common name	Botanical name (Family)	Traditional Use	[ref]
Juniper	*Juniperus communis*	spice, antiseptic, digestive;	Selim (2011)
Eucalyptus	*Eucalyptus globulus*	antiseptic, anti-infectious, respiratory decongestant;	Kesbi (2011)
Tarragon	*Artemisia dracunculus* L	antibacterial, anti-fermentative, antiallergic;	György (2010)
Lemon	*Citrus limon*	anti-infectious, antinauseant, anxiolytic;	Ferhat *et al.* (2016)
Lavender	*Lavandula angustifolia*	analgesic, antidepressant, cardio-tonic;	Chemloul (2014)
Rosemary	*Rosmarinus officinalis L.*	antibacterial, anticancer, hypoglycemic;	Sharma (2016)
Ginger	*Zingiber officinale*	antimicrobial, antioxidant, anticancer, stimulation of the immune system.	Hassan *et al.* (2017)

The antibacterial activity of essential oils is summarized in Table2. Results revealed that the seven selected essential oils showed antibacterial activity with varying magnitudes. An inhibition diameter above 8 mm was taken as positive result (Duraffourd *et al.*, 1990).

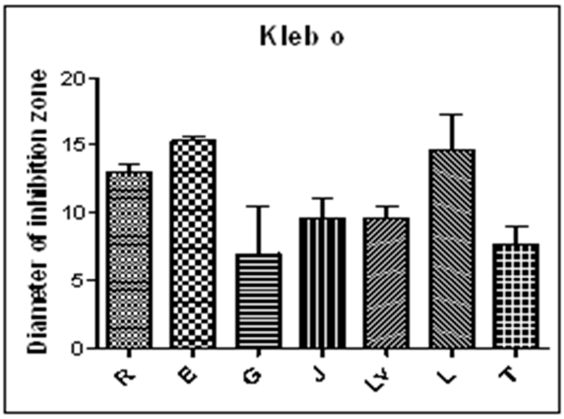

Fig. 1. Antibacterial activity of Eos against *Klebsiella oxytoca*.

A marked bacterial inhibition of the tested Eos was reported where the ginger oil presented the highest activity.

It was more effective on Gram negative bacteria especially *E. coli* ESBL, *Acinetobacter sp*, *Morganella moganii*, *Serratia sp* and *Proteus sp* with an inhibition zone of 28.43mm, 27.03 mm, 24.33mm, 21.33mm and 18,43 mm respectively.

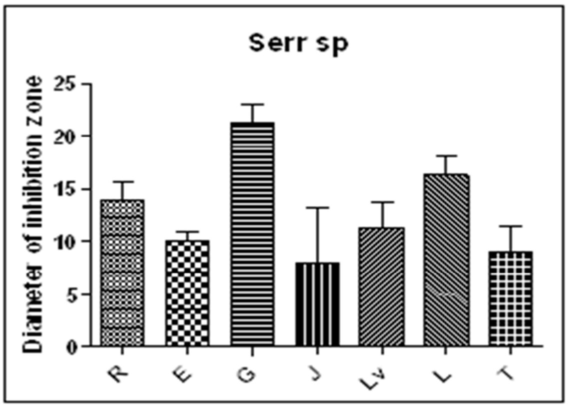

Fig. 2. Antibacterial activity of Eos against *Serratia sp*.

Lemon citrus essential oil showed the strong activity against *E coli*. However, Gram negative *Enterobacter sp* and S aureus MRSA were resistant (2, 34 mm and 8.94 mm respectively).

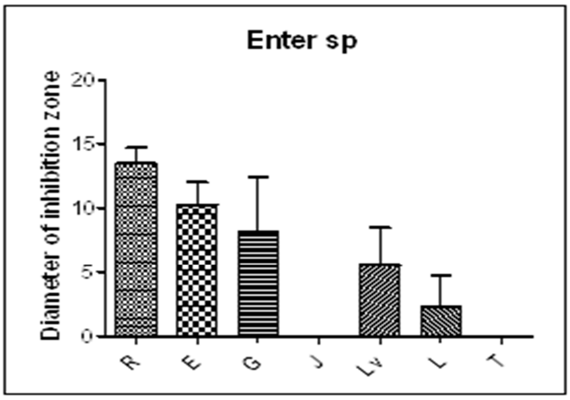

Fig.3. Antibacterial activity of Eos against *Enterobacter sp*.

Rosmarinus officinalis was more effective towards Gram negative bacteria. *Proteus sp* was the more resistant bacterium (9.65 mm) while *E. coli* the most sensitive one (22.32 mm).

Eucalyptus globulus essential oil used in our study inhibited moderately all tested bacteria. *Ps. Aeruginosa* MBL showed a strong resistance (4,24 mm). Eucalyptus oil exhibited a highest activity against *Klebsiella oxytoca* with an inhibition diameter of 15,33 mm.

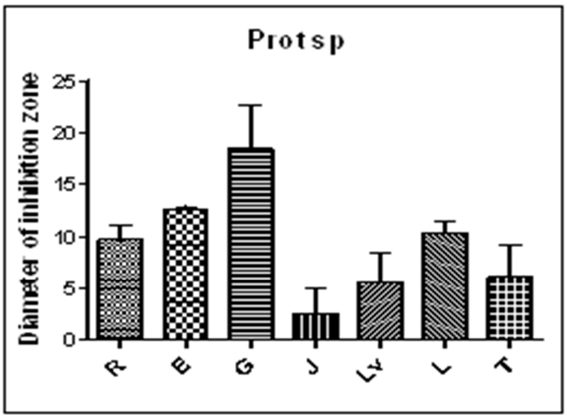

Fig. 4. Antibacterial activity of Eos against *Proteus sp*.

Comparatively, Juniper, Lavender and Tarragon oils were the less effective against all tested bacteria.

Testing synergy of essential oils and ginger oil

In determining synergism and antagonism, combinations of 50% of the chosen essential oils solutions were used. Synergism is registered when the activity of the combined substances is higher than the sum of the individual activities. In contrast, the antagonistic effect is registered when the activity of components in combination is inferior in comparison when they are applied separately. An additive effect is observed when the combined effect is equal to the sum of the individual effects (Faleiro, 2011).

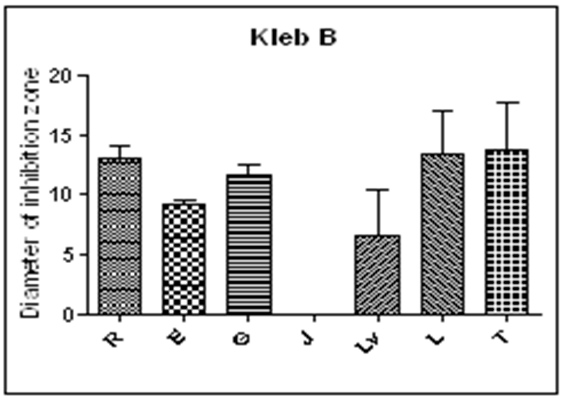

Fig. 5. Antibacterial activity of Eos against *Klebsiella pneumoniae* EBSL.

In the present research, binary combination of the essential oils exhibited some synergic and antagonist effects against examined microorganisms (Table 3).The strongest synergic activity was related to the combination of ginger and rosemary EOs against *S.*

aureus ATCC(36,36 mm) and *E. coli* ATCC (34,53 mm) and finally by the blend of ginger and lavender EOs against *Ps. aeruginosa* ATCC(14,49 mm).

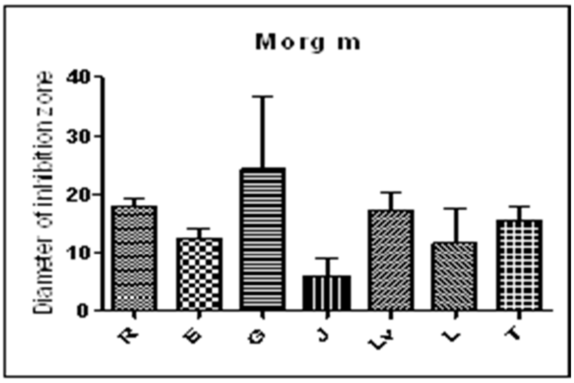

Fig.6. Antibacterial activity of Eos against *Morganella morganii.*

Surprisingly, some mixture of essential oils exhibited synergic effect on tested bacteria whereas none of these essential oils showed antibacterial activity alone. Effectively, all the EOs mixture except that of ginger and lemon showed a synergy effect on *Ps. aeruginosa, S. aureus* and *E. coli*. The combination of ginger-rosemary showed the highest synergetic effect followed by that of ginger-juniper on the studied bacteria.

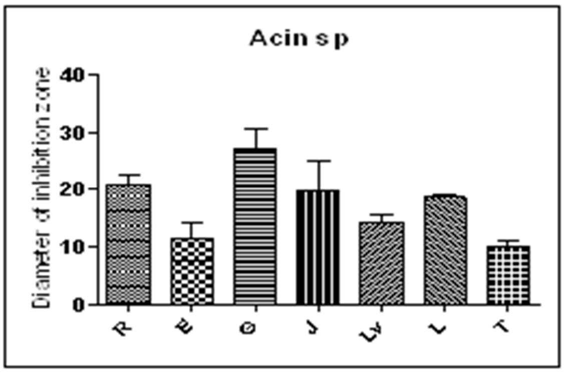

Fig.7. Antibacterial activity of Eos against *Acinetobacter sp.*

The antagonist effect was observed for mixture of ginger-tarragon for *S. aureus*, ginger-eucalyptus for *E. coli* while the additive effect was observed in a single case of ginger-lavender on *S. aureus*.

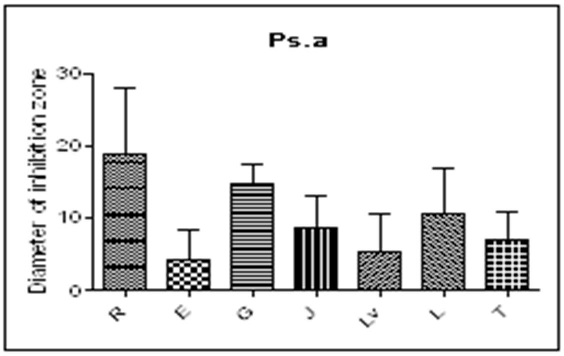

Fig. 8. Antibacterial activity of Eos against *Ps. aeruginosa* MBL.

Discussion

Antibacterial activity

Sharma *et al.* (2016) reported that microorganisms were more sensitive to ginger EO than Tetracycline and Fluconazole used as positive control. The high activity of ginger essential oil may be due to the presence of phenolic compounds as it is well known that the major pungent compounds of ginger are gingerone and gingerol which have strong inhibitory activity against pathogenic bacteria. However, antimicrobial activity (bioactivity) of essential oils was dependent not only on the major components but also on the chemical structures of these components (Sharma *et al.*, 2016).

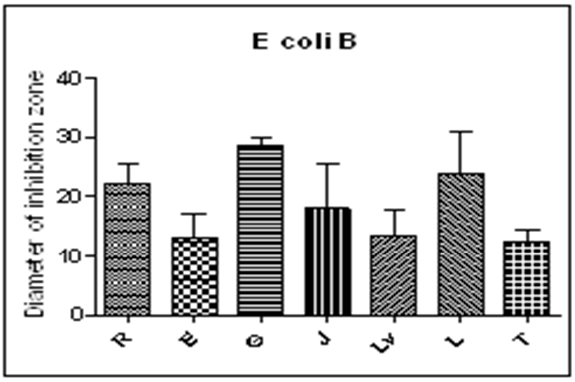

Fig. 9. Antibacterial activity of Eos against *E. coli.*

Our findings differ from those of Nader *et al.* (2009) which reported effectiveness of ginger EO on Gram positive bacteria. However, Hassan *et al.* (2017) demonstrated that the Gram negative bacterium *Pseudomonas aeruginosa* was more sensitive than *S. aureus* and *Bacillus subtilis* to ginger oil. Previous studies indicated that, *E. coli*, *Campylobacter coli* and *C. jejuni* are not inhibited by ginger oil probably since the cell membranes of Gram negative bacteria and fungi are more complex than those of Gram positive bacteria and yeasts (Sa-Nguanpuag *et al.,* 2011). *S. aureus* showed a weak sensitivity to ginger oil which is in agreement with Ionica *et al.* (2016), the inhibition growth zones were much smaller than Gram negative bacteria except for *Enterobacter sp* which was the most resistant strain.

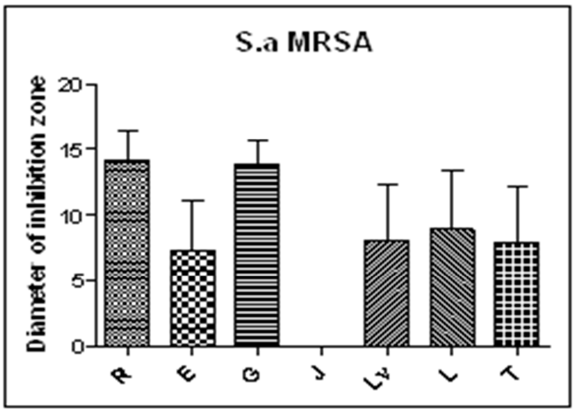

Fig. 10. Antibacterial activity of Eos against *S. aureus* MRSA.

For *Lemon* EO, our results did not agree with those of Elumalai *et al.* (2010) for *K. oxytoca* with > 90.0 mm but it was the same finding for *Enterobacter* strain. The same investigation reported a strong effectiveness of this oil against *S. aureus* but our extract seemed to be inactive on *S. aureus*.

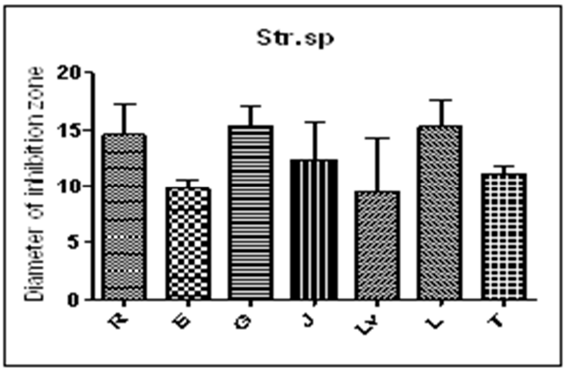

Fig.11. Antibacterial activity of Eos against *Streptococcus sp.*

Frouhat and Lahcini (2013) reported for *Rosmarinu* soil: *E.coli* (12 mm) was effectively the most sensitive bacterium same as our study followed by *S. aureus* (9 mm) and *Streptococcus thermophilus* (8 mm).

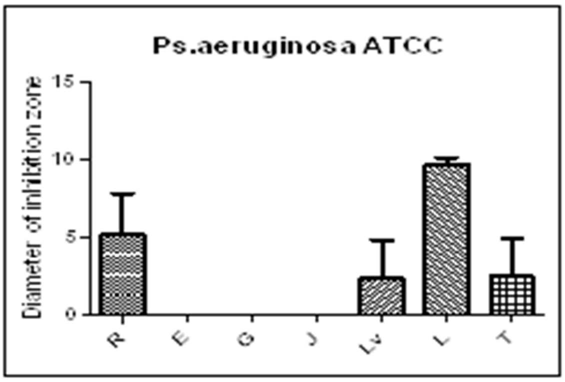

Fig. 12. Antibacterial activity of Eos against *Ps. Aeruginosa* ATCC (27853).

Mota *et al.* (2015) exhibited the same result for *Eucalyptus* EO but Kesbi (2011) reported the effectiveness of this oil on *Ps. aeruginosa* in comparison with the other rested organisms.

The low efficiency of Eos is probably due to the losses of the volatile compounds of essential oil during storage and / or extraction and the evaporation of the volatile components during the incubation period, which would reduce the EOs concentration, and thus their antibacterial activity (Chemloul, 2014). Generally, essential oils are poorly soluble in water, which induced many problems to study their antibacterial activity, as reported by Kezzouna (2015). Angioni *et al.* (2003) reported that the antimicrobial activity of *Juniperus communis* oils was generally non-significant while Glisic*et al.* (2007) showed a low antimicrobial activity to all the investigated species which is in agreement with our results. *Acinetobacter sp* was the most susceptible bacterium (19.92 mm) while *Enterobacter sp*, *Klebsiella sp* ESBL and *Staphylococcus aureus* MRSA were not inhibited by *Juniperus communis* EOs.

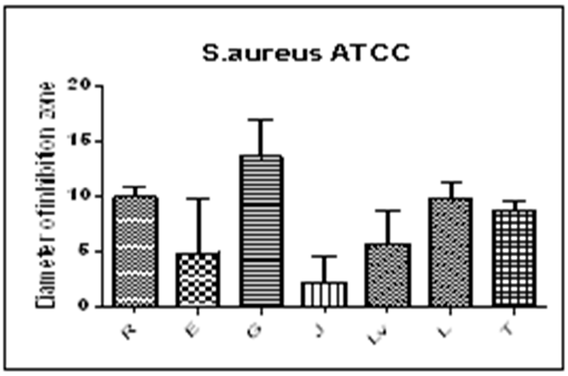

Fig. 13. Antibacterial activity of Eos against *S. aureus* (25923).

In the study of Chemloul (2014), *Lavandula officinalis* did not show a high activity against tested bacteria where *S. aureus* was the most sensitive followed by *E. coli*. Our result showed the predominance of *M. morganii* followed by *E. coli.*

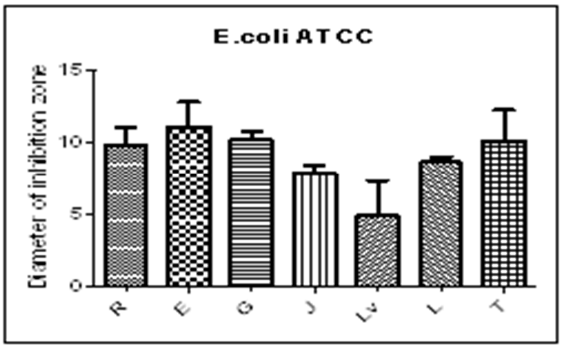

Fig. 14. Antibacterial activity of Eos against *E. coli* ATCC (25922).

According to our study, *Artemisia dracunculus* essential oil exhibited a lower antibacterial effect, in comparison with the other oils. Kordali *et al.* (2005) showed no activity on *Enterobacter cloacae*, *Enterobacter intermedius*, *Escherichia coli* and *Staphylococcus aureus* ATCC 29213. *Enterobacter sp* was totally inhibited, *S. aureus* was resistant (7.98 mm) and *E. coli* presented a weak inhibition diameter (12.47 mm).

From these results, it can be seen that ginger oil was the most effective oil against all tested bacteria followed by rosemary and lemon essential oils. In the other hand, juniper Eo was the weakest one. *Enterobacter sp, K pneumoniae, S. aureus, Proteus sp, Ps. aeruginosa* and finally *K. oxytoca* were the most resistant bacteria to all the tested essential oils. Many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites (György, 2010).

Plant essential oils have been shown to be active against several bacteria. The extent of the sensitivity of a test organism varies with the studied strain, the type of chemical constituents present in the essential oil, the imposed environmental conditions, and the structural differences in the cell membrane compositions of Gram-positive and Gram-negative bacteria (Swamy *et al.,*2016).

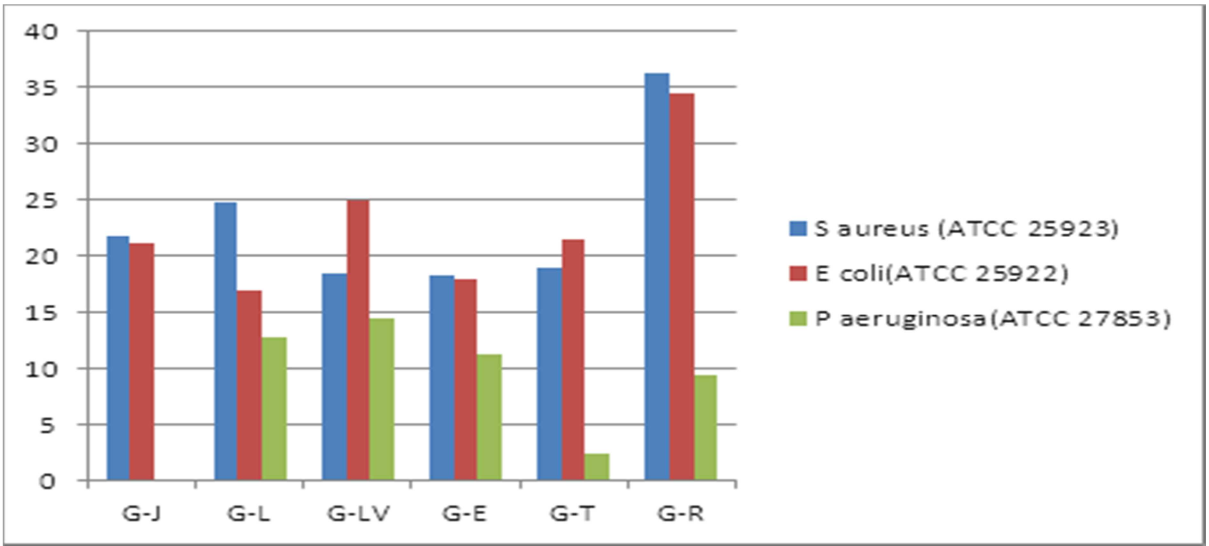

Fig. 15. Antibacterial effect of the EOs combinations.

Testing synergy of essential oils and ginger oil

Pseudomonas aeruginosa ATCC was the most resistant bacterium for the combination of EOs which is in consisting with Fahimi *et al.* (2015).

According to our results, ginger-rosemary combination showed the highest synergic effect; however, Bassolé and Juliani (2012) reported that only the combination with rosemary oil yielded synergistic effects.

In contrast to our finding, Padalia *et al.* (2015) reported that lavender oil combinations with others EOs showed 26.7% synergistic effect and 48.9% additive effect.

There are some accepted mechanisms of antimicrobial interaction that produce synergism: the inhibition of a common biochemical pathway, inactivation of microbial enzyme, leaking of cell membrane and increasing the membrane permeability (Padalia *et al.*,2015). Mechanisms of antimicrobial interaction that produce antagonism are less known, although they include combinations of bactericidal and bacteriostatic agents, use of compounds that act on the same target of the microorganism and chemical interactions (direct or indirect) among compounds (Fahimi *et al.*, 2015).

Conclusion

The present study enabled us to evaluate and compare the antibacterial potential of *Juniperus communis, Eucalyptus globulus, Artemisia dracunculus* L., *Citrus limon, Lavandula angustifolia, Rosmarinus officinalis* L., *Zingiber officinale* essential oils individually and in binary combinations with ginger oil against some pathogenic bacteria. The ginger-rosemary mixture has proved to be the most effective one. It was reported that the combination of some particular oils presented synergy resulting of the combined activities of two or more constituents of essential oils. As pathogens cannot easily acquire resistance to multiple components of two or more essential oils, therefore, essential oils can be used as strong antimicrobial agents and raise industrial interest in naturally produced preservatives.

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