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The effect of some herbal essential oils on pathogenic bacteria

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Abstract

Nowadays, many antibiotics are being used to control infectious diseases. Inappropriate use of antibiotics leads to emergence of resistant bacteria and problems such as a prolonged course of treatment. Medicinal plants and their derivatives, as a good source of treatment, are effective against drug resistance. In this study we have evaluated the antimicrobial activity of herbal essential oils of four medicinal plant *Cuminum cyminum*, *Satureja hotensis* L, *Citrus limon* and *Mentha piperita*. For preparation of herbal essential oils clevenger apparatus was used, and the active components of the herbal essential oils were determined by GC-MS. In order to observe the effects of the essential oils, they were examined on *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* using disc diffusion method in vitro. Also, the Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC) of each essential oil was measured and compared with commonly used antibiotics. Obtained results showed that all of the essential oils possess inhibitory and antibacterial effects, but the *Mentha piperita* essential oil showed a better effect in comparison to other used essential oils. It was concluded that all the herbal species demonstrate antibacterial properties, but the level of bacterial growth inhibition induced by plant materials, shown to be dependent on herbal source and bacterial strain.

Introduction

Infectious diseases are the cause of early death, and they make thousands of people die all around the world (Beg & Ahmad., 2000). Various antibiotics are being used to control these diseases. Using the antibiotics for a long time can reveal bacteria which are resistant to some drugs, it causes some clinical challenges in treatment of the infectious diseases (Singh et al., 2002). Hence, investigation on discovering new antimicrobial components, from some sources like plants, is important. Certainly, using medicinal plants is the oldest way to treat the disease, and there has been a close relation between human and the plants through the human civilizations. Considering the drug resistance and side effects of chemical antimicrobial drugs, the trends toward using natural resources in recent decades, and antimicrobial effects of the plants are being investigated (Gavanji et al., 2014). The plants, through their primary metabolism, produce many compositions with different molecular structures, some of which possess antimicrobial property (Souza et al., 2005). Secondary metabolites are stored as passive pre-existing structures in plant tissues and they are released in response to environmental stresses. These structures include some compositions such as phenolic compositions, flavonic compositions, flavonoids, glycosides, alkaloids, and polyacetilene (Han et al., 2000; Lanciotti et al., 2004; Leitner., 2000). The compositions, due to their inhibitory effects toward microorganism, are in the center of attention (Lanciotti et al., 2004).

Today, efforts are being made for discovering and producing of those herbal components possessed antimicrobial property. For a long period of time herbal extracts have been used for treatment of some diseases. *Cuminum cyminum* is an aromatic plant in Apiaceae family. The plant is used for tasting the foods, making the perfumes, and medical applications. Its fruits are yellow to brown, and

crescent like (Jazani et al., 2008). *Cuminum cyminum* is used for treatment of emphysema, cough and pulmonary diseases, and it facilitates the process of digestion (De et al., 2003). *Satureja hotensis* L is a member of Labiatae family; its aerial organs are used in traditional medicine for treatment of gastric pains, qualm, indigestion, squirt and infectious diseases (Güllüce et al., 2003). There are significant similarities between chemical compositions of extract of *Satureja hotensis* L and *Thymus vulgaris* extract. In *Satureja hotensis* L, like *Thymus vulgaris*, the most important compositions are monoterpenoid group, and compositions Thymol and carvacrol are the two typical compositions (Sefidkon et al., 2005). *Citrus limon* is a member of Rutaceae family; its fruit is used for treatment of many infections in traditional medicine (Burt., 2004; Rodrigues et al., 2000). Another plant is *Mentha piperita*, which is in Lamiaceae family. This plant is used in traditional medicine, and it possesses high amounts of menthol and tannin (Sivropoulou et al., 1996). The aim of the present study is to examine the effect of herbal extracts of *C. cyminum*, *S. hotensis* L, *C. limon* and *M. piperita* on some pathogenic bacteria, and to compare it with the effect of common antibiotics.

Materials and Methods

- **Plant materials**

Fresh aerial parts of herbs including *C. cyminum*, *S. hotensis* L, *C. Limon* and *M. piperita* were collected from the Lorestan, Hormozgan, and Chaharmahal provinces (Iran) in 2012. The herbs were dried at room temperature for 3 days. The dried herb samples (500 g) were ground and subjected to hydro distillation using a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4 and stored at 4 °C in a sealed amber vial until use.

- **Oil analysis procedure**

Analysis was performed using GC-MS with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 m). The carrier gas was helium at a flow rate of 0.8 ml/min. The column temperature was kept at 50 °C for 2 minutes, and it was programmed to 200 °C at a rate of 3°C /min and kept constant at 200 °C for 10 minutes. The injection was performed in split mode with a ratio of 50:1 at 250 °C. The compounds were identified by comparison of RRI (Relative Retention Indices) with those reported in the literature, and also by comparison of their mass spectra with published mass spectra (Adams., 2005; Sparkman., 1997). The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standards (Van Den Dool & Kratz, 1963).

- **Microorganisms**

For bacterial strains, the species are as follows: *S. aureus* (PTCC 1112), *E. coli* (PTCC 1074) and *S. typhi* (PTCC 1639) which were prepared from Traditional Medicine Institute of Isfahan (Iran). To prepare fresh culture and activation of microbial strains, micro-agar Macconkey for *E. coli*, as well as Brain-Hart Agar (BHA) for *S. typhi*, and Nutrient agar for *S. aureus* were used. Also to carry out tests in all stages (MBC, disk diffusion method and MIC), medium Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) were used which is manufactured by MAST company in England.

Antibacterial assay

- **Disc diffusion method**

This method is the most common form of antimicrobial substance evaluation and it is known as Kirby-Bauer test (Gavanji et al., 2014). In order to perform this test moler hinton agar was added to a sterile Petri dish (5mm thickness), and the bacteria samples was pick up by applicator from the basic culture medium and was inoculated to culture. All these steps took place in asptic conditions to prevent contamination of the medium by the bacteria in the environment. Then the prepared disks, containing concentrations 0.08 up to 100 µgml-1 of herbal essential oils, was placed in culture medium by sterile pence in certain intervals of each other. Also the standard of antibiotic Gentamicin and Amikacin disks were used as positive control for comparison with the mentioned essential oils.

Finally, the inoculated Petri dishes were placed in incubator at temperature 37°C for 24 h, and after 24h the diameter of the no growth zones formed around the discs were measured using a caliper.

- **Evaluation of essential oils antimicrobial activity using Broth Dilution Susceptibility method**

Dilution method was used to determine the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericide Concentration (MBC). In order to determine the MIC, the suspensions of bacterial strain (cultured in 12h) were prepared and compared with turbidity equal to 0.5 Mac Farland. Essence was prepared in the 6 dilutions and, added to Micro plates containing the liquid culture medium. Few Micro plates containing Gentamicin, and Amikacin, antibiotic with concentrations of 0.001 to 0.04 µg/µl were used as positive control, then 96 wells Micro plate was shaken for 20 minutes, and placed in an incubator at a temperature of 37 °C for 24 h. MIC was determined (after the mentioned period of time) according to both the viewing micro plates turbidity, and changes occurred to liquid culture medium

turbidity. In fact, the first micro plate without turbidity was considered as the MIC and measurement of the MBC essential oils, and antibiotic was determined according to the MIC results. 5 microliter of the well, in which bacterial growth was completely stopped, was added to the plates containing culture medium Muller Hinton Agar (MHA), and was kept at the temperature of the incubator for 24 h. No growth bacterial concentrations were reported as the MBC values (Celiktas et al., 2007). For determination of MIC, the suspensions of bacterial stain were cultured in 12h and compared with turbidity equal to 0.5 Mac Farland. 6 concentrations of essential oil were prepared, and they were added to microwells comprising culture medium. Some of the microwells were filled with Gentamicin and Amikacin (concentrations from 0.001 to 0.04).

Results

Data were analyzed using SPSS version 20 by ANOVA method, and performed averages compared with Tukey method, the effect of different concentrations of *S. hotensis* L were statistically surveyed on *S. aureus*, *E. coli* and *S. typhi* at 24h, 48h and 72h. The results showed that 100 µg/µl has more efficiency than lower concentrations in each 3 bacteria ($P < 0.0001$). In this concentration, the extract of *S. hotensis* L possessed the highest inhibitory effect on *E. coli*, and it showed the lowest effect on *S. typhi* (Table 1).

Table 1. Anti-bacterial activity of different concentrations of *S. hotensis* L herbal essence against three bacteria using disk diffusion method (zone of inhibition)

	Staphylococcus aureus			*Escherichia coli*			*Salmunella typhi*		
S. hotensis (µg/ml)	Mean±SE			Mean±SE			Mean±SE		
	24	48	72	24	48	72	24	48	72
0.08	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.16	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.31	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.63	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1.25	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2.5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.23±0.14^{a}	0.27±0.14^{a}	0.27±0.14^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	0.43±0.07^{a}	0.57±0.14^{ab}	0.57±0.14^{ab}	1.20±0.20^{a}	1.57±0.30^{a}	1.70±0.25^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
10	1.40±0.21^{a}	1.83±0.12^{b}	1.83±0.12^{b}	3.00±0.35^{b}	3.50±0.29^{b}	3.60±0.30^{b}	0.40±0.21^{a}	0.40±0.21^{a}	0.40±0.21^{a}
20	3.40±0.21^{b}	4.00±0.17^{c}	4.20±0.11^{c}	4.40±0.40^{b}	5.03±0.58^{b}	5.57±0.70^{b}	2.70±0.43^{b}	4.00±0.35^{b}	4.10±0.32^{b}
40	4.40±0.38^{b}	5.17±0.22^{c}	5.47±0.32^{c}	6.93±0.54^{c}	7.33±0.33^{c}	7.50±0.29^{c}	5.53±0.37^{c}	7.33±0.40^{c}	7.50±0.29^{c}
60	6.37±0.88^{c}	7.37±0.35^{d}	7.50±0.40^{d}	10.77±0.38^{d}	11.67±0.33^{d}	11.97±0.32^{d}	7.10±0.40^{c}	8.53±0.87^{c}	8.77±0.75^{c}
80	10.97±0.32^{d}	12.00±0.58^{e}	12.27±0.72^{e}	13.80±0.53^{e}	14.63±0.58^{e}	14.83±0.50^{d}	10.30±0.81^{d}	12.43±0.52^{d}	12.60±0.50^{d}
100	15.07±0.73^{e}	16.00±0.58^{f}	16.27±0.82^{f}	16.70±0.51^{f}	19.13±0.59^{f}	19.33±0.67^{f}	13.00±0.36^{e}	15.27±0.37^{e}	15.50±0.45^{e}

Different letters on every column represent meaningful difference ($p<0.0001$)

The effects of different concentrations of *M. piperita* were statistically surveyed on *S. aureus*, *E. coli* and *S. typhi* at 24h, 48h and 72h. The results showed that the concentrations of 80 µg/µl and 100 µg/µl have more efficiency than lower concentrations on *S. aureus* and *S. typhi* in the first 24h. But over the time, 100 µg/µl showed a better performance on *S. typhi* than 80 µg/µl ($p<0.0001$). Generally, two concentrations of 80 and 100 µg/µl of *M. piperita* extract were respectively more effective on *E. coli* and *S. aureus* (Table 2).

Table 2. Anti-bacterial activity of different concentrations of *M. piperita* herbal essence against three bacteria using disk diffusion method (zone of inhibition)

	Staphylococcus aureus			*Escherichia coli*			*Salmunella typhi*		
M. piperita	Mean±SE			Mean±SE			Mean±SE		
(µg/ml)	24	48	72	24	48	72	24	48	72
0.08	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.16	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.31	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.63	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.57±0.07^{a}	0.67±0.09^{a}	0.67±0.09^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1.25	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.50±0.29^{a}	1.60±0.30^{a}	1.77±0.23^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2.5	0.53±0.07^{a}	0.53±0.07^{a}	0.53±0.07^{a}	4.27±0.18^{b}	4.87±0.13^{b}	5.03±0.09^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	2.77±0.23^{b}	2.83±0.17^{b}	2.97±0.14^{b}	6.90±0.38^{c}	7.17±0.60^{c}	7.50±0.50^{d}	0.40±0.06^{a}	0.73±0.09^{a}	0.73±0.09^{a}
10	7.73±0.27^{c}	7.83±0.33^{c}	8.23±0.40^{c}	9.30±0.62^{d}	10.00±0.58^{d}	10.07±0.52^{e}	2.10±0.06^{b}	3.93±0.35^{b}	4.30±0.35^{b}
20	10.17±0.44^{d}	10.70±0.15^{d}	10.70±0.15^{d}	13.03±0.37^{e}	13.83±0.44^{e}	13.97±0.43^{f}	6.27±0.38^{c}	8.53±0.37^{c}	8.73±0.40^{c}
40	11.90±0.46^{e}	12.90±0.38^{e}	13.13±0.35^{e}	16.00±0.26^{f}	16.33±0.33^{f}	16.60±0.30^{g}	9.37±0.63^{d}	12.37±0.58^{d}	12.50±0.50^{d}
60	13.97±0.58^{f}	15.23±0.37^{f}	15.33±0.42^{f}	18.67±0.60^{g}	19.10±0.58^{g}	19.53±0.57^{h}	11.83±1.17^{e}	15.17±0.44^{e}	15.20±0.42^{e}
80	19.23±0.50^{g}	19.60±0.46^{g}	19.23±0.50^{g}	23.07±0.58^{h}	23.93±0.47^{h}	24.43±0.47^{i}	15.13±0.18^{f}	18.10±0.49^{f}	18.47±0.32^{f}
100	19.90±0.58^{g}	20.33±0.88^{g}	20.60±0.78^{g}	26.20±0.30^{i}	26.90±0.10^{i}	27.07±0.12^{j}	16.37±0.48^{f}	20.63±0.58^{g}	20.83±0.61^{g}

Different letters on every column represent meaningful difference ($p<0.0001$).

The effect of different concentrations of *C. cyminum* extract on 3 species of bacteria at 24, 48 and 72h, showed that the extract has a weak inhibitory effect ($p<0.0001$), but its highest effect was on *E. coli*, and the lowest was observed on *S. aureus* and *S. typhi* (Table 3).

Table 3. Anti-bacterial activity of different concentrations of *C. cyminum* herbal essence against three bacteria using disk

	Staphylococcus aureus			*Escherichia coli*			*Salmunella typhi*		
C. cyminum (µg/ml)	Mean±SE			Mean±SE			Mean±SE		
	24	48	72	24	48	72	24	48	72
0.08	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.16	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.31	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.63	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1.25	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2.5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.23±0.23^{a}	0.23±0.23^{a}	0.23±0.23^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
10	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	2.50±0.50^{b}	3.00±0.29^{b}	3.20±0.25^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
20	1.10±0.30^{a}	1.10±0.30^{a}	1.23±0.37^{a}	3.70±0.15^{b}	4.90±0.26^{c}	5.03±0.26^{c}	0.33±0.17^{a}	0.33±0.17^{a}	0.33±0.17^{a}
40	2.83±0.17^{b}	2.83±0.17^{b}	2.93±0.12^{b}	5.60±0.38^{c}	6.07±0.52^{c}	6.27±0.43^{c}	2.10±0.46^{b}	3.27±0.43^{b}	3.50±0.32^{b}
60	5.33±0.33^{c}	5.47±0.29^{c}	5.83±0.20^{c}	7.90±0.85^{d}	8.53±0.75^{d}	8.77±0.63^{d}	3.83±0.42^{b}	5.27±0.50^{c}	5.47±0.44^{c}
80	8.33±0.33^{d}	8.60±0.46^{d}	8.93±0.58^{d}	11.00±0.50^{e}	12.13±0.38^{e}	12.33±0.44^{e}	6.87±1.10^{c}	8.73±0.27^{d}	8.87±0.24^{d}
100	11.57±0.78^{e}	11.83±0.93^{e}	12.13±0.77^{e}	13.53±0.52^{f}	14.83±0.44^{f}	15.00±0.40^{f}	8.77±0.95^{c}	11.77±0.39^{e}	12.03±0.43^{e}

diffusion method (zone of inhibition).

Different letters on every column represent meaningful difference ($p<0.0001$).

The effect of different concentrations of *C. limon* extract on 3 species of bacteria at 24, 48 and 72h, showed that the extract, similar to *M. piperita*, has a strong inhibitory effect in concentrations 80 and 100 µg/µl ($p<0.0001$) (Table 4).

Table 4. Anti-bacterial activity of different concentrations of *C. limon* herbal essence against three bacteria using disk diffusion method (zone of inhibition)

	Staphylococcus aureus			*Escherichia coli*			*Salmunella typhi*		
C. limon	Mean±SE			Mean±SE			Mean±SE		
(µg/ml)	24	48	72	24	48	72	24	48	72
0.08	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.16	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.31	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.33±0.33^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
0.63	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
1.25	0.00±0.00^{a}	0.20±0.11^{a}	0.20±0.11^{a}	0.53±0.03^{ab}	0.53±0.03^{ab}	0.67±0.09^{ab}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2.5	1.00±0.29^{ab}	1.20±0.20^{ab}	1.27±0.27^{ab}	2.00±0.58^{bc}	2.37±0.58^{bc}	2.47±0.32^{bc}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
5	1.83±0.44^{ab}	2.37±0.35^{b}	2.60±0.30^{b}	3.60±0.38^{c}	4.30±0.43^{c}	4.57±0.34^{c}	067±0.17^{ab}	067±0.17^{a}	067±0.17^{a}
10	3.27±0.27^{b}	5.33±0.49^{c}	5.33±0.49^{c}	6.87±0.66^{d}	7.00±0.58^{d}	7.07±0.52^{d}	2.03±0.37^{b}	3.60±0.78^{b}	3.80±0.64^{b}
20	7.23±0.64^{c}	8.47±0.29^{d}	8.60±0.35^{d}	8.23±0.89^{d}	8.67±0.67^{d}	8.77±0.62^{d}	5.47±0.44^{c}	7.60±0.15^{c}	7.77±0.19^{c}
40	10.30±0.65^{d}	10.70±0.44^{e}	10.70±0.44^{e}	12.27±0.38^{e}	13.17±0.60^{e}	13.17±0.60^{e}	8.97±0.84^{d}	10.80±0.42^{d}	10.97±0.37^{d}
60	10.57±0.30^{d}	10.93±0.52^{e}	11.50±0.50^{e}	15.80±0.42^{f}	16.33±0.33^{f}	16.77±0.39^{f}	10.63±0.19^{e}	12.17±0.60^{d}	12.37±0.54^{d}
80	13.93±0.07^{e}	14.53±0.29^{f}	15.27±0.55^{f}	18.57±0.72^{g}	19.33±0.33^{g}	19.53±0.29^{g}	14.03±0.38^{f}	15.10±0.59^{e}	15.27±0.50^{e}
100	17.50±1.12^{f}	18.50±0.50^{g}	19.07±0.40^{g}	20.17±0.93^{g}	21.20±0.92^{g}	21.37±0.82^{g}	16.13±0.07^{g}	16.50±0.06^{e}	17.03±0.48^{f}

Different letters on every column represent meaningful difference ($p<0.0001$).

- **Determination of antibacterial activity by using Disc Diffusion**

Results of Inhibition Zone (IZ) showed that the highest effect was related to *M. piperita* extract (100 µg/µl) on *E. coli* with a diameter of 27mm, and the lowest effect was observed for *C. cyminum* with a diameter of 14mm (Figure 1). In *S. aureus*, the highest effect was observed for *M. piperita* extract with diameter of 20mm (Figure 2). Also IZ results from the effect of *C. cyminum* on *S. aureus* and *S. typhi* showed the lowest effect among all used extracts with the diameters of 11 and 12mm, respectively (Figure 2 and 3). The IZ of herbal extracts on *S. typhi* revealed that *M. piperita* possesses the best inhibitory effect showing a diameter equal to 20mm (Figure 3). Generally, the Disc Diffusion assay showed that the extracts of *M. piperita* and *C. limon* possess higher inhibitory effects compared to other used extracts.

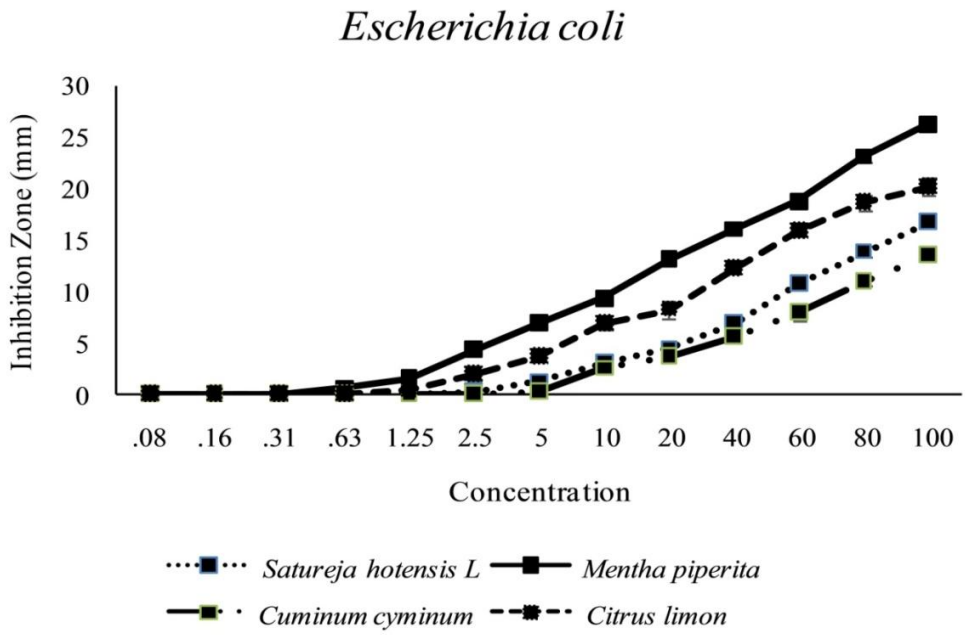

Fig1: Effect of some essential oils on *E. coli*

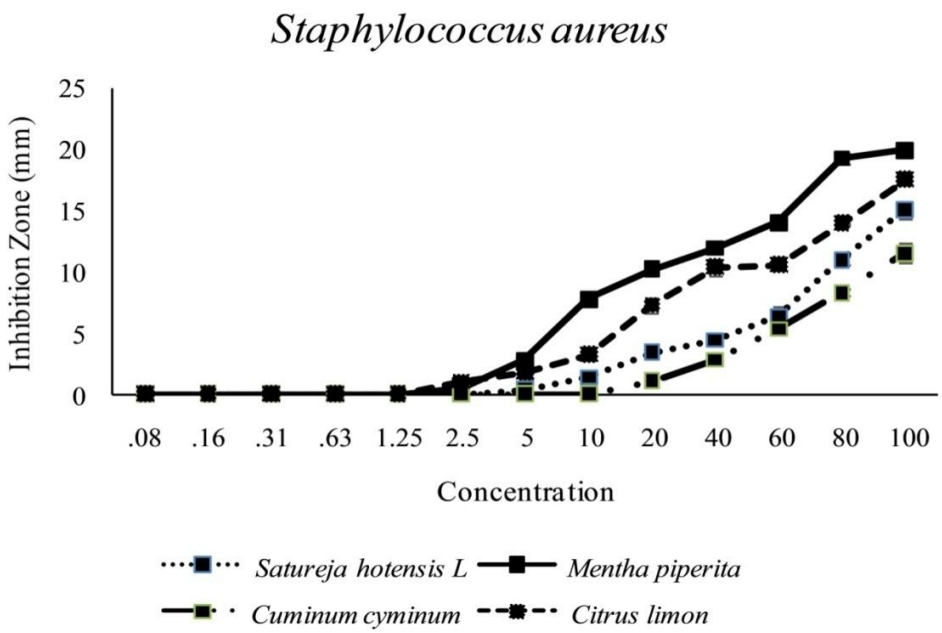

Fig2: Effect of some essential oils on *S. aureus*

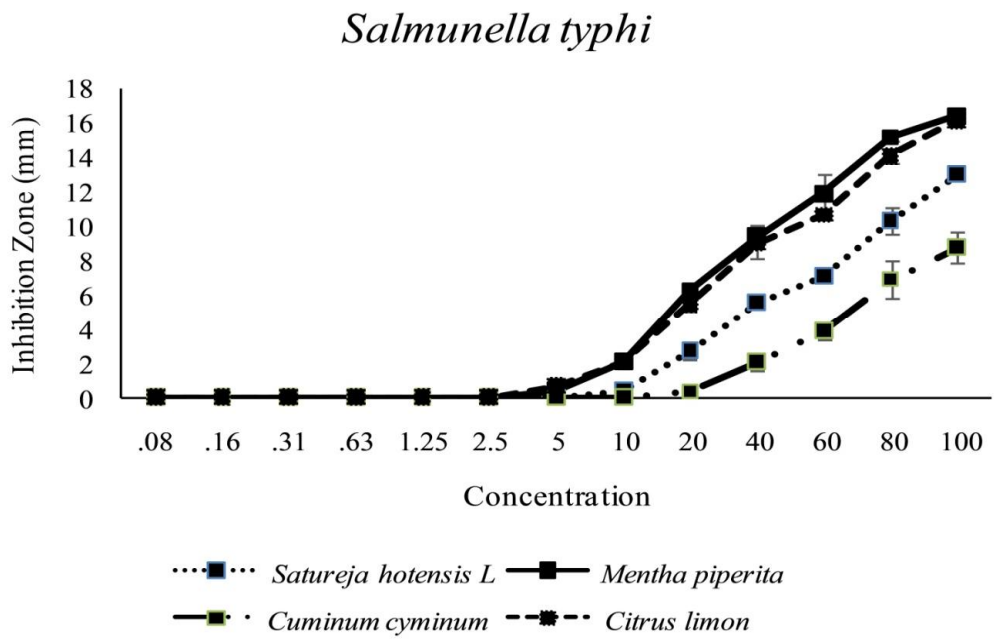

Fig3: Effect of some essential oils on *S. typhi*

- **The comparison of the effects of the extracts and common antibiotics on pathogens**

The most effective concentration of the extracts (100 ppm) was compared with common synthetic antibiotics in 24, 46 and 72h. Results showed that Gentamicin possesses the highest effect on *S. aureus* in all times, and the extract of *M. piperita* was the most effective on *S. aureus*, when compared with the other extracts and Amikacin. Also the extracts of *S. hotensis* L and *C. cyminum* were observed to have a similar and lower effects with Amikacin in all times, respectively on *S. aureus*. The effect of *M. piperita* and *C. limon* extracts were similar to Gentamicin on *S. typhi* bacteria in the first 24h, but in 48 and 72h, Gentamicin showed the highest inhibitory effect on the bacteria. The lowest effect was related to *C. cyminum* extract in all time intervals. Generally, the comparison of the effects of all extracts with mentioned antibiotics on the bacteria, revealed that Gentamicin possesses the best inhibitory effect on 3 species of bacteria, and also *M. piperita* extract was the best between the other used extracts (Table 5).

Table 5. Comparison of different herbal essential oils concentrations and antibiotics effect on *S. aureus*, *E. coli* and *S. typhi*

Treatment (100µg/ml)	*Staphylococcus aureus*			*Escherichia coli*			*Salmunella typhi*		
	Mean±SE			Mean±SE			Mean±SE		
	24	48	72	24	48	72	24	48	72
S. hotensis L	15.07±0.73^{bc}	16.00±0.58^{b}	16.27±0.82^{b}	16.70±0.51^{ab}	19.13±0.59^{b}	19.33±0.67^{b}	13.00±0.36^{b}	15.27±0.37^{b}	15.50±0.45^{b}
M. piperita	19.90±0.58^{d}	20.33±0.88^{c}	20.60±0.78^{c}	26.20±0.30^{d}	26.90±0.10^{c}	27.07±0.12^{d}	16.37±0.48^{c}	20.63±0.58^{c}	20.83±0.61^{c}
C. cyminum	11.57±0.78^{a}	11.83±0.93^{a}	12.13±0.77^{a}	13.53±0.52^{a}	14.83±0.44^{a}	15.00±0.40^{a}	8.77±0.95^{a}	11.77±0.39^{a}	12.03±0.43^{a}
C. limon	17.50±0.40^{cd}	18.50±0.50^{bc}	19.07±0.40^{b}c	20.17±0.93^{c}	21.20±0.92^{b}	21.37±0.82^{bc}	16.13±0.07^{c}	16.50±0.06^{b}	17.03±0.48^{b}
Gentamicin (50)	22.70±0.30^{e}	33.50±0.76^{d}	34.00±0.58^{d}	19.07±0.58^{bc}	29.33±0.88^{c}	30.33±0.33^{e}	16.10±0.59^{c}	25.67±0.40^{d}	26.00±0.58^{d}
Amikacin (3)	13.07±0.52^{ab}	15.43±0.30^{b}	16.27±1.01^{b}	14.03±1.03^{a}	21.17±0.73^{b}	22.60±0.83^{c}	11.73±0.14^{b}	14.40±0.87^{b}	14.70±0.66^{b}

Different letters on every column represent meaningful difference ($p<0.0001$)

- **Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)**

Results obtained from MIC and MBC of extracts plus antibiotics effects on *S. aureus*, showed that *M. piperita*, with MIC and MBC equal to 76 and 93 µg/ml respectively, possesses the highest inhibitory effect, but the antibiotics were better in this respect (MIC and MBC of Amikacin were 4 and 5 µg/ml, respectively) (Table 6). In *E. coli*, the best effect was also related to *M. piperita* extract with MIC and MBC of 49 and 72 µg/ml, respectively, and then the extracts of *S. hotensis* L and *C. limon* were shown to be effective. MIC and MBC of Gentamicin were 5.5 and 7 µg/ml, respectively (Table 7). Results from MIC and MBC of the extracts on *S. typhi*, revealed that the highest effects were related to *M. piperita*, *C. limon* and *S. hotensis* (respectively from high to low), and *C. cyminum* extract had the lowest effect (MIC and MBC of 100 and 127 µg/ml, respectively) (Table 8).

Table 6. MIC and MBC of the herbal extracts on *S. aureus*

NO	Extract	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)
1	*S. hotensis L*	93	103
2	*M. piperita*	76	93
3	*C. cyminum*	96	109
4	*C. limon*	81	100
5	Gentamicin	8	9
6	Amikacin	4	5

Table 7. MIC and MBC of the herbal extracts on *E. coli*

NO	Extract	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)
1	*S. hotensis L*	64	98
2	*M. piperita*	49	72
3	*C. cyminum*	68	101
4	*C. limon*	77	93
5	Gentamicin	5.5	7
6	Amikacin	3	3.4

Table 8. MIC and MBC of the herbal extracts on *S. typhi*

NO	Extract	MIC ($\mu g\ ml^{-1}$)	MBC ($\mu g\ ml^{-1}$)
1	*S. hotensis L*	97	120
2	*M. piperita*	71	97
3	*C. cyminum*	100	127
4	*C. limon*	89	104
5	Gentamicin	10	13
6	Amikacin	8	10

- **GC-MS of the essential oils**

The amounts of the essential oils were assessed by distillation with cold water based on the weight of dried sample. Results from the analysis of *C. cyminum* essential oil using GC-MS showed 24 compositions (this comprises 98.75% of the whole compositions) in the extract in which the most common compositions were Cuminic alcohol (30.32%), γ-Terpinene (25.32%) and β-Pinene (15.94%)(Table 9). 20 compositions were identified in *S. hortensis* L(this comprises 94.25% of the whole compositions) in which the most common compositions were Carvacrol (32.38%), gamma-Terpin (31.96%), Thymol (9.96%), p-Cymene (6.62%), and α-Terpinene (4.31%)(Table 10). GC-MS of *M. piperita* led to identification of 21 compositions (this comprises 94.30% of the whole compositions) such as Menthol (39.21%), Isomenthone (15.73%), and Menthyl acetate (7.02%) which were the most common compositions (Table 11). GC-MS of *C. limon* showed 45 compositions (this comprises 98.75% of the whole compositions) include Limonene (33.36%), p-Menth-1-en-8-ol (10.31%), and β-Pinene (6.72%)(Table 12).

Table 9. Chemical composition of the essential oil of *C. cyminum*

No	Compositions	%	RI
1	*α*-Thujene	0.39	929
2	*α*-Pinene	1.04	941
3	Sabinene	1.13	974
4	*β*-Pinene	15.94	978
5	*β*-Myrcene	1.11	988
6	*α*-Phellandrene	0.96	1006
7	Δ-3-Carene	0.06	1011
8	*α*-Terpinene	0.23	1016
9	*p*-Cymene	6.22	1028
10	1,8-Cineole	0.2	1030
11	*β*-Phellandrene	0.84	1032
12	γ-Terpinene	25.32	1056
13	α-Terpinolene	0.08	1082
14	Linalool	0.11	1098
15	cis-Sabinene hydrate	0.06	1100
16	Terpin-4-ol	0.22	1173
17	*α*-Terpienol	0.05	1186
18	Cuminic aldehyde	11.15	1225
19	Safranal	2.91	1274
20	Cuminic alcohol	30.32	1282
21	*γ*-Elemene	0.09	1394
22	Myrtenol	0.14	1402
23	*β*-Caryophyllene	0.08	1412
24	trans-β-Farnesene	0.1	1427
Total		**98.75**	

Table 10. Chemical composition of the essential oil of *S. hotensis* L

No	Compositions	%	RI
1	α-Thujene	0.88	931
2	α-Pinene	1.32	937
3	Camphene	0.14	951
4	Sabinene	0.07	974
5	β-Pinene	0.57	979
6	β-Myrcene	1.45	993
7	α-Phellandrene	0.39	1007
8	DELTA-3-Carene	0.1	1012
9	α-Terpinene	4.31	1016
10	p-Cymene	6.62	1025
11	Limonene	1.63	1032
12	1,8-Cineole	0.25	1030
13	β-Ocimene Z	0.15	1038
14	gamma-Terpinene	31.96	1057
15	α-Thujone	2.17	1087
16	Borneol	0.3	1062
17	α-Terpineol	0.22	1086
18	Thymol	9.96	1285
19	Carvacrol	32.38	1285
20	β-Caryophyllene	0.26	1412
Total		**94.25**	

Table 11. Chemical composition of the essential oil of *M. piperita*

No	Compositions	%	RI
1	α-Pinene	1.08	934
2	Sabinene	0.26	973
3	β-Pinene	0.74	977
4	Myrcene	0.21	991
5	p-Cymene	0.11	1024
6	Limonene	3	1028
7	1,8-CINEOL	0.19	1032
8	α-Terpinolene	0.08	1086
9	Linalool	0.24	1099
10	Menthone	20.3	1157
11	Isomenthone	15.73	1161
12	Menthol	39.21	1180
13	Neo-Iso Menthol	1.09	1184
14	α-Terpineol	0.98	1190
15	Pulegone	1.58	1235
16	Piperitone	1.2	1250
17	Menthyl acetate	7.02	1294
18	β-Bourbonene	0.32	1378
19	β-Caryophyllene	0.76	1413
20	Germacrene-D	0.11	1475
21	Caryophyllene oxide	0.09	1588
Total		**98.75**	

Table 12. Chemical composition of the essential oil of *C. limon*

No	Compositions	%	RI
1	α-Thujene	0.08	931
2	α-Pinene	1.45	937
3	Camphene	0.47	951
4	Sabinene	0.05	974
5	β-Pinene	6.72	980
6	β-Myrcene	0.58	994
7	α-Phellandrene	0.47	1008
8	α-Terpinene	1.55	1018
9	p-Cymene	4.18	1026
10	Limonene	33.36	1032
11	1,8-Cineole	0.5	1033
12	β-Ocimene Z	0.19	1039
13	γ-Terpinene	7.29	1058
14	α-Terpineol	2.69	1082
15	Linalool	0.86	1098
16	Fenchon	0.99	1099
17	Terpinen-1-ol	0.53	1106
18	Borneol	0.98	1163
19	Terpinen-4-ol	2.95	1173
20	p-Menth-1-en-8-ol	10.31	1192
21	γ-Terpineol	0.97	1207
22	trans-Carveol	0.14	1219
23	Carvol	0.81	1238
24	Geraniol	0.16	1250
25	E-Citral	0.13	1252
26	trans-Anethole	0.35	1254
27	Thymol	1.42	1282
28	Carvacrol	1.13	1295
29	Δ-Elemene	0.31	1339
30	Thymol acetate	0.09	1352
31	Neryl acetate	0.19	1360
32	Carvacryl acetate	0.07	1370
33	Geranyl acetate	0.28	1380
34	β-Elemene	0.55	1391
35	β-Caryophyllene	2.15	1401
36	α-Farnesene	2.31	1409
37	α-Humulene	0.4	1454
38	Germacrene D	0.43	1479
39	α-Selinene	0.56	1495
40	trans-α-Bisabolene	0.33	1508
41	Δ-Cadinene	0.1	1525
42	cis-α-Bisabolene	0.22	1537
43	Germacrene B	0.39	1558
44	(+) spathulenol	0.23	1576
45	α-Bisabolol	0.25	1580
	Total	**87.83**	

Discussion

This study discovered significant differences in presenting the antimicrobial potential of the tested herbal provisions. All the herbal species demonstrated antibacterial properties, but the level of bacterial growth inhibition induced by plant materials, shown to be dependent on herbal source, and bacterial strain. By using disc method, we detected that herbal essential oils determined different inhibition zones on experienced bacteria. However, it was established that herbal essential oils of different species had antibacterial effects both on Gram/positive (*S. aureus*) and on Gram/negative bacteria (*E. coli, S. typhi*). *C. cyminum, S. hotensis* L, *C. limon* and *M. piperita* exposed similar antimicrobial potentials. Previous studies have already shown the growth inhibitory activity on different microorganisms by *C. cyminum* essence (Jirovetz et al., 2005; Li & Jiang., 2004). Studies in 2005 showed that the *C. cyminum* herbal essential oils have antibacterial activity against Gram-positive and Gram-negative bacterial species (Iacobellis et al., 2005). Singh & Goswami and Singh with co-worker reported that *C. cyminum* oil (in very low concentrations) is similarly effective or even more effective when it is compared with standard antibiotics (Singh & Goswami., 1998; Singh et al., 2002). *S. hotensis L* is another plant studied in this experiment. Several studies revealed that the herbal essential oils of *Satureja* species are among the most effective herbal essential oils with respect to antimicrobial properties (Mihajilov-Krstev et al., 2010; Skočibušić & Bezić., 2004; Skočibušić et al., 2006). In 2010, Mihajilov-Krstev., et al examined that the herbal essence of *S. hortensis L.* has a significant activity against a wide range of Gram negative bacteria (MIC/MBC=0.025–0.78/0.05-0.78 µl/ml) and Gram positive bacteria (MIC/MBC=0.05–0.39/0.05-0.78 µl/ml) (Mihajilov-Krstev et al., 2010). In a Similar study, herbal essence of *S. hortensis* L was tested by micro-well dilution method for investigation its activity against 10 different pathogenic bacteria. The oil showed antibacterial activity against all tested strains. MIC/MBC values were in the range of 0.75- 25 µl/ml. Another medicine plant that its herbal essence antimicrobial activity has been demonstrated in this study, was *C. limon*. The herbal essence of various species of *Citrus* has effective antibacterial activities against many Gram-negative and positive pathogenic bacteria such *as S. oureus* and *E. coli* (Espina et al., 2011) Chanthaphon. et al., studied on the peels of *Citrus* spp. for their antimicrobial activities against food related microorganisms by micro dilution assay. The MIC and MBC values of the *Citrus* against *S. aureus* were 1.13 mg/ml, and 1.13 mg/ml, respectively. However, all Gram-negative tested including *Salmonella* sp. and *E. coli* were resistant to all citrus extracts at the concentration tested (Gavanji et al., 2014). The high antimicrobial activity of herbal essential oils explained primarily by their rich of phenolic compounds. Most of the studies on the mechanism of phenolic compound presented in herbal essential oils focused on its properties on cellular membranes which alters its function and, in some instances, structure (Friedman et al., 2002;

Kalemba & Kunicka., 2003). Generally, Gram positive bacteria were more sensitive to herbal essential oils than Gram-negative bacteria, due to their outer membrane barriers (Burt., 2004).

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