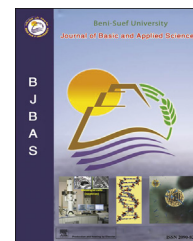


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Comparative chemical and antimicrobial study of nine essential oils obtained from medicinal plants growing in Egypt

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ABSTRACT

Essential oils are one of interesting natural products group that are used in different aspects of life due to their various biological activities. This study investigate the antimicrobial activities of 9 herbal essential oils on survival and growth of selected pathogenic and spoilage microorganisms. Essential oils were obtained by hydrodistillation method and were analyzed using GC/MS technique. The oils were tested for their antimicrobial activity against 2 Gram +ve, *Staphylococcus aureus* (*S. aureus*) and *Listeria innocua* (*L. innocua*), 2 Gram –ve, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Salmonella Typhi* (*S. Typhi*) as well as 2 Fungi, *Aspergillus niger* (*A. niger*) and *Candida albicans* (*C. albicans*), using agar dilution method. Minimum inhibitory concentration (MIC) was determined. The antibiotic susceptibility test was performed against the test organisms by disc diffusion method. Results showed that Cinnamon oil was found effective against all the tested strains (MIC \leq 1 μ l/ml). Peppermint, lemon grass, caraway, anise, fennel and clove showed activity at (MIC \leq 1 μ l/ml) with all the tested organisms except for *P. aeruginosa*. Lavender oil exhibited antimicrobial activities against 4 strains (*S. aureus*, *L. innocua*, *A. niger* and *C. albicans*) with MIC (\leq 1 μ l/ml) while geranium oil was inhibitory at (MIC \leq 1 μ l/ml) against *S. aureus*, *S. Typhi*, *A. niger* and *C. albicans* and with MIC \sim 2 μ l/ml against *L. innocua*. Although Gram –ve organisms had shown high resistance toward different essential oils, they were found to be susceptible to cinnamon oil even at lower concentration. Cinnamon oil is effective against drug resistant organisms. It can be suggested to use essential oils/constituents as potential natural preservatives and would be helpful in the treatment of various infections.

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1. Introduction:

Essential oils obtained from spices, herbs and medicinal plants by distillation, expression or solvent extraction are well-known in traditional medicine that are considered to be an area of interest as a potential source of antimicrobial agents. They are characterized by a broad-spectrum activity, including antifungal, antibacterial and antiviral activities. Besides, antimicrobial activities of essential oils, they used in food preservation, food industry as flavoring, pharmaceuticals, in cosmetics as fragrances and alternative medicine (Hussain et al., 2010). The proportions of the major and minor constituents specify the chemical composition of each EO and furthermore chemotypes can be recognized according to the levels of the major characterizing components. The antimicrobial effectiveness is not only assessed through the main component but also a synergistic effect may occur by the other components (Faleiro et al., 2003). EO is more efficient than various artificial antimicrobial agents that used for air disinfection due to its low toxicity level and high volatility specific property that is not found in other antimicrobial agents (Inouye et al., 2003). In addition to that, natural food preservatives has been widely used and accepted by the consumers, who prefer natural and healthy products with low synthetic additives (Militello et al., 2011).

EO antimicrobial efficiency of a same plant species is often affected with harvest time, weather conditions during growth and harvest, genotype and different geographic locations where plants are grown (Militello et al., 2011). The wrong and excessive dose of antibiotics is a serious problem in antimicrobial chemotherapy which causes resistance and ineffective antimicrobial treatment (Ang et al., 2004).

The antimicrobial activities of EOs are used by people all over the world for several years from popular commercially available herbal and medicinal plants: lemon grass (*Cymbopogon citrates*), Fennel (*Foeniculum vulgare*), peppermint (*Mentha piperita*), geranium (*Geranium dissectum*), caraway (*Carum carvi*), lavender (*Lavandula officinalis*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum cassia*) and anise (*Pimpinella anisum*), those have been used to treat bacterial and fungal infections (Prabuseenivasan et al., 2006). In the present study antimicrobial potential of 9 different plant essential oils was assessed against pathogenic strains i.e., *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Listeria innocua* (*L. innocua*), *Salmonella Typhi* (*S. Typhi*), *Aspergillus niger* (*A. niger*) and *Candida albicans* (*C. albicans*) in various antimicrobial assays.

The aim of the present study was to determine the chemical composition of essential oils obtained from 9 different plant species cultivated in Egypt and to evaluate their antimicrobial activity against 2 fungal and 4 bacterial species that may cause food poisoning and spoilage. Data obtained in this study could aid the identification of potential essential oils to be applied as food preservatives.

2. Material and methods

2.1. Plant material

Different plant organs mentioned in Table 1 used in this study was collected at May 2012 from different farming in Egypt. The

Table 1 – Plant species and main constituents of the respective essential oils.^a

Common name	Botanical name	Family	Plant organ
Clove	<i>Syzygium aromaticum</i>	Myrtaceae	Flower bud
Cinnamon	<i>Cinnamomum cassia</i>	Lauraceae	Bark
Lavender	<i>Lavandula officinalis</i>	Lamiaceae	Flowers
Lemon grass	<i>Cymbopogon citrates</i>	Poaceae	Herb
Fennel	<i>Foeniculum vulgare</i>	Apiaceae (Umbelliferae)	Fruits
Caraway	<i>Carum carvi</i>	Apiaceae	Fruits
Anise	<i>Pimpinella anisum</i>	Apiaceae	Fruits
Peppermint	<i>Mentha piperita</i>	Lamiaceae	Leaves
Geranium	<i>Geranium dissectum</i>	Geraniaceae	Leaves

^a All essential oils were obtained by hydrodistillation of the aerial parts of the plants.

systematic identification of the plant materials was kindly verified by Dr. Hossam M. Hassan, Faculty of Pharmacy, Beni-Suef University, Egypt. Plant materials were stored cool and in dry place for further investigation.

2.2. Extraction of essential oils

Half kg of each sample were collected and then subjected to hydrodistillation using Clevenger apparatus for 4 h and evaporate the solvent under reduced pressure at 40 °C using rotary evaporator. The essential oils obtained were separately dried over anhydrous sodium sulfate and stored at low temperature (–20 °C) till analysis by gas chromatography–mass spectrometry (GC–MS) or their usage in bioassays (Bhuiyan et al., 2008). All the tested herbal oils or extracts were sterilized by filtration using Millipore cellulose filter membrane (0.45 µm pore diameter).

2.3. Analysis of volatile oil extracts

2.3.1. Chemical composition of volatile oils

The volatile oil samples were analyzed by using Gas Chromatography/Mass Spectrophotometer (GC/MS) Agilent 6890 apparatus.

2.3.2. Physical properties of volatile oils

2.3.2.1. *Specific gravity bottle.* For determination of specific gravity of the different oil samples.

2.3.2.2. *Abbe's refractometer.* For measuring the refractive indices of the different volatile oil samples.

2.4. Microbial strains and growth conditions

2.4.1. Preparation of inoculum

All bacterial isolates were subcultured on Brain Heart Infusion agar (B.H.I.A.) and incubated at 37 °C for 24 h. Three bacterial

colonies were selected and suspended in 5 ml saline, standardized at 350 nm (equivalent to half McFarland) which gave a stock suspension of microorganisms equal to 1×10^6 CFU/ml saline.

Candida albicans was subcultured on Sabouraud dextrose agar (SDA) for 24 h at 30 °C. As previously mentioned with bacterial pathogens, a suspension of 1×10^5 CFU/ml saline was prepared. Concerning *A. niger*, it was subcultured on SDA and incubated at 25 °C for 4 days. With a pair of dissecting needles, a small portion of the colony to be examined was digged out and suspended in saline before use (Koneman et al., 1992).

2.4.2. Test microorganisms

The essential oils were tested against two fungal strains and four bacterial strains: Two strains of Gram-negative bacteria *P. aeruginosa* (ATCC 9022) and *S. Typhi* (ATCC 35664) and two

strains of Gram-positive bacteria *S. aureus* (ATCC 9027) and *L. innocua* (LMGT 2708) were used in the antibacterial assay.

Fungal strains are *C. albicans* (ATCC 60193) and *A. niger* (ATCC 1718109). All of these strains were kindly supplied from Microbiology Department, Faculty of Pharmacy, Beni-Suef University.

2.5. Antibacterial assay

The Agar-diffusion method was employed for screening the antimicrobial properties of isolated volatile oils (Jacobes and Appelbaum, 1995). A stock solution of the tested compounds was prepared in a concentration of 200 µl/ml in DMSO and final test concentrations in the range of 1–16 µl/ml were achieved in 10 ml Muller Hinton agar media. Controls were prepared using the same quantities of DMSO as blank. The mixtures were mixed, poured into sterile petri dishes and allowed to harden at room temperature. The agar surface was inoculated with 10 µl of a standardized suspension of the test organisms using multiple inoculators.

A positive control plate was made using DMSO. Each experiment was done in duplicates. All plates were incubated at 35 °C for 48 h.

The result of growth was recorded knowing that the MIC is defined as the lowest concentration of antifungal agent giving no visible growth or causing almost complete inhibition of growth.

2.6. Antibiotic susceptibility testing

The antibiotic susceptibility test was performed by using Disc Diffusion Method (Finegold and Martin, 1990). Six different forms of sensitivity discs with variable concentrations were used for studying the in-vitro sensitivity of standard isolates: Enrofloxacin (5 mg); Ciprofloxacin (5 mg); Doxycycline (30 mg); Amoxicillin (10 mg); Spiramycin (30 mg); Cefotaxime sodium (30 mg). These discs were obtained from "Oxoid". The results were interpreted according to CLSI (2010).

Table 2 – Physical and chemical properties of tested essential oils.			
Oil sample	Physical properties		Chemical properties Main compounds (%) by GC/MS
	Optical rotation	Density	
Cinnamon	+0.6	1.226	Trans-caryophyllene (17.18%), followed by eugenol (14.67%), linalool L (14.52%), trans-cinnamyl acetate (13.85%), cymol (11.79%) and cinnamaldehyde (11.25%)
Lavender	+0.1	1.238	Delta-3-carene (17.14%), followed by α -fenchene (16.79%), diethyl phthalate (13.84%), Linalyl acetate (6.9%), Camphor (6.12%), Linalool L (5.97%), α -Pinene (4.75%), Nopyl acetate (3.77%), β -Citronellol (3.72%) and α -Terpinenyl acetate (3.03%)
Lemon grass	+0.1	1.206	Geranial (47.34%), followed by β -myrcene (16.53%), Z-citral (8.36%), Geranyl acetate (7.89%) and Pulegone (3.29%)
Peppermint	+2.6	1.258	Menthone (40.82%), followed by Carvone (24.16%), β -isophorone (9.37%) and 1,8-cineol
Caraway	+2.1	1.226	DL-limonene (53.35%), followed by β -selinene (11.08%), β -elemene (10.09%) and Caryophyllene oxide (9.85%)
Anise	+0.2	1.260	Anethole (64.82%), followed by gamma-himachalene (9.29%), methoxyphenyl acetone (2.6%), DL-limonene (1.82%) and β -Bisabolene (1.39%)
Fennel	+0.1	1.245	Trans-anethole (33.3%), followed by DL-limonene (19.66%), carvone (12.03%), fenchyl acetate (7.12%) and p-allyl anethole (6.11%)
Geranium	+1.9	1.292	β -citronellol (25.45%), followed by geraniol (13.83%), 1-menthone (9.46%), L-linalool (8.2%) and α -gurjunene (9.08%)
Clove	+1.1	1.298	Eugenol (84.07%), followed by isoeugenol (10.39%)

3. Results

3.1. Essential oils composition

3.1.1. Physical characters

As shown in Table 2, it was found that all the essential oils under investigation had similar density in range of (1.206–1.260) and were dextrorotatory (+0.1 to +2.6).

3.1.2. Chemical composition

The chemical composition of the essential oils was determined by the GC–MS analysis technique. The identification of the unknown compounds was based on their relative retention time and their mass spectra in comparison with those observed by standards. Some compounds were tentatively identified, by using the NBS75K library data of the GC–MS system and literature data. The results are presented in Table 2.

Cinnamon, lavender, lemon grass and peppermint essential oils were characterized by the presence of delta-3-carene,

Table 3 – Antibiotic susceptibility.

Chemo-therapeutic disc	Antimicrobial code	Disk content µg/disk	Source	<i>P. aeruginosa</i>	<i>S. Typhi</i>	<i>L. innocua</i>	<i>S. aureus</i>
Ciprofloxacin	CIP	5	Oxoid	S	S	S	R
Doxycycline	DO	30	Oxoid	I	S	S	I
Enrofloxacin	ENR	5	Oxoid	R	S	S	R
Spiramycin	SP	30	Oxoid	R	R	I	R
Amoxicillin	AML	10	AML	R	R	S	R
Cefotaxime sodium	CRO	30	Oxoid	S	S	S	S

R: resistant, I: intermediate, S: susceptible.

geranial, trans-caryophyllene and menthone. Trans-caryophyllene was found as the main compound in cinnamon (17.18%), delta-3-carene was the major constituent in lavender (17.14%), geranial was found as the main component in lemon grass (47.34%), while menthone represented the major component in peppermint as (40.82%). The analysis of anise oil gave a large numbers of constituents (forty-eight compounds representing (99.77%) of the total oil) which participated in the mixture in variable percentages. Among them anethole was detected as the main component (64.82%), followed by gamma-himachalene (9.29%), DL-limonene (1.82%), P-allyl anethole (0.54%), β-Selinene (0.5%) and estragole (0.49%). In geranium essential oil, thirty-seven compounds representing (99.97%) of the geranium oil were identified and the main component was β-citronellol (25.45%), followed by geraniol (13.83%). DL-limonene give a different profile in fennel and caraway essential oil composition. Trans-anethole was the main constituent in fennel as (33.3%), followed by DL-limonene (19.66%). In caraway oil, the predominant component was DL-limonene (53.35%), followed by β-Selinene (11.08%). In case of clove oil, eugenol was the main constituent. Isoeugenol (10.39%), eugenol (84.07%) and α-caryophyllene (2.42%) consisted (99.16%) of the total clove oil.

3.2. Antimicrobial activity

The MICs of 9 plant essential oils obtained by the agar dilution method are shown in Table 4. Cinnamon was the only essential oil which showed an effective antimicrobial activity against all the tested strains at the lowest used concentration (≤ 1 µl/ml). Cinnamon was the only essential oil which showed an active antibacterial activity against *P. aeruginosa* at the lowest used concentration (≤ 1 µl/ml).

Peppermint, lemon grass, caraway, anise, fennel and clove had the same antimicrobial activity at the used concentration against the same used strains where *S. aureus*, *L. innocua*, *S. Typhi*, *A. niger* and *C. albicans* were very susceptible to the tested oil, as MIC values obtained were very low. The MIC value for all tested microbes was ≤ 1.0 µl/ml, while *P. aeruginosa* was very resistant against the tested oil even at the highest used concentration (16 µl/ml). Lavender showed antimicrobial activity against all the tested strains at the lowest concentration (≤ 1 µl/ml) except *P. aeruginosa* and *S. Typhi* which were resistant to lavender oil at the highest used concentration (16 µl/ml). MIC value in geranium oil was obtained lowest 1 µl/ml against *S. aureus*, *S. Typhi*, *A. niger* and *C. albicans*, while at MIC about 2 µl/ml, this essential oil had shown inhibitory effect against *L. innocua*. However, in case of *P. aeruginosa*, geranium oil didn't show any inhibitory activity toward geranium oil.

3.3. Antibiotic susceptibility test

Table 3 showed the sensitivity of different microbial strains to various chemotherapeutic agents, the in vitro sensitivity of *P. aeruginosa* was done against 6 chemotherapeutic agents. *P. aeruginosa* was highly sensitive to ciprofloxacin and cefotaxime sodium. The same strain was highly resistant to amoxicillin, spiramycin and enrofloxacin. On the other hand, the tested strain showed intermediate sensitivity to doxycycline. The tested *S. Typhi* strain was highly sensitive to ciprofloxacin, doxycycline, cefotaxime sodium and enrofloxacin. On the other hand, it was highly resistant to amoxicillin and spiramycin. It was noticed that, *L. innocua* was highly sensitive to ciprofloxacin, doxycycline, amoxicillin, cefotaxime sodium and enrofloxacin. On the other hand, the tested strain showed

Table 4 – Antimicrobial activity of various concentrations of selected essential oils (from 1 µl/ml to 16 µl/ml) against the tested strains.

The tested essential oils	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. Typhi</i>	<i>L. innocua</i>	<i>C. albicans</i>	<i>A. niger</i>
Lavender oil	≤ 1	>16	>16	≤ 1	≤ 1	≤ 1
Cinnamon oil	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1
Lemon grass oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1
Peppermint oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1
Caraway oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1
Anise oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1
Fennel oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1
Geranium oil	≤ 1	>16	≤ 1	2	≤ 1	≤ 1
Clove oil	≤ 1	>16	≤ 1	≤ 1	≤ 1	≤ 1

intermediate sensitivity to spiramycin. In case of *S. aureus*, this strain was highly sensitive to cefotaxime sodium. On the other hand, it was highly resistant to ciprofloxacin, spiramycin, amoxicillin and enrofloxacin. The same strain assayed an intermediate sensitivity to doxycycline.

4. Discussion

The antimicrobial efficiency may be influenced by the EO solubility and the preparing method of the essential oil stock solution where there is no standardized test method or format for reporting assay results of antimicrobial activity of essential oils (Friedman et al., 2002).

From Table 2, it was noticed that lavender oil which its chemical composition results were not coincided with published one (Tadtong et al., 2012; Zhejzakov et al., 2012) who showed that the main constituents of lavender oil were Linalyl acetate (38.23%) and Linalool (35.01%), on the other hand was not like reported by (Abdel-Reheem et al., 2012) where it was reported that the major components were 1,8-cineol and Linalool was highly effective against *S. aureus*, *L. innocua*, *C. albicans* and *A. niger* and completely un effective against *P. aeruginosa* and *S. Typhi*. Similar or nearly similar results were obtained by (Gomez-Estaca et al., 2010).

It is very interesting to concluded from Table 2 that cinnamon oil which its GC/MS analysis verified that it was completely different from that reported by Li et al. (2006) who showed that cinnamaldehyde was the major component comprising (85%), this difference may be due to environmental conditions and time of collection. Antimicrobial results indicated that it was the only EO which was effective at its lowest concentration (1 µl/ml) against all tested bacterial and fungal strains. CIN is an aromatic aldehyde and it had been suggested that the carbonyl group of aldehydes can bind to metal ions, sulfhydryl groups, amino groups and proteins (Bowles and Miller, 1993). Similar or nearly similar results were recorded by (Bouhdid et al., 2010).

Concerning lemon grass oil (Table 2), its chemical composition data was going with that published by (Piaru et al., 2012) while was different from what reported by Tyagi and Malik (2010) who showed that the major components were: Myrcene (3.5%), Limonene (30.3%), Camphene (6.5%), α -Citral (17.6%), β -Citral (11.3%), 6-Me hepten-2-one (14.6%) and linalool (1.5%). LGO was highly effective against all strains except *P. aeruginosa*. These results were going with that reported by Tyagi and Malik (2010) and Mickiene et al. (2011) but these were completely different from that verified by Onawunmi (1988) who showed that the combined use of LGO and phenoxyethanol would increase the spectrum of activity of phenoxyethanol whose activity was mainly against *P. aeruginosa*.

Peppermint oil which its chemical composition results were similar to some extent with that reported by Schmidt et al. (2009), but on other hand it was different from what verified by Marotti et al. (1994) who indicated that menthol was the main component in peppermint oil. Some components were isolated as minor amounts such as: α -terpineol (2.82%), trans-caryophyllene (1.99%) and β -bourbonene (1.62%). It showed activity against all tested strains but it was ineffective

against *P. aeruginosa*. These antimicrobial results were completely similar to that revealed by Li et al. (2011) and Matan et al. (2011).

Caraway oil that its GC/MS analysis result was different from what indicated by Chowdhury (2002) who reported that carvone was the main constituent which represented as (81.5%). Minor constituents were also identified as: β -Patchoulene (3.05%), Limonene glycol (2.44%) and Trans-caryophyllene (2.36%). It was highly effective against all strains except *P. aeruginosa*. These data were like that was assayed by Mohamed and Saad (2010), but on the other hand was different from what revealed by Rosangela et al. (2005) who found that caraway oil exhibited antimicrobial agent against *P. aeruginosa*.

It was found that the data of GC/MS analysis of anise oil was consisting with that reported by Ritter et al. (2012). Anise oil (Table 4), showed good antimicrobial activity toward all the tested strains except *P. aeruginosa*, these results were completely similar with that published by Matan et al. (2011) and Yutani et al. (2011), while it was nearly similar with Gurdip et al. (2006) whose results were going with our results.

Chemical analysis results of fennel oil were like that was assayed by Qiu et al. (2012) who showed that Trans-anethole was the major constituent, whereas was different from Dawidar et al. (2008) who indicated that DL-limonene was the major constituent. On other hand Coelho et al. (2003) referred that fenchone was the major one. Table 4 demonstrated that fennel oil showed high inhibitory effect against all the used strains except *P. aeruginosa*. These results were not coincided with published one (Gulfraz et al., 2008).

It was indicated that the chemical composition of geranium oil was different from (Lis-Balchin and Roth, 2000) who indicated that Methyl eugenol was high in *Pelargonium odoratissimum* and *Pelargonium fragrans*; fenchone and limonene was common in *P. odoratissimum*, *Pelargonium exstipulatum* and *P. fragrans*, while thujene and α -pinene were high in *P. exstipulatum* and *P. fragrans*. These differences were referred to the different species under investigation. Minor amounts of seven other constituents could also be identified: α -pinene, neryl acetate, bicycloelemene, α -cubebene, α -amorphene, δ -cadinene and geranyl tiglate. Geranium oil (Table 4), *S. aureus*, *S. Typhi*, *C. albicans* and *A. niger* were very sensitive to all concentration, *L. innocua* was only sensitive to 2 µl/ml concentration. On the other hand, *P. aeruginosa* was completely resistant against all concentration used. This data was coinciding with that reported by Bigos et al. (2012) and Singh et al. (2012).

In regard with clove oil, the chemical analysis data was going with that reported by Joseph and Sujatha (2011). From Table 4, it was noticed that clove oil was very effective against all the used strains except *P. aeruginosa*. This result was like that was assayed by Joseph and Sujatha (2011) and Ahmad et al. (2011). On the other hand, these results were nearly similar with Vasanti and Shrutika (2009) whose results were going with our results.

The data obtained from the in-vitro antibacterial susceptibility of the studied bacterial pathogens performed in the present study showed variable sensitivity of both Gram positive and Gram negative to some chemotherapeutic agents.

The results of antimicrobial susceptibility illustrated in Table 3 revealed that *S. aureus* was resistant to ciprofloxacin, enrofloxacin, spiramycin and amoxicillin while it was sensitive to cefotaxime sodium only and gave moderate sensitivity to doxycycline. Concerning ciprofloxacin, our result was opposed to that obtained by Mamache et al. (2011) and Hussain et al. (2012) who found that *S. aureus* was sensitive to ciprofloxacin. Also our result of enrofloxacin was opposite to that obtained by Hussain et al. (2012). On the other hand, our results of spiramycin were the same to that of Jana et al. (2009) but opposed to that of Mamache et al. (2011). Belonging to the amoxicillin results, our results was similar to that reported by Mamache et al. (2011) and Shaheen et al. (2013) while opposite to that recorded by Hussain et al. (2012). Opposed to our results, Sina et al. (2011) reported that *S. aureus* was resistant to cefotaxime sodium.

According to Table 3, *P. aeruginosa* was resistant to enrofloxacin, spiramycin and amoxicillin while it was sensitive to ciprofloxacin and cefotaxime sodium only and gave moderate sensitivity to doxycycline. Concerning enrofloxacin, our result was similar to that of Harada et al. (2012) who reported that the degree of resistance to enrofloxacin was greatly affected by efflux pump inhibitors, indicating over expression of efflux pump contributed to these resistances. They also observed enrofloxacin resistance even in isolates without mutations. On the other hand this result was opposite to that obtained by Harry et al. (2009) who found that *P. aeruginosa* was sensitive to enrofloxacin. Moreover, our result of spiramycin resistance was opposed to that of Grunt et al. (1978). Belonging the amoxicillin results, our results was similar to that reported by Gehan et al. (2011) and Akano et al. (2013) while opposite to that recorded by Nkang et al. (2009). Concerning our result of *P. aeruginosa* sensitivity to ciprofloxacin, it was similar to that of Akano et al. (2013) but opposed to that Gehan et al. (2011) as well as Aseel et al. (2013) who extracted a large individual DNA-plasmid from all resistant *P. aeruginosa* isolates. Moreover, our result of cefotaxime sodium sensitivity was similar to that of Huai et al. (2012) while Harada et al. (2012) obtained opposite results.

Regarding *S. Typhi*, results illustrated in Table 3 revealed that *S. Typhi* was sensitive to all the tested antimicrobial drugs except spiramycin and amoxicillin; it was resistant. Concerning ciprofloxacin, our result was the same to that of Nkang et al. (2009) but opposed to that of Adabara et al. (2012). Meanwhile the results of enrofloxacin and cefotaxime sodium sensitivities were the same to that obtained by Boris et al. (2010). On the other hand, our results concerned with the resistance of *S. Typhi* to amoxicillin was similar to that of Adabara et al. (2012) but opposite to that obtained by Nkang et al. (2009) and Ajayi and Egbebi (2011).

Moreover, data illustrated in Table 3 revealed that *L. innocua* was sensitive to all the tested antimicrobial drugs except spiramycin; gave moderate sensitivity. Wang et al. (2012) obtained the same result of amoxicillin while Akano et al. (2013) obtained a similar result with ciprofloxacin but obtained an opposite result with amoxicillin. Moreover, Gupta and Sharma (2013) reported opposite results to that of cefotaxime sodium where they found that *L. innocua* isolates were resistant.

5. Conclusion

Our results confirm that many essential oils possess in-vitro antimicrobial activity against pathogens. Moreover, in this study the effect of some essential oils against spoilage bacteria was ascertained, suggesting that they may be used for the development of novel systems for food preservation. However, further studies are necessary to investigate the possible interaction between oils and food components for their use in food.

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