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Essential oil analysis and Antibacterial activities of some medicinal plants

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Abstract

The extraction of medicinal plants has been used traditionally against pathogenic bacteria that caused infectious disease in human and Microbial spoilage of food and have been used safely in herbal medicine as antimicrobial compounds. In the present study, The antibacterial activities of the oils were evaluated against human and animals pathogenic bacteria; the three gram positive bacteria; *Staphylococcus aureus*, *Listeria monocytogenes, Streptococcus pneumonia* and four gram negative bacteria; *Pseudomonas aeruginosa*, *Klebsiella pneumoni*, *Escherichia coli* and *Salmonella typhi*. In this assay, the selective plants were reported ethnobotanical uses traditionally and also referenced in some herbal medicine text. The essential oil of *Stachys pubescens*, Coriandrum sativum and *Cinnamomum zelanicum, Bupleurum falcatum* were prepared by hydrodistillation. The oils were analyzed by GC/MS. The number of 6, 10, 15 and 23 components was identified in *C. sativum*, *C. zelanicum, B. falcatum and S. pubescens* respectively. The MIC and MBC of oils determined with broth microdilution and agar diffusion method on bacterial strains. Results from the antibacterial testing indicated that *B. falcatum*, *S. pubescens* and *C. zelanicum* essential oils showed high activities and inhibited the growth all of the selected bacteria. While the essential oil of C. sativum displayed the moderate potential activity. Our finding supported the notion that plant essential oils composition or total extract may have a role as pharmaceuticals and preservatives effects as safely and effective drugs with low resistance against microorganisms. Therefore, these essential oils could be used for management of these pathogens as a potential source of sustainable eco-friendly botanical bactericides.

Keywords: Antibacterial, Bupleurum falcatum, Stachys pubescens, Coriandrum sativum, Cinnamomum zelanicum, GC/MS.

Introduction

Herbal medicine has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources as a good choice, because these natural resources have ordinarily fewer side effects [1,2]. The medicinal plants have been proved effective in the treatment of infectious diseases simultaneously decrease many of the side effects [3]. Also they are costless and effective against a broad spectrum of antibiotic resistant microorganisms and they have very potent natural biologically active agents [4]. In many parts of the world, the extracts and essential oil of medicinal plants with active biological compounds are used for their antimicrobial and antiviral properties [5] that have been used in folk medicine. The increasing occurrence of antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine [6,7]. For this reason, there is a growing interest in the antimicrobial screening of extracts and essential oils from plants in order to discover new antimicrobial agents. Nowadays, about 25 percent of the drugs prescribed worldwide come from plants and 252 of them are considered as basic and essential by the World Health Organization (WHO). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs in developing countries. Infectious diseases are the second

leading cause of death worldwide [8]. From the time of the ancient Iranian, the plants were considered to protect against diseases. Iran has a very honorable past in traditional medicine, which goes back to the time of Babylonian - Assyrian civilization. One of the most significant ancient heritages is sophisticated experience of people who have tried over millennia to find useful plants for health improvement, with each generation adding its own experience to this tradition [9]. Based on literature search 18% of the plant species are used for medicinal purposes in Iran. Treatment of infections continues to be a problem in modern time because of side effects of some drugs and growing resistance to antimicrobial agents. To investigate for novel, safer and more potent antimicrobials is a pressing need. Herbal medicines have received much attention as a source of new antibacterial with low side effect and significant activity [8]. In the present study, the biological activities of the *Bupleurum falcatum*, *Stachys pubescens*, Coriandrum sativum, *Cinnamomum zelanicum* was evaluated. Nowadays, there is a considerable research interest towards the compositional analysis of essential oil and extract. It has been reported that essential oil yield and their components in plants is related to genetic [10], climate, elevation, topography [11,12] and genotype (G), growing conditions (E) and their interaction (G x E) [13,14]. Recent studies have shown that herbal medicine especially these selected species have strong biological activity [15-21]. The antibacterial activity of *S. pubescens* doesn't evaluate and it carried out for the first time in this study. Concerning the *B. falcatum* worked only in one research [15]. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of bacterial resistant [22]. Although there were low levels of preexisting antibiotic resistant bacteria before the widespread use of antibiotics evolutionary pressure from their use has played a role in the development of multidrug resistance varieties and the spread of resistance between bacterial species [23]. Biological cost or metabolic price is a measure of the increased energy metabolism required to achieve a function. Drug resistance has a high metabolic price [24] in pathogens for which this concept is relevant [25]. Although several strategies have been proposed to overcome and control this situation. However, a clear solution has not yet been elucidating due to the antibiotic resistance, consequences, and side effects of antimicrobial drugs. Many plants are used in Iran in the form of oils and crude extracts, infusion or plaster to treat common infections without any scientific evidence of efficacy. Pharmacological studies carried out on essential oils of some aromatic plants' species that were obtained in central regions of Iran, have shown antimicribial activity which is coherent with the use of these plants in folk medicine. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. In the present study were selected the three medicinal plants which are widely used in the folk medicine in our region. All of them have been used in the treatment of infectious diseases with different geographical area [26,27]. The aim of this study was to evaluate the antibacterial potential of the essential oils derived from *B. falcatum*, *S. pubescens*, *C. sativum* and *C. zelanicum* which growth of the wild in the central part of Iran against standard bacterial strains. The selected strains; *Staphylococcus aureus* (PTCC: 1431), *Listeria monocytogenes* (PTCC:1163), *Streptococcus pneumoniae* (PTCC:1240), *Pseudomonas aeruginosa* (PTCC:1430), *Klebsiella pneumoniae* (PTCC:1053), *Escherichia coli* (PTCC:1329) and *Salmonella typhi* (PTCC:1609) purchased from Iranian Research Organization for Science and Technology (IROST). The antimicrobial potential was performed by disc diffusion (DD) and broth microdilution method (BMD) to determine the Minimum Inhibitory Concentration (MICs) and minimum Bactericidal Concentration (MBCs).

Materials and methods

Collection of plant materials and essential oil extraction

The plants were collected from their wild habitat in Semnan city in the central part of Iran between April and June 2011 which are shown Geographical and environmental conditions in Table-1. Plants were identified by experts of the University of Applied Science and Technology (UAST) Education Center in Semnan branch. The flowers of *B. falcatum* and the leaves of *S. pubescens, C. sativum, C. zelanicum* were collected to determine their antibacterial activity. A voucher specimen for each plant has been deposited in the herbarium of Medicinal Plants Research UAST. Air-drying of plant material was performed in a shady place at room temperature for 4 days. Grinding and dried leaves of plants (100 g) were subjected to hydro-distillation for 3 hr, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

Gas chromatography/ mass spectrometry (GC/MS) analysis

The essential oils were analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. A fused

silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) was used for the separation. Helium was used as a carrier gas at a flow rate of 1ml/min. The oven temperature was programmed at 60°C (4 min), and then rising to 300°C at 4°C/min. The injector and detector temperature were kept at 250 °C and 300 °C, respectively. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy with MS transfer line at temperature of 300 ºC was used. Ion source and interface temperatures were 200 °C and 250 °C, respectively. The split ratio was 1:50. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250 °C. The column was programmed as follows: 60°C for 2 min and then increased by 3°C/min up to 300°C. Volume of injected samples was 0.5µl. Identification of components was based on the comparison of retention times (RT) and the computer mass spectra libraries using Wiley 275 GC/MS Library (Wiley, New York), those found in the literature [28,29] and the mass spectrometry data bank (NIST). The percentage composition of the essential oil was computed by the normalization method from the GC peak areas measurements (Table-4).

Table-1: Geographical and environmental conditions

NO	**plant**	**Region**	**Altitude (m asl[1])**	**latitude**	**Longitude**
1	*Coriandrum sativum*	Garmsar North Eyvanakey	2100	35.432670	53.256050
2	*Cinnamomum zelanicum*	Shahrood Mayamey Bekran	2150	36.43520	54.376050
3	*Bupleurum falcatum*	Semnan Fullad mahaleh	2650	35.78527	53.32405
4	*Stachys pubescens*	Shahrood, Semnan	2315	36.32415	54.35316

Table 2: MIC and MBC (μg/ml) values for different essential oils of plants

Bacteria	Gentamicin		Ciprofloxacin		*C. zelanicum*		*B. falcatum*		*C. sativum*		*S. pubescens*	
	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]
S. p	1	2	0.5	0.5	0.5	0.5	1	1	1	2	1	1
S. a	0.5	1	1	2	0.5	1	0.5	1	4	4	0.5	1
P. a	1	1	0.125	0.5	1	2	2	4	1	2	1	2
E. c	2	4	0.5	1	2	2	8	8	8	8	1	2
S. t	1	2	0.5	0.5	4	8	4	4	16	32	4	8
L. m	2	2	1	1	8	16	16	32	32	32	8	8
K. p	2	4	1	2	8	16	16	64	-	-	8	16

MIC = Minimum Inhibitory Concentration **MBC**= Minimum Bactericidal Concentration

"-" No growth inhibition. E.c=*Escherichia coli*, P.a= *Pseudomonas aeruginosa*, S.a=*Staphylococcus aureus*, S.t=*Salmonella typhi*, S.p=*Streptococcus pneumonia*, K.p=*Klebsiella pneumoniae*, L.m=*Listeria monocytogenes*.

Table-3: Antibacterial activity screening of antimicrobial agents by zone of inhibition (mm diameter) in disc diffusion method

Bacterial strains	D.D OF N.C	D.D OF P.C		*C. zelanicum*	*B. falcatum*	*C. sativum*	*S. pubescens*
		G	C	D.D[a]	DD[a]	DD[a]	DD[a]
Str. pneumoniae	-	18	25	27	25	21	26
Sta. aureus	-	24	28	25	27	18	25
P. aeruginosa	-	19	22	23	15	20	21
E. coli	-	14	16	17	10	13	20
S. typhi	-	17	21	16	19	11	13
L. monocytogenes	-	16	15	10	8	5	11
K. pneumoniae	-	15	18	8	7	-	8

DD= Diameter of inhibition zone (mm) including of disc diameter of 6mm. **a**= tested at a concentration of 20 μg /disc. **N.C**= Negative Control. **P.C**= Positive Control (**G**= gentamicin, **C**= ciprofloxacin,). "**-**" No growth inhibition. **E**= Escherichia, **P**= Pseudomonas, **Sta**= Staphylococcus, **S**= Salmonella, **Str**= Streptococcus. **K**= Klebsiella, **L**= Listeria.

Table 4: Chemical analyses of essential oils

	Cinnamomum zelanicum			*Coriandrum sativum*			*Bupleurum falcatum*			*Stachys pubescens*		
NO	**Components**	**P.A (%)**	**RT**	**Components**	**P.A (%)**	**RT**	**Components**	**P.A (%)**	**RT**	**Components**	**P.A (%)**	**RT**
1	Benzaldehyde	4.50	5.845	α-Pinene	1.22	5.479	α-Pinene	3.5	5.240	β-Pinene	1.8	4.840
2	Phenylmethanal	2.50	5.879	Benzene	1.96	6.881	Pinocarvone	4	5.285	1,4-Cyclohexadiene	0.4	5.412
3	Benzene acetaldehyde	0.56	7.184	γ-Terpinene	1.40	7.413	α-Cubebene	8.1	5.432	Myrcene	0.9	6.124
4	Acetophenone	0.73	7.539	Cyclopropane	1.24	7.544	Pinocamphone	1.7	6.641	α-Terpinene	2.7	6.529
5	Benzaldehyde dimethyl acetal	1.00	8.214	Linalool L	91.54	8.036	Heptanal	4.2	7.405	Benzene	0.9	6.790
6	Benzeneacetic acid	0.82	10.262	Geranyl acetate	2.64	12.042	cis-Verbenol	1.5	7.910	Limonene	6.3	6.893
7	2-Propenal	54.28	10.680	-	-	-	Myrtenal	2.9	10.470	(*E*)-β-Ocimene	2.8	7.115
8	Cinannamaldehyde Dimethylc Acetale	18.57	12.345	-	-	-	Thyopsene	3.1	10.958	γ-terpinene	1.2	7.430
9	2-Propenoic acid	16.24	12.883	-	-	-	trans-Pinocarveol	4.1	11.690	3-Cyclohexen-1-ol	1.5	9.280
10	Ortho Metthoxy Cinnamic Aldehyde	0.79	13.999	-	-	-	Trans-Verbenol	0.3	11.825	Linalool	9.7	9.987
11	-	-	-	-	-	-	Cuparene	2.8	11.931	2,6-Octadien	11.5	10.420
12	-	-	-	-	-	-	Torilenol	39.1	11.965	Octen-1-ol acetate	1.6	10.560
13	-	-	-	-	-	-	Spathulenol	19.6	12.560	2,6-Octadienal	2.1	10.602
14	-	-	-	-	-	-	α-Calacorene	2.4	12.940	Linalyl acetate	1.2	10.742
15	-	-	-	-	-	-	Pentacosane	1.5	13.850	δ-Elemene	5.4	11.124
16	-	-	-	-	-	-	-	-	-	β-Bourbonene	0.2	12.247
17	-	-	-	-	-	-	-	-	-	δ-Cadinene	19.7	13.905
18	-	-	-	-	-	-	-	-	-	Naphthalene	1.2	13.926
19	-	-	-	-	-	-	-	-	-	β-Gurjunene	0.3	13.945
20	-	-	-	-	-	-	-	-	-	Bicyclogermacrene	1.8	14.364
21	-	-	-	-	-	-	-	-	-	Caryophyllene oxide	1.3	14.821
22	-	-	-	-	-	-	-	-	-	Spathulenol	0.8	14.834
23	-	-	-	-	-	-	-	-	-	Germacrene	22.4	15.248
Total	-	99.99	-	-	100	-	-	98.8	-	-	97.7	-

Microorganisms, inoculums and antibacterial assay

Bacterial strain

In the present study, a total of 7 standard isolates were obtained from IROST in 2011. Bacterial strains used in this study were four gram-negative bacteria: *Pseudomonas aeruginosa* (PTCC: 1430), *Klebsiella pneumoniae* (PTCC: 1053), *Escherichia coli* (PTCC: 1329), *Salmonella typhi* (PTCC: 1609) and three gram-positive bacteria: *Staphylococcus aureus* (PTCC: 1431), *Listeria monocytogenes* (PTCC: 1163), *Streptococcus pneumonia*e (PTCC: 1240), that were grown in Müeller–Hinton (MH) agar (Oxoid) and incubated for 24 hr at 37°C. Cultures were used for making bacterial suspensions, and turbidity was adjusted to 0.5 McFarland and confirmed using a spectrophotometer (UV-VIS 1650, Shimatzu, Japan).

Preparation of inoculums

The inocula of the bacterial strains were prepared by suspending one isolated colony from MH agar plates in 5mL of MH broth (Oxoid) and overnight broth cultures. The suspensions were adjusted in 0.5 McFarland standard turbidity to obtain final inoculums of 5×10^5 to 5×10^6 CFU/mL after 24 h of growth at 37°C and confirmed using a spectrophotometer. The essential oils were dissolved in dimethyl sulfoxide (DMSO, 25 mg/ml) and diluted to MH broth for antibacterial tested. All strains were tested by broth microdilution (BMD) and disk diffusion (DD) techniques according to the National Committee for Clinical Laboratory Standards(NCCLS) [30,31].

Serial dilution Method

MICs and MBCs of essential oils were determined by using BMD method as described by the NCCLS [30] in flat-bottomed 96-well clear plastic tissue-cultured plates (Greiner, 650161). The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained twofold dilutions of antimicrobial agents at the concentration ranges: 0.5 to 64µg/ml (25%, v/v). These dilutions were used to dispense 100 µL into each of the sterile 96-wells and an equal volume of bacterial inoculums was added to each well on the microtiter plate. After incubation for 24 h at 37 °C, the microdilution trays were checked with unaided eyes to detect the growth inhibition of the bacteria, and then the MICs were determined with spectrophotometer. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The final concentration of DMSO in the assays did not interfere with the bacterial proliferation which is used as a control. Negative controls were prepared with non-inoculated medium with oils, and one non-inoculated well, without antimicrobial agents, was also included to ensure medium sterility. The commercial antimicrobials Ciprofloxacin (Sigma) and Gentamicin (Merk) were included as positive controls. One inoculated well was included to allow control of the broth suitability for organism growth. To determine the MBCs, the suspensions (20µl) were taken from each well without visible growth and inoculated in MH agar for 24 h at 37ºC. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganisms are completely killed. Tests were performed in triplicate for each test concentration ($P > 0.05$).

Disc diffusion method

Agar diffusion method was carried out for the assessment of the essences antibacterial activity which as recommended by NCCLS [31]. The potential activity of oils were confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone (IZ) compared to known standard antibiotics. Essential oils were diluted in DMSO to different test concentrations. 50 µl of standardized inoculums according to 0.5 McFarland turbidity standard solutions (10^5 to 10^6 CFU/ml) of the selected strains were spread on to the surface of Mueller Hinton (MH) agar and kept for 2 h at 4ºC for absorption. Sterilized paper discs (Whatman,6 mm diameter) containing approximately 20 µL of the essential oils were impregnated with different amount of essential oils (0.5, 1, 2, 4, 8, 16, 32 and 64 µg/ml). The prepared discs of the oils and standard antibiotics were placed on the surface of MH agar media. The inoculated plates were incubated at 37ºC for 24 hr and the resulting zone of inhibition (diameter) was measured in millimeters by comparing the different concentrations of oils and the standard antibiotics. The MIC was defined as the lowest concentration, resulting in a clear zone of growth inhibition around the disc after incubation period. Gentamicine (Merk) and Ciprofloxacin (Sigma) discs were applied over the test plates as a positive control. Negative controls were prepared using the solvent to dissolve the essential oil solution. All experiments were performed in triplicate.

Statistical analysis

Comparison of data was performed using the one way ANOVA or the unpaired Student's t-test and is presented as

mean ± standard deviation. Comparison of MIC and MBC values, tests were made in triplicate for quantification. Values of $p < 0.05$ were considered significant.

Results and Discussion

All essential oils showed effective antibacterial activities on the selected pathogenic bacteria. Antibacterial activities of essential oils were investigated by broth microdilution and the Disc Diffusion methods. The MICs and MBCs and diameter of inhibition zone (DD) of the selected oils on the bacteria are shown in Tables 2 and 3. The results showed that essential oil of the plants were active against all the pathogenic bacteria species with different degree in the following range of concentrations: Essential oil of *S. pubescens and C. zelanicum* had the best antibacterial effect and its MIC value was between 0.5 to 8μg/ml. *B. falcatum* is in the second degree with MIC values between 0.5 to 16μg/ml. Otherwise, *C. sativum* had a lowest antibacterial effect comparison to above essences and its MIC value was 1 to 32μg/ml. Ciprofloxasin and Gentamicin used as positive control as well as DMSO as a negative control which didn't show any inhibition against the pathogens bacteria. MIC range of standard antibiotics "Ciprofloxasine and Gentamycin" were 0.5 - 1μg/ml and 0.5 – 2μg/ml respectively. Even at low concentrations, the plant's species showed antibacterial activity more or nearly equal to the commercial antibiotics agents. All of the oils had the best inhibitory activities against *Str. Pneumonia*, *S. aureus* and *P. aeruginosa*. The weakest activity was observed against *L. monocytogenes* and *K. pneumoniae* with the highest MIC and MBC, and *K. pneumoniae* was resistance against *L. angustifolia*. The results of the chemical analyses using GC/MS of the essential oils were listed in Table 4. Number of indentified constituents In C. sativum, *C. zelanicum, B. falcatum and S. pubescens* were 6,10,15 and 23 respectively. Also, analysis of data with creditable library shows that, the main components of *S. pubescens* were: Germacrene(22.4%),δ-Cadinene (19.7%), 2,6-Octadien(11.5%), Linalool (9.7%); in *C. zelanicum* were*:* 2-Propenal(54.28%), Cinannamaldehyde Dimethylc Acetale(18.572) and Propenoic acid(16.24); in *Bupleurum falcatum* were: Torilenol (39.1%), Spathulenol(19.6) and α-Cubebene(8.1%), and C. sativum were include Linalool L(91.54%) and Geranyl acetate(2.64%). In this study was attempted to purify the selected plant's oils that are native in our region in order to identify their essential oils as antibacterial properties. In addition, components of plants were determined and comparison the result with other studies. This is due to several reasons, namely, conventional medicine can have side effects, high coast, abusive or incorrect usage of synthetic drugs result in complications, and the large percentage of world's population do not have access to conventional pharmacological treatment. The best antibacterial activities were seen in *S. pubescens* , *C. zelanicum and B. falcatum*, whilst *C. sativum* displayed a moderate response against bacterial species. In comparison to the standard drugs, these data showed *S. pubescens* and *C. zelanicum* had the highest activity; *B. falcatum* had the lower activity but with the lowest different, while the different properties of C. sativum was more. The results were confirmed the antimicrobial potency of these plants, especially about the *S. pubescens and B. falcatum, that* evaluated for the first time *in the world and Iran*. The two gram positive (*Staphylococcus aureus, Streptococcus pneumonia*) and two gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*) showed the high sensitivity against the essential oils with lowest MICs and MBCs. In other studies concerning the antimicrobial activity of these plants, inhibition effects of C. sativum on the some microorganisms such as *Bacillus megaterium, Klebsiella ozaenae*, *Proteus mirabilis*, *Salmonella. paratyphi* A, *Salmonella paratyphi* B and *Serratia marcescens* was studied but did not show any antibacterial activity against Gram negative urinary pathogens [17-19]. In the previous studies *C. zelanicum* showed the antimicrobial activity against some of standard and clinical microorganisms [20,21]. The above finding supports their traditional usage of these oils as antibiotic and antiseptic [2,32]. Briefly, the results of this study showed that the essential oils of these plants have a very broad spectrum of antibacterial activities against both Gram-positive and Gram-negative bacteria with notable MICs and MBCs, which are near or lower than dose synthetic drugs. These plants could safely be used as organic preservatives to replace synthetic antibiotics in the prevention and cure of some human and animal infectious disease as well as food industrial preservatives [2,6]. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. In comparison to the other studies, the main components of selected plants were different to the main components in this study. Against to our result, in previous studies on *C. zelanicum* showed that the main components of the oil were eugenol, linalool and piperitone [33], there are any of these components in our main components. Eugenol as the first main component is known as an antimicrobial constituent [35]. Concerning the *S. pubescens*, there are a few studies which determined the chemical composition. *Many studies have not been conducted so far***;** Iranian researcher reported; germacrene D, (*Z*)-β-Ocimene and Bicyclogermacrene as main components [35]. Since, each of Germacrene,δ-Cadinene , 2,6-Octadien and Linalool alone was not considerable amount in the total extract,

therefore, it was suggested its antibacterial activity should be also belonging to the mixture of whole components, with attention to other its components were less than 9.7%. Since C. sativum showed the antibacterial activities, then it was suggested the Linalool L(91.54%) which alone was considerable amount in the total extract, alone or mix to another components (synergic effect) play a major antimicrobial role. In another study, the components of 2-decenoic acid, E-11-tetradecenoic acid and capric acid determined as main components [16]. Previous studies related to the chemical composition of *B. falcatum,* α-pinene reported as main component which are not similar to our result [15]. Since, *B. falcatum* display the high antimicrobial activity, therefore, we concluded that the most main components as Torilenol and Spathulenol have an antimicrobial activity alone or mix to other components. In the present study the similarity of composition to other studies is low. Nonetheless, all of them have a good antimicrobial activity and this subject describe that the antimicrobial potency of this plant is yield the activity of components alone or mix. Although these plants are in the same region, but their main component is different, and this variety in biological activity is related to their composition. It was suggested these differences in components could be due to the variety of the ecotype system that reported by other scientists and references [36]. Further studies are needed to determine the antibacterial activities of the bioactive compounds responsible for the observed potential value.

Conclusion

Plant-derived Natural Products may be a source of new alternative active compounds. In attention to, in the present study, most isolates showed a less difference concentrations of essential oils between bacteriostatic and bactericidal values. Suggesting that the essential oils of selected plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms and also in the search for novel antibacterial agents with the potential application of some major or minor constituents alone, mixed of presented essences or in combination with antibiotics for the treatment and prevention of pathologies associated with multi resistant bacteria. However, the mechanism of inhibitory effects of these plant's oils against infectious bacteria is still unclear. Further investigations regarding the in vitro and in vivo should be conducted in order to clear mechanisms pathway and develop such products.

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