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Antibacterial activity of essential oil of *Trachyspermum ammi* (L.) Sprague ex Turrill against isolated and standard bacteria

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Abstract
The essential oil of *Trachyspermum ammi* (L.) Sprague ex Turrill (Apiaceae) fruits was extracted by hydrodistillation. The residue of the fruit treated with hydrochloric acid and again hydrodistilled to extract volatile compounds. The components of obtained oils were identified using GC and GC-MS. Antibacterial activity of the oils and pure thymol were examined against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* as well as two isolated bacteria, *Salmonella typhimurium* and *E. coli*, from diseased hen, using disk diffusion and microdilution methods. The fruit of the plant yielded 3.5 and 2.4 mL oils, with thirteen and twenty-four identified compounds in the oil before and after acid hydrolysis, respectively. Thymol, *p*-cymene, and γ-terpinene were the major components of the both oils. All the tested bacteria sufficiently suppressed by the oils with inhibition zone (IZD) diameters ranging 26.6-50.3 mm with minimum inhibitory concentration (MIC) of <0.02 µL/mL. Thymol inhibited growth of the bacteria with concentration of 100 mg/mL. The results of present study revealed that the fruit of *T. ammi* could serve for production of supplements or therapeutic agents for poultry industries.

Keywords: *Trachyspermum ammi*, antibacterial, poultry disease, acid hydrolysis

1. Introduction
Trachyspermum ammi (L.) Sprague ex Turrill (synonym: *T. copticum* (L.) Link, *Carum copticum* (L.) Benth. & Hook. f.) is an annual plant belongs to Apiaceae family growing in the center, south and southeast of Iran. The plant also found in Afghanistan, Pakistan, India, and north of Africa [1]. The most utilized part of the plant is the small, brown seed like fruit, which is popular for its dietary and medicinal characteristics. The fruit traditionally have been used for its antispasmodic, stimulant, tonic and carminative properties [2]. Oil of the fruit was evaluated in previous works resulting in identification of its chemical composition. Thymol, carvacrol, γ-terpinene, cymene, limonene were the main constituents of the plant oil with different amount in the most previous studies [3-12]. In addition, recently some experiments have been performed to investigate other biological and pharmacological activities of the plant. The studies have been confirmed antioxidant, antiviral, antifilarial, antifungal, nematicidal, anthelmintic, insecticidal, scolicidal, anti-inflammatory, wound healing, analgesic, antinociceptive, antipyretic activities of the plant [4, 5, 13-20]. Antimicrobial activity of the fruit oil was evaluated against several bacteria and fungi with different origin in several investigations [3-7, 21-26]. The fruit oil and different extracts of the plant suppressed the growth of gram-negative and gram-positive food spoilage and food born bacteria by hampering of cytoplasmic membrane of the tested bacteria in previous analysis [2]. Investigations of new classes of antimicrobial agents from natural sources have been designed to overcome increase of bacterial resistance to conventional antibiotics. Antibacterial activity of medicinal plants especially of those have been used traditionally are increased to treat infectious diseases [27].
The aim of this study was to demonstrate chemical composition and antibacterial activity of essential oils of the fruit of *T. ammi* before and after acid treatment against two isolated pathologic strains of bacteria in poultry along with four standard pathogen strains. Due to the best of our knowledge, there is no report about composition of hydrolyzed volatile compounds of fruit. Besides, susceptibility of two isolated pathogen bacteria from diseased hens were tested in the present work.

2. Methods and Materials

2.1 Plant Materials and Hydro-distillation

The dried fruit of *T. ammi* were purchased from local medicinal plant shop in 2013. The fruit (100 g) were subjected to obtain essential oil using Clevenger type apparatus for 4 h. Afterwards, hydrochloric acid (Merck, Darmstadt, Germany) (10 mL, 1N) was added to the fruit residue over night at room temperature and again subjected to extraction of volatile compounds using Clevenger type apparatus for 4 h [28]. As a result of acid treatment in the mixture, hydrolyzing procedure of glycosidic components was successfully facilitated. The obtained oils (before and after acid treatment) were separately dried by anhydrous sodium sulfate (Merck, Darmstadt, Germany) and stored in sealed dark glass vials in refrigerator (4 ºC) until further investigations.

2.2 Gas chromatography analysis (GC)

The analysis of the oils were carried out using a Dani Master GC (Dani, Italy) with OV1 column (SE54CB, 25 m×0.25 mm i.d., 0.25 µm film thickness), nitrogen as carrier gas, split ratio of 1:20, and flame ionization detector (FID). Temperature programming was performed from 75 ºC (42 min) to 250 ºC (14 min) at 15 ºC/min, injector and detector temperatures were 250 and 260 ºC, respectively.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The essential oils were also analyzed by GC-MS method on Agilent 6890 with MS instrument (Agilent, U.S.) equipped with a BPX5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was raised from 50 °C to 300 °C at a rate of 3 °C/min for 75 min. The oven temperature was held for 5 min at 50 °C and transfer line temperature was 290 °C. Helium was used as a carrier gas at a flow rate of 0.8 mL/min with a split ratio equal to 1/30. The quadrupole mass spectrometer was scanned over ionizing voltage of 70 eV and an ionization current of 150 µA. The retention indices for all the components were calculated using retention times of *n*-alkanes (C8-C25) injected at the same temperature and conditions, after the essential oil. The compounds were identified by comparison of retention indices with those reported in the literature together with comparison of their mass spectra with the Wiley, Adams and NIST libraries.

2.4 Antibacterial Test

Antibacterial activity of the oils were tested against some standard bacteria strains including *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 as well as two isolated bacteria from diseased hen, *Salmonella typhimurium* and *E. coli*. Microdilution method was employed to evaluate minimum inhibitory concentration (MIC) of both essential oils [29, 30]. The suspensions of the strains were prepared in normal saline and the turbidity adjusted to 0.5 McFarland with absorbance of 0.08-0.1 at 600 nm. The suspension of bacteria diluted 1:100 (v/v) in Mueller-Hinton broth. Briefly, bacterial suspensions (1×10^6 CFU/mL) were added to serial dilutions of the oils in a 96-well microtiter plate with concentrations ranged from 100-0.02 µL/well. The MICs were defined as the lowest concentration that showed clear against a black background (no visible growth). Moreover, the disc diffusion method was applied for evaluation of inhibition zone diameters (IZD). Sterile cotton swab was used to spread microbial suspension on Muller-Hinton agar and all plates were incubated for 16 h at 37 °C. Sterile paper discs (6 mm in diameter) were impregnated with 10 µL of oil placed on the inoculated plates and incubated at 37 °C for 24 h. Thymol was used as positive control. All tests were performed in triplicate and data were presented mean ± standard deviation (SD).

3. Results &Discussion

The fruit of the plant yielded 3.5 mL yellow-colored oil with density of 0.97 mg/mL before acid treatment. Additionally, the treated fruit with acid yielded 2.4 mL yellow-colored oil with density of 0.85 mg/mL. Thirteen compounds were successfully identified in the oil before acid, which comprise 99.1% of all. Thymol (74.2%), *p*-cymene (16%), and γ-terpinene (7.1%) were the major components of the oil of *T. ammi* fruit before acid treatment (Table 1). Twenty-four compounds were characterized in the oil after hydrolysis yielding 98.8% of total oil and the most abundant compounds were thymol (67.7%), *p*-cymene (14%), and γ-terpinene (7.2%). Although, the oils were similar in their main components, the oil after hydrolysis constituted some additional components like 1-terpineole (1.5%), 1,4-cineole (1.1%), *p*-cymenene (0.8%), and 1,8-cineole (0.6%) (Table 1). Both oils only contained aromatic monoterpenes, monoterpene hydrocarbons, and oxygenated monoterpenes. The amount of aromatic monoterpenes in the oil before hydrolysis was more than oil after acid treatment (90.7 and 84.04, respectively). However, monoterpene hydrocarbons and oxygenated monoterpenes were higher in the oil after acid hydrolysis.

The obtained oils were tested against some pathogen strains. The growth of all the examined bacteria was effectively suppressed with the essential oils, while thymol has not showed inhibition activity toward the tested bacteria with concentrations of 1 and 10 mg/mL. The most susceptible strains to the oil before hydrolysis and oil after hydrolysis were *P. aeruginosa* (50.3 ± 0.6) and *S. aureus* (46.0 ± 0.0), respectively. Isolated strain from hen, *S. typhimurium*, with IZD of 28.7 ± 0.6 was the most resistant strain. Antibacterial examination showed that standard strains in the present study were more sensitive to the essential oils in comparison with those isolated pathogens with inhibition zone diameter ranging between 30.0-50.3 and 26.6-30.7 mm, respectively (Table 2). Except for two gram-negative pathogens, *E. coli* and *E. faecalis*, other tested organisms were more susceptible to the oil before acid treatment. The MIC values of both oils for all microorganisms was successfully assessed as <0.02 µL/mL, as well. The growth of the bacteria was totally suppressed in the presence of thymol with concentration of 100 mg/mL with IZD of >80 mm.

The fruit of the plant yielded higher amount of oil before acid treatment than that after acid hydrolysis with different density. Monoterpenes including aromatic monoterpenes, monoterpene hydrocarbons, and oxygenated monoterpenes are constituents of the oils. However, low amounts of sesquiterpenes have been reported in the oil of the fruit in other studies [2, 3, 31, 32]. Although the composition of essential oil after acid treatment showed some differences in presence of minor compounds, major compounds of the oil were characterized same as those identified in the oil before acid treatment with nearly the same concentrations. The amounts of main compounds of the oils were approximately similar to each other. Concerning the identified chemical constituents of oils of the plant fruit in the previous studies, general similarity is obvious in the main components of *T. ammi* oil with quantitative differences. The results of the present study indicated that thymol, *p*-cymene, and γ-terpinene dominated the main parts of both oils, which are reported in several studies as predominant parts of the fruit oil [3-12, 23, 33]. The oil of fruit before acid hydrolysis consisted of

74.2% of thymol in the present study. However, different amount of thymol were reported in the previous studies ranging from 17% up to 71% [4, 5, 13-16, 18-20, 29]. Moreover, dominating compound of the oil after acid hydrolysis was also characterized thymol (67.7%). In addition to those freely occurring monoterpenes in the fruit, further volatiles are formed through acid hydrolysis [34]. Acid treatment may help glycosidically-bound compounds to be hydrolyzed, since enzyme and acid both hydrolyze glycoside bonds [35, 36]. Some compounds that were identified in the oil after acid treatment like 1-terpineole, 1,4-cineole, *p*-cymenene, and 1,8-cineole were absent in the oil before acid hydrolysis. On the other hand, the most abundant compounds of both oils were similar; it probably can be result of the cell wall damage by acid treatment through hydrolysis of major hydrocarbons of the fruit cell walls like cellulose and hemicellulose facilitating release of remnant volatile compounds [37] from cells. Therefore, acid treatment can be assumed a procedure to enhance the amount of the oil of *T. ammi* through hydrolysis of glycosidically bound compounds and damaging cell wall of fruit.

Antibacterial activities of extracts of various parts of *T. ammi* were examined against several strains of microorganisms, which are summarized in Table 3. According to the previous data, the MIC values of the plant oil against gram-positive, gram-negative, and fungi are ranging 0.06-6, 0.06-60, and 0.25-8 µL/mL, respectively (Table 3). Gram-negative bacteria due to their protein outer membrane usually are less sensitive to essential oil comparing to gram-positive bacteria [38]. However, the results of our study revealed that all the gram-positive and gram-negative strains are susceptible to the oils. The MIC values for all microorganisms were successfully assessed <0.02 µL/mL in the present study, that is lower than those calculated in the other studies. Standard strains were more sensitive to the essential oil in comparison with those isolated pathogen strains with inhibition zone diameter ranging between 30.0-50.3 and 26.6-30.7 mm, respectively.

Oil of the fruit impaired membrane structure causing the intracellular ATP leakage, loss of cytoplasmic materials and potassium as well as alteration of cell surface morphology resulting unbalanced metabolism [2]. Thymol, one of the major components of the oils, damages membrane integrity with changes of pH hemostasis and equilibrium of inorganic ions. *p*-Cymene, a hydrophobic compound, enhances antimicrobial activity of thymol through dissolution in the cytoplasmic membrane of the bacterial cell [39,40]. The results of some studies have indicated that whole essential oil displays stronger antibacterial activity in comparison with the mixture of major components [41, 42], suggesting synergistic effect or potentiating activity of minor compounds of the oils [15]. Antibacterial activity of the oil in the present study indicated a role of major components and synergistic activity of minor parts of the oil against examined bacteria, since the oils of fruit inhibited growth of bacteria more sufficiently with lower amount than thymol. Thymol has not showed antibacterial activity in concentration of 1 and 10 mg/mL. Therefore, antimicrobial activity of oils was generally regarded to the phenolic compounds like thymol and hydrocarbons such as γ-terpinene and *p*-cymene [43]. Although, results of a study using thin layer chromatography (TLC) bioautographic method indicated that thymol is responsible for antibacterial activity of the oil of *T. ammi* [32], our results suggested that synergistic effect between minor components of oil and thymol, main compound of the oils, are crucial for antibacterial activity of the plant oil.

The results of the present work are encouraging as using acid hydrolysis can be assumed as a procedure for enhancement of the fruit oil yield. Both oils of the fruit of *T. ammi* inhibited the growth of gram-negative and gram-positive bacteria. The growth of isolated microorganisms from diseased hen including *S. thyphimurium* and *E. coli*, in particular, suppressed in presence of the oils suggesting that *T. ammi* fruit may serve for production of supplements or therapeutic agents for poultry industries.

3.1 Tables

Table 1: Chemical composition of essential oils of *T. ammi* before acid treatment and after acid treatment.

NO.	compound	RT[1]	KI[2]	percent (%)		MI[5]
				BA[3]	AA[4]	
1	α-thujene	11.3	926	0.1	-	a
2	β-pinene	14.06	981	0.3	0.05	a, b
3	β-myrcene	14.62	992	0.1	0.07	a
4	1,4-cineole	16.02	1019	-	1.1	a
5	α-terpinene	16.11	1021	0.1	1.1	a
6	*p*-cymene	16.63	1031	16	14	a, b
7	limonene	16.78	1033	0.1	0.1	a
8	β-phellandrene	16.89	1036	0.1	-	a
9	1,8-cineole	16.97	1037	-	0.6	a, b
10	γ-terpinene	18.32	1063	7.1	7.2	a
11	α-terpinolene	19.71	1090	-	0.5	a
12	*p*-cymenene	20.14	1098	-	0.8	a
13	*exo*-fenchol	21.64	1128	-	0.2	a
14	1-terpineol	22.43	1144	-	1.5	a
15	*cis*-β-terpineol	23.20	1160	-	0.2	a
16	borneol	23.92	1174	-	0.3	a
17	terpinen-4-ol	24.71	1190	0.2	0.4	a
18	α-terpineol	25.50	1206	0.1	1	a, b
19	γ-terpineol	25.69	1210	-	0.3	a
20	carvone	28.04	1260	0.2	0.1	a, b
21	thymol	30.19	1306	74.2	67.7	a, b
22	*p*-cymen-7-ol	30.37	1310	-	0.5	a
23	carvacrol	30.46	1312	0.5	0.6	a
24	piperitenone	32.33	1354	-	0.04	a
25	myristicin	40.03	1438	-	0.04	a
26	dillapiole	43.78	1634	-	0.2	a
monoterpenes hydrocarbons				7.9	9.02	
oxygenated monoterpenes				0.5	5.74	
aromatic monoterpenes				90.7	84.04	
total				99.1	98.8	

1: Retention time, 2: Kovats index, 3: Before acid treatment, 4: After acid treatment, 5: Method of identification, a: GC-MS, b: GC.

Table 2: Antibacterial activity of the essential oil of *T. ammi* against some standard and isolates strains of bacteria from diseased hen.

Microorganisms						
IZD ± SD (mm)						
Samples	***S. aureus***	***E. coli***	***P. aeruginosa***	***E. faecalis***	***S. typhimurium****	***E. coli****
oil before acid	47.3 ± 0.5	30.7 ± 0.6	50.3 ± 0.6	30.0 ± 0.0	28.7 ± 0.6	30.7 ± 0.6
oil after acid	46.0 ± 0.0	40.0 ± 0.0	33.0 ± 0.8	40.3 ± 0.4	26.6 ± 0.4	27.3 ± 0.4
thymol**	>80.0 ± 0.0	>80.0 ± 0.0	>80.0 ± 0.0	>80.0 ± 0.0	>80.0 ± 0.0	>80.0 ± 0.0

IZD: Inhibition zone diameter, SD: Standard deviation.*: The isolated strains from diseased hen, **: Tested with concentration of 100 mg/mL.

Table 3: Results of antimicrobial activity of extracts and essential oil of *T. ammi* in previous studies.

Extract	Microorganism	MIC (µL/mL)	IZD (mm)	Main components (%)	Ref.
96% ethanol extract of leaves	*Acinetobacter lwoffii* [1]	-	20 ± 1.3	-	[44]
	Pseudomonas aeruginosa[1]	-	11 ± 1.3		
	Staphylococcus aureus[1]	-	15 ± 1.6		
	Bacillus subtilis[1]	-	11 ± 0.6		
essential oil	*Staphylococcus aureus*	1	-	thymol (45.9%), γ-terpinene (20.6%), *o*-cymene (19.0%)	[3, 44]
	Staphylococcus epidermidis	2	-		
	Staphylococcus saprophyticus	2	-		
	Enterococcus faecalis	2	-		
	Enterococcus faecium	4	-		
	Streptococcus salivarius	0.06	-		
	Streptococcus sanguis	1	-		
	Escherichia coli	0.5	-		
	Enterobacter aerogenes	0.5	-		
	Proteus vulgaris	0.25	-		
	Salmonella typhimurium	1	-		
	Pseudomonas aeruginosa	8	-		
	Serratia marcescens	1	-		
	Shigella flexneri	0.06	-		
	Shigella dysenteriae	0.06	-		
	Klebsiella pneumoniae	0.125	-		
	Candida albicans	0.25	-		
	Candida glabrata	0.25	-		
	Aspergillus flavus	0.25	-		
	Aspergillus niger	0.25	-		
	Aspergillus parasiticus	0.5	-		
essential oil	*Lactobacillus acidophilus*	-	0.0 ± 0.0	thymol (55.3%), limonene (20.5%), α-terpinene (18.9%)	[3]
	Escherichia coli	-	14.3 ± 0.03		
	Micrococcus luteus	-	5.2 ± 0.03		
	Staphylococcus aureus	-	9.2 ± 0.04		
	Aspergillus oryzae	-	14.4 ± 0.03		
	Aspergillus niger	-	12.2 ± 0.03		
	Penicillium digitatum	-	6.4 ± 0.03		
	Mucor	-	9.3 ± 0.04		
essential oil	*Salmonella typhimurium*	0.3	22	thymol (36.7%), γ-terpinene (36.5%), *p*-cymene (21.1%)	[6]
	Pseudomonas aeruginosa	10	0		
	Escherichia coli	0.3	21		
	Staphylococcus aureus	0.1	23		
essential oil	*Staphylococcus aureus*	6	27	thymol (43.7%), *p*-cymene (17.7%)	[7]
	Enterococcus faecalis	6	25		
	Escherichia coli	6	20		
	Pseudomonas aeruginosa	6	11		
	Proteus vulgaris	60	23		
	Klebsiella pneumoniae	60	26		
	Salmonella sp.	6	30		
	Candida albicans	6	27		
essential oil	MRSA	3.56	-	γ-terpinene (48.1%), *p*-cymene (33.7%), thymol (17.4%)	[21]
	MSSA	4	-		
	VR *E. faecalis*	8	-		
	VR *E. faecium*	1.41	-		
	VS *E. faecalis*	1.18	-		
	Streptococcus pyogenes	2	-		
	TGC Resistant *Escherichia coli*	7	-		
	TGC Sensitive *Escherichia coli*	3	-		
	Enterohemorrhagic *Escherichia coli*	2	-		
	Pseudomonas aeruginosa(MR)	21.11	-		
	Pseudomonas aeruginosa(SS)	20.15	-		
	Shigella flexneri	0.5	-		
	Salmonella enterica	1	-		
	Candida albicans	1.63	-		
	Candida tropicalis	0.5	-		
	Candida parapsilosis	8	-		
	Candida glabrata	0.5	-		
	Candida krusei	4	-		
	Aspergillus flavus	2	-		
	Aspergillus fumigatus	1	-		
ethanol extract	*Escherichia coli*[2]	-	17.67 ± 0.04	cymene, terpinene, terpineol, carvacrol, emersol, ethyl ester, phenol 4-methoxy, 2,3,6-	[22]
	Klebsiella pneumoniae[2]	-	15.72 ± 0.58		
	Citrobacter amalonaticus[2]	-	18.67 ± 0.57		

	Citrobacter diversus[2]	-	17.33 ± 0.39	trimethyl	
	Pseudomonas aeruginosa[2]	-	15.33 ± 0.67		
	Proteus mirabilis[2]	-	18.67 ± 0.63		
	Proteus vulgaris[2]	-	19.84 ± 0.52		
	Escherichia coli[3]	-	15.33 ± 0.57		
	Klebsiella pneumoniae[3]	-	15.33 ± 0.57		
	Citrobacter amalonaticus[3]	-	15.33 ± 0.57		
	Pseudomonas aeruginosa[3]	-	16.33 ± 0.57		
essential oil	*Pseudomonas syringae*	-	28.3	-	[45]
	Bacillus subtilis	-	33.3		
	Escherichia coli	-	32.1		
	Staphylococcus sp.	-	35.3		
essential oil	*Bacillus cereus*	0.5	35	thymol (27.6%), γ-terpinene (15.9%), β-ocimene (14.1%), *p*-cymene (14.1%)	[46]
	Staphylococcus aureus	0.5	33.5		
	Listeria monocytogenes	0.5	36		
	Salmonella typhimurium	0.5	34		
	Escherichia coli	0.5	39		
	Penicillium citrinum	2	>80		
	Penicillium chrysogenum	2	>80		
	Aspergillus flavus	2	>80		
	Aspergillus niger	1	>80		
	Aspergillus parasiticus	3	>80		
essential oil	*Escherichia coli*	2.5-10[4]	-	-	[46]
	Klebsiella sp.	1.25-10[4]	-		
	Staphylococcus aureus	2.5-5[4]	-		
essential oil	*Bifidobacterium bifidum*	2.75	-	-	[24]
	Bifidobacterium lotigum	2.75	-		
	Bacteroides fragilis	1.3	-		
	Candida albicans	1.3	-		
	Clostridium difficile	1.3	-		
	Clostridium perfringens	1.3	-		
	Enterococcus faecalis	2.75	-		
	Escherichia coli	5.5	-		
	Eubacterium limosum	5.5	-		
	Lactobacillus acidophilus	22	-		
	Lactobacillus plantarum	22	-		
	Peptostreptococcus anaerobius	2.75	-		
essential oil	*Bacillus cereus*	2 ± 0.4	25 ± 0.11	thymol (45.6%), γ-terpinene (20.0%), *p*-cymene (11.0%)	[21]
	Bacillus subtilis	2.5 ± 0.4	15 ± 0.21		
	Staphylococcus aureus	NA	NA		
	Shigella shiga	5.5 ± 0.5	17 ± 0.02		
	Escherichia coli	5.5 ± 0.1	12 ± 0.5		
	Shigella sonnei	4.4 ± 0.12	24 ± 0.5		
	Pseudomonas aeruginosa	NA	NA		
	Candida parapsilosis	1.5 ± 0.11	30 ± 0.21		
	Aspergillus niger	2 ± 0.2	21 ± 0.41		
	Aspergillus fumigatus	3.5 ± 0.78	24 ± 0.5		
essential oil	*Bacillus cereus*	2	-	γ-terpinene (48.1%), *p*-cymene (33.7%), thymol (17.4%)	[47]
	Streptococcus sobrinus	4	-		
	Enterococcus faecalis	2	-		
	Streptococcus salivarius	1	-		
	Streptococcus mutans	1	-		
	Streptococcus pyogenes	0.5	-		
	Streptococcus sanguinis	2	-		
	Staphylococcus aureus	0.5	-		
	Staphylococcus aureus	1	-		
	Pseudomonas aeruginosa (MR)	4	-		
	Pseudomonas aeruginosa (SS)	2.66	-		
	Salmonella paratyphi	1	-		
	Pseudomonas aeruginosa	4	-		
	Escherichia coli	1	-		
essential oil	*Staphylococcus aureus*	2.5	-	-	[26]
	Bacillus subtilis	2.5	-		
	Escherichia coli	2.5	-		
	Pseudomonas aeruginosa	2.5	-		
extract	*Helicobacter pylori*	31.25-125	25-40	-	[48]

1: Isolated strains from patient, 2:Extended spectrum β-lactamase (ESBL) producer, 3: Metallo- β-lactamase (MBL), 4: mg/mL, 5: µg/mL, Conc.: Concentration, MIC: Minimum inhibitory concentration, IZD: Inhibition zone diameter, MRSA: Methicillin resistant *S. aureus*, MSSA: Methicillin sensitive *S. aureus*, VR: Vancomycin resistant, VS: Vancomycin sensitive, TGC: Third generation Cephalosporin, NA: Not active, MR: Multidrug-resistant, SS: Sensitive strain.

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