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Investigating the antibacterial and phytochemical effect of methanol and acetone extracts of the *Cupressus sempervirens* and *Juniperus excelsa* on some important foodborne diseases

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ABSTRACT

Background & Aim: The unfettered usage of antibiotics has engendered a mounting resistance of bacteria to these agents, thereby necessitating the discovery and development of novel compounds and medications to a greater extent than previously. The focal point of this research was to explore the chemical constituents of acetone and methanol-extracted samples of *Cupressus sempervirens* and *Juniperus excelsa*, as well as their potential inhibitory actions against a prevalent food-borne pathogen.

Experimental: This experimental investigation was conducted on standard strains of *Staphylococcus aureus* (PTCC 1430), *Bacillus cereus* (PTCC 1431), *Listeria monocytogenes* (PTCC 1298), *Escherichia coli* (PTCC 1399), and *Shigella dysentery* (PTCC 1188). Following the preparation of methanol and acetone extracts derived from *Juniperus excelsa* and *Cupressus sempervirens* using Clevenger apparatus, the antimicrobial efficacy was assessed by both qualitative agar well diffusion method and quantitative macrodilution method. The active constituents present in the methanol and acetone extracts of the plants were identified by gas chromatography-mass spectrometry (GC/MS) analytical method. Means were compared with Duncan's test at the 5% probability level.

Results: The findings of this investigation pertaining to the antimicrobial potency of the extracts, ascertained via the qualitative agar well diffusion method, indicated its efficacy against Gram-positive strains including *S. aureus* and *B. cereus*. The methanol extracts of the *J. Excelsa* were found to produce smaller inhibition zones on the tested bacteria compared to other plant extracts. The highest sensitivity to the acetone extract of *C. sempervirens* and *J. excelsa* observed in *S. aureus* and *B. cereus*. The extracts obtained from the two plant did not demonstrate any discernible impact on the Gram-negative bacteria that are commonly associated with foodborne pathogens. The findings obtained through gas chromatography-mass spectrometry (GC/MS) indicated the presence of efficacious components such as Benzene 1,2,4,5-tetramethyl, and Cyclopropane cyclopenta in *J. excels* extract. The acetone extract of *J. excelsa* showed more potent antimicrobial constituents than its methanolic counterpart. It is anticipated that in forthcoming times, the acetone extract derived from this botanical specimen may be employed to prevent bacterial-induced foodborne illnesses.

Recommended applications/industries: Duo to the existence of a range of bioactive compounds in the acetone extracts obtained from *C. sempervirens* and *J. excels*, these extracts have the potential to be used against the development of foodborne infections and diseases caused by bacterial agents.

1. Introduction

The proliferation of pathogenic bacteria in foodstuffs represents a significant public health concern, as it contributes to a high incidence of diseases, many of which are prevalent on a global scale (Abebe *et al.*, 2020; Dhama *et al.*, 2013). Enterotoxins produced by *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Yersinia* spp., and *Clostridium* spp. are responsible for poisoning the digestive system and causing gastrointestinal symptoms (Bintsis *et al.*, 2017; Tod *et al.*, 2014; Kadariya *et al.*, 2014; Akbar *et al.*, 2011). Foodborne illnesses manifest in two forms; a subset of these illnesses comprises food poisoning, whereby symptoms arise from the consumption of food contaminated with pathogenic agents such as bacteria and viruses (as a result of non-observance of hygiene principles) and indicate their symptoms quickly (Tod *et al.*, 2014; Zhao *et al.*, 2014; CDC, 2013).

Amongst the various illnesses that afflict humans, we can mention that are accompanied by diarrhea and vomiting. The second classification arises as a consequence of food contamination by several chemicals, fungal toxins, pesticides, heavy metals, and insecticides (Lebelo *et al.*, 2021; Alengebawy *et al.*, 2021). The manifestation of symptoms associated with prolonged consumption of contaminated food is often observed, leading to adverse health outcomes like neurological impairment, congenital anomalies, and diverse forms of cancer (Rather *et al.*, 2017). Unseemly use of anti-microbial agents not only causes medicate resistance but also have side effects. The development of planting medicinal plants can be of great help in this field, as these plants have been used since ancient times to treat infections, as well as to flavor and preserve food (Miller *et al.*, 2022; Hashempour-Baltork, 2019). These substances have been ascertained as benign and salubrious and traditional utilization devoid of any documentation of detrimental outcomes, along with supporting toxicological scrutiny (Shane *et al.*, 2009; Parasuraman, 2011). *Juniperus excelsa* is an evergreen tree or shrub from the *Cupressaceae* family that grows in the countries of the Balkan region, Turkey, Syria, Lebanon, Georgia, Armenia, Azerbaijan, and the eastern part of Iran near Turkmenistan, as well as in the Northeastern areas of the coasts of the Aegean Sea (Asili *et al.*, 2008; Mozaffarian, 1996., Darvishi *et al.*, 2016).

Juniprus excelsa is considered as a medicinal plant that was used in the past to treat painful menstruation, cough, bronchitis and colds, jaundice, tuberculosis and to stimulate menstruation (Fujita *et al.*, 1995). *Cupressus sempervirens* is a species of evergreen trees, belonging to *Pinidae* subclass, the *Pinales* order, and *Cupressaceae* family. *C. sempervirens* is an attractive tree with a height of 20 to 30 m spread throughout North America, the Northern Mediterranean zone and Asia (Orhan and Tumen, 2015; Rawat *et al.*, 2009; Selim *et al.*, 2014). The wood of this tree is recognized safe to rot and insect's intrusion. The plant *C. sempervirens* has special botanical characteristics, counting resilience in dry and cold conditions, resistance to diverse climatic conditions, and compatibility in acidic and antacid soils (Auria *et al.*, 2020; Baldi, 2011).

In medicinal application, it has been suggested that the ingestion of the leaf decoction of this tree holds potential in treating conditions such as urinary infections, diabetes as well as intestinal and gastric ulcers. Additionally, the gargling of the mentioned decoction is useful in cases of toothache, gum ulcers and insufficient gum health. The desiccated seeds are utilized in the management of various types of wounds, contusions, cutaneous papules, and acne. The essential oil derived from the foliage of plant has been utilized for the treatment of acute and chronic colds as well as bronchitis (Al-Snafi *et al.*, 2016; Milos *et al.*, 2002; Tumen *et al.*, 2012; Selim *et al.*, 2014; Boukhris *et al.*, 2012). A limited number of studies have addressed the antibacterial properties of the aforementioned botanical specimen. *C. sempervirens* a native plant species in Iran, has been known for its numerous medicinal properties and investigation on its antibacterial properties can provide valuable information (Frezza *et al.*, 2022). Therefore, in the present study, the effects of methanol and acetone extracts of these plants were investigated on some important standard food born pathogenic bacteria including *Staphylococcus aureus* (PTCC 1430), *Bacillus cereus* (PTCC 1431), *Listeria monocytogenes* (PTCC 1298), *Escherichia coli* (PTCC 1399), and *Shigella dysentery* (PTCC 1188) using both qualitative (agar well diffusion) and quantitative (macrodilution) techniques. Moreover, the main composition of the essential oil obtained from the aerial parts of *C. sempervirens* and *J. excelsa* was determined.

2. Materials and Methods

2.1. Extraction of methanolic extract of Francoeuria Undulata

In this study, *Francoeuria Undulata* was collected at two periods from Ardestantown in northeastern Isfahan: a) September 2014 and b) September 2015.

Aerial parts of the plant were dried out at room temperature in the shade. Dried-out samples were powdered using a grinder and kept at 4ºC (Nabipour, 2015). Then, powder was soaked in 80% methanol for 72 hours to prepare the extract. For this purpose, 50 grams of the powder was mixed with 500 ml of 80% methanol. The mixture was shaken to mix the powder with the solvent. Then, the flasks containing the mixture were covered with aluminum taps. The flasks were shaken at 150rpm for 72 hours at room temperature. These extracts were filtered with a filter paper and transferred to sterilized plates at ambient temperature until they were dried out. Then, the extracts were condensed with 10%dimethyl sulfoxide (DMSO) solvent. Then, the extracts were prepared at 62.5, 125, 250 and 500 mg/ml concentrations (Cowan, 1999; Skocibusic *et al.*, 2004).

The bacteria were isolated from 100 inpatients and outpatients aged from 20 to 50 who visited the Shahid Beheshti Gynecologic Clinic in Isfahan.

2.2. Antibacterial assay

Blood Agar and EMB (Daruash-Tehran) media were used for primary culture of the isolates. Biochemical and confirmatory tests were used to detect the bacteria (Sokmen *et al.*, 2003; Mardane, 2012).

Bacterial genus and species were detected and confirmed using the above tests (Naghsh, 2013). Antibiogram patterns were determined using antibiotic discs (5µg trimethoprim, 30µg ceftazidime, 30µg cefotaxime, 5µg ciprofloxacin, 10µg gentamicin, 30µg cefalotin, 5µgcefixime, 30µg tetracycline, 10µg streptomycin, 10µg penicillin). Ciprofloxacin was identified as a positive control given antibiotic sensitivity and resistance of each bacterium to various antibiotics and common sensitivity tociprofloxacin.

Isolated bacteria were glycerolizedin a freezer at 20°C. All isolates were revived by standard methods. Fresh cultures of multi-colony bacteria were transferred to Mueller-Hinton broth to prepare bacterial suspension. Turbidity of bacterial suspension was set in accordance with McFarland Standard as1.5×10^6cfu/ml. Antimicrobial effects of methanol extracts of aerial parts of *F. undulata*at two harvest times on several gram-positive and gram-negative aerobic bacteria causing vaginal infections were studied using two methods of serial dilutions and well-plate. Various concentrations of the extract were prepared for both old and new harvest times.

In well-plate method, 100 ml of microbial suspension with concentration of 1.5×10^6cfu/ml was evenly spread in Mueller-Hinton agar medium. Then, three wells with 6mm diameter at 2.5cm distance were created in each medium. Then, 100ml of each concentration was transferred to each well. The wells were sealed (bottom of the wells were closed). Negative control was determined as 10% DMSO and 10% Tween80. Positive control was determined as 5µg ciprofloxacin. All media were incubated for 24h at 37°C. Then, bacterial cultures were evaluated in terms of presence or absence of inhibition zone around the wells (Rajabpour *et al.*, 2013). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extracts were determined using tube dilution method (macro-dilution) (Talebi, 2014).

To determine the MICs, 62.5, 125, 250 and 500 mg/ml dilutionsof methanol extracts were prepared. In addition, one ml bacterial suspension with a concentration of 1.5×10^6cfu/ml was added to every dilution. Tubes of positive control (with bacterial medium) negative control (without bacterial medium) were prepared. The tubes were incubated for 24 h at 37°C. After incubation, the tubes were studied in terms of turbidity and growth of inoculated bacteria.

2.3. Statistical analysis

Each test was repeated 3 times. Then, mean and standard deviation were calculated. Data analysis was performed using SPSS20, ANOVA and LSD. The difference between groups was determined at $P<0.001$ significance level.

3. Results and discussion

3.1. The antimicrobial effects of the extracts by well agar diffusion

The present study investigated the zone of inhibition observed with different concentrations of the acetone and methanol extracts derived from *C. sempervirens*

and *J. excelsa* plant against bacterial colonies. The diameter of the inhibition zone resulting from the acetone extract of *J. excels* at a concentration of 500 mg/mL against two standard food bacteria, *Staphylococcus aureus* and *Bacillus cereus*, exhibited inhibition zone diameters measuring 28.66 and 19.6 mm, respectively, while for the methanolic extract of the same plant, 28.33 mm and 18.66 mm were obtained, respectively. The acetone extract of *C. sempervirens* at a concentration of 500 mg/mL was effective against *S. aureus* and *B. cereus* with the inhibition zone diameters of 26 mm and 16.33 mm, respectively, while the methanol extract effectively produced inhibition zones of 25 mm and 16 mm for these two bacteria, respectively. The results of this study showed that *S. aureus* and of *B. cereus* were the most sensitive strain to the acetone extract of *C. sempervirens* and *J. excelsa* while the diameter of the inhibition zones on these bacteria was smaller by the methanol extracts of plant. *Listeria monocytogenes* exhibited increased susceptibility to the extracts relative to the two Gram-negative bacterial strains. The results revealed that in the case of *L. monocytogenes*, only a reduction in the bacterial growth was evident upon treatment with the two extracts. The zone of inhibition for different concentration of the methanolic plant extract of *J. excelsa* on bacterial growth, as depicted in millimeters, are illustrated in Tables 1-4. The employment of botanical extracts in the management of illnesses dates back to antiquity (Petrovska, 2012; Pan *et al.*, 2014). Given the potential compatibility of these substances with the human body and their advantageous therapeutic properties, it is imperative to explore the antibacterial properties of plants commonly utilized in traditional medicinal practices (Vaou *et al.*, 2021; Bittner *et al.*, 2021). In this research, by performing two qualitative and quantitative methods, the methanol and acetone extracts of *C. sempervirens* and *J. excelsa* exhibited significant antimicrobial activity against several Gram-positive pathogenic bacteria commonly found in food. According to agar well diffusion method, the growth inhibition zone diameter on the two studied bacteria, *S. aureus* and *B. cereus*, was expanded by the higher concentration of acetone and methanol extracts of both plants. Furthermore, the tested extracts were not effective on Gram-negative bacteria, *E. coli* and *Shigella dysentery* and showed little effects on *L. monocytogenes*. *S. aureus*, having a larger diameter of growth inhibition zone by different concentrations of the extracts and a smaller MIC value compared to *Bacillus cereus*, was more sensitive to the methanol and acetone extracts of *C. sempervirens* and *J. excelsa*. According to the findings of present investigation, disparities in the cellular wall configuration of Gram-positive and Gram-negative bacteria may precipitate variations in their reactions towards *C. sempervirens's* acetone and methanol extracts. These observations are in line with the findings of other researchers who reported the greater sensitivity of Gram-positive bacteria to the plant extract (Ouattara *et al.*, 1997; Das *et al.*, 2012; Koohsari *et al.*, 2015; Elisha *et al.*, 2017). It has been documented that not only the presence of external lipopolysaccharide in the cell walls of Gram-negative bacteria can act as an external barrier against toxic particles, but also due to the abundant lipid in the different layers of the cell wall of these bacteria such as LPS, PL and LP layers, the entry of hydrophilic non-polar compounds into the bacterial cell is prevented (Gaunt *et al.*, 2005). The zone diameters of inhibition exhibited by the acetone and methanolic extracts were found to be significantly distinct from those of the other extracts, with a statistical significance level of $P<0.05$.

Table 1: Zone of inhibition (mm) for different concentration of the acetone extract of *Cupressus sempervirens* on tested bacteria.

Concentration mg/mL	3.125	6.25	12.5	25	50	100	125	250	500	Control (Tetracycline 20 µg/mL)
Staphylococcus aureus (PTCC1430)	0.00^{a}±8.00	0.00^{a}±10.00	0.33^{a}±11.33	0.33^{a}±12.66	0.33^{a}±15.33	0.00^{a}±17.00	0.33^{a}±18.66	0.00^{a}±22.00	0.00^{a}±26.00	0.33^{a}±38.33
Bacillus cereus (PTCC1431)	0.33^{b}±7.33	0.00^{b}±9.00	0.33^{b}±10.66	0.33^{b}±11.33	0.33^{b}±12.33	0.33^{b}±13.66	0.33^{b}±13.66	0.33^{b}±14.66	0.33^{b}±16.33	0.33^{a}±32.33
Listeria monocytogenes (PTCC1298)	-	-	-	-	-	-	-	-	-	0.33^{b}±15.66
Escherichia coli (PTCC1399)	-	-	-	-	-	-	-	-	-	0.33^{c}±9.33
Shigella dysentery (PTCC1188)	-	-	-	-	-	-	-	-	-	0.33^{b}±11.66

All the values are mean ± SEM of 3 determinations; (-): No zone of inhibition
Control: Antibiotic (Tetracycline) at a concentration of 20 µg/ml as positive control
* a,b,c: The values with different letters in a column are significantly different ($P<0.05$)

Table 2: Zone of inhibition (mm) for different concentration of the methanolic extract of *Cupressus sempervirens* on bacteria.

Concentration mg/mL	3.125	6.25	12.5	25	50	100	125	250	500	Control (Tetracycline 20 µg/mL)
Staphylococcus aureus (PTCC1430)	0.00^{a}±8.00	0.33^{a}±9.33	0.00^{a}±11.00	0.33^{a}±12.33	0.00^{a}±15.00	0.33^{a}±15.66	0.33^{a}±18.33	0.33^{a}±20.66	0.00^{a}±25.00	0.33^{a}±38.33
Bacillus cereus (PTCC1431)	0.33^{b}±3.33	0.33^{b}±8.33	0.33^{b}±9.33	10.33^{b}	0.33^{b}±11.33	0.33^{b}±13.33	0.33^{b}±13.33	0.33^{b}±14.33	16^{b}	0.33^{a}±32.33
Listeria monocytogenes (PTCC1298)	-	-	-	-	-	-	-	-	-	0.33^{b}±15.66
Escherichia coli (PTCC1399)	-	-	-	-	-	-	-	-	-	0.33^{c}±9.33
Shigella dysentery (PTCC1188)	-	-	-	-	-	-	-	-	-	0.33^{b}±11.66

All the values are mean ± SEM of 3 determinations; (-): No zone of inhibition
Control: Antibiotic (Tetracycline) at a concentration of 20 µg/mL as positive control
* a,b,c: The values with different letters in a column are significantly different (P<0.05).

Table 3: Zone of inhibition (mm) for different concentration of the acetone extract of *Juniperus excelsa* on bacteria.

Concentration mg/mL	3.125	6.25	12.5	25	50	100	125	250	500	Control (Tetracycline 20 µg/mL)
Staphylococcus aureus (PTCC1430)	0.33^{b}±8.66	0.33^{a}±10.66	0.00^{a}±12.00	0.00^{a}±14.00	0.33^{a}±15.66	0.33^{a}±17.66	0.33^{a}±20.66	0.33^{a}±24.66	0.33^{a}±28.66	0.33^{a}±38.33
Bacillus cereus (PTCC1431)	0.33^{a}±9.66	0.33^{a}±10.66	0.33^{a*}±11.66	0.33^{b}±12.66	0.33^{b}±13.66	0.33^{b}±15.66	0.33^{b}±15.66	0.33^{b}±16.66	0.00^{b}±19.00	0.33^{a}±32.33
Listeria monocytogenes (PTCC1298)	-	-	-	-	-	-	-	-	-	0.33^{b}±15.66
Escherichia coli (PTCC1399)	-	-	-	-	-	-	-	-	-	0.33^{c}±9.33
Shigella dysentery (PTCC1188)	-	-	-	-	-	-	-	-	-	0.33^{b}±11.66

All the values are mean ± SEM of 3 determinations; (-): No zone of inhibition
Control: Antibiotic (Tetracycline) at a concentration of 20 µg/mL as positive control
* a,b,c: The values with different letters in a column are significantly different (P<0.05)

Table 4: Zone of inhibition (mm) for different concentration of the methanolic extract of *Juniperus excelsa* on bacteria.

Concentration mg/mL	3.125	6.25	12.5	25	50	100	125	250	500	Control Tetracycline 20 µg/ml
Staphylococcus aureus (PTCC1430)	0.00^{b}±7.00	0.00^{a}±10.00	0.33^{a}±12.33	0.33^{a}±11.66	0.33^{a}±15.33	0.00^{a}±15.00	0.33^{a}±18.66	0.00^{a}±21.00	0.00^{a}±24.00	0.33^{a}±38.33
Bacillus cereus (PTCC1431)	0.33^{a}±9.66	0.00^{b}±8.00	0.33^{b}±10.66	0.33^{b}±11.33	0.33^{b}±13.33	0.33^{b*}±14.66	0.33^{b}±14.66	0.33^{b}±15.66	0.33^{b}±17.33	0.33^{a}±32.33
Listeria monocytogenes (PTCC1298)	-	-	-	-	-	-	-	-	-	0.33^{b}±15.66
Escherichia coli (PTCC1399)	-	-	-	-	-	-	-	-	-	0.33^{c}±9.33
Shigella dysentery (PTCC1188)	-	-	-	-	-	-	-	-	-	0.33^{b}±11.66

All the values are mean ± SEM of 3 determinations; (-): No zone of inhibition
Control: Antibiotic (Tetracycline) at a concentration of 20 µg/mL as positive control
* a,b,c: The values with different letters in a column are significantly different (P<0.05).

3.2. The MIC and MBC

The MIC and MBC values of two acetone and methanol extracts of *C. sempervirens* and *J. excelsa* using quantitative macrodilution method are shown in Table 5. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanol and acetone extracts against *S. aureus* were 125 mg/mL and 250 mg/mL, respectively. The results of MIC and MBC of the methanol and acetone extracts of two plants against *B. cereus* were 250 mg/mL and 500 mg/mL, respectively.

Table 5. MIC and MBC methanolic and acetone extracts of *Cupressus sempervirens* and *Juniperus excelsa* (mg/mL)

Standard bacterial strains	Acetone extracts		Methanolic extracts	
	MIC	MBC	MIC	MBC
Staphylococcus aureus (PTCC1430)	125	250	125	250
Bacillus cereus (PTCC1431)	250	500	250	500
Listeria monocytogenes (PTCC1298)	-	-	-	-
Escherichia coli (PTCC1399)	-	-	-	-
Shigella dysentery (PTCC1188)	-	-	-	-

The significance level of the test (P<0.05) for data interpretation.

3.3. The bioactive compounds of extracts using GC-MS

Considering that the acetone extract of both plants showed more antimicrobial effects, so the chemical composition of this extract was evaluate and the results are shown in Tables 6 and 7. The results of the present research revealed that the acetone extract of *C. sempervirens* contains effective compounds such as Sabinene, Gamma-Terpinene, Germacrene D, Caryophyllene, and Delta Cadinene. Among the identified compounds, the highest concentration in the acetone extracts of *J. excelsa* and *C. sempervirens* were related to the (Benzyloxy)-6, 11-dimethoxy-1, 2, 3 (14.08%) and Cyclopropane cyclopenta (6.9%), respectively. These compounds have physiological effects in addition to antioxidant, antibacterial, anti-inflammatory, and antifungal properties (Zahed *et al.*, 2013). Selim et al (2014) identified α-pinene (48.6%), δ-3-carene (22.1%), limonene (4.6%) and α-terpinolene (4.5%) as the main components comprising 79.8% of the oil. El Hamrouni-Aschi et al (2013) were reported α-Pinene, δ-car-3-ene, limonene, carvacrol methyl ether, α-humulene, and α-amorphene in *C. sempervirens* L. var. *numidica* TRAB oils using GC/MS analyses. Therefore, the antibacterial properties of methanol and acetone extracts of *C. sempervirens* and *J. excelsa* can be attributed to the presence of effective compounds in these extracts.

Table 6: Chemical compounds identified in the acetone extract of *Cupressus sempervirens.*

Line	Percentage	Composition	Inhibition index
1	1.44	Diacentone alcohol	3.740
2	0.59	α - thujene	5.540
3	0.76	Sabinene	6.861
4	1.48	D-allose	21.995
5	0.97	Propylpyrocatechol	24.456
6	1.50	Mome inositol	27.082
7	1.70	Isomytilitol	27.435
8	1.74	Tetradecanoic acid	27.578
9	3.01	Neophytadiene	28.954
10	0.96	Neophytadiene	29.836
11	1.98	Palmitic acid	32.652
12	1.45	Isopimaradiene	32.567
13	1.21	Dehydroabiteane	33.618
14	2.93	β- irone	34.541
15	1.07	Ethyl linoleolate	34.998
16	1.15	Durene	35.321
17	1.32	Totarol	37.632
18	1.94	Isopimaradiene	38.360
19	4.46	Ferruginol	38.435
20	6.69	Benzene,1,2,4,5-tetramethyl	39.073
21	0.94	β-androstane	40.686
22	2.09	Bis[(3-methyl-5-phenyl)-2-furyl]methane	40.851
23	6.95	Cyclopropane cyclopenta	41.826
24	3.05	Germacrene-B	49.765

Table 7. The results of the identification of compounds in the acetone extract of *Juniperus excels.*

Line	Percentage	Composition	Inhibition index
1	0.12	Sabinene	7.123
2	0.09	γ-terpinene	11.852
3	0.15	β-elemene	13.821
4	0.14	α.-Humulene	14.783
5	0.10	β-Cubebene	14.853
6	0.76	Germacrene-D	15.707
7	0.44	α -Muurolene	15.284
8	2.55	Delta-Cadinene	15.522
9	0.27	α -Amorphene	15.714
10	2.10	Elemol	15.938
11	3.51	1,6-Germacradien-5-ol	16.330
12	0.53	α -Cadinol	17.261
13	0.32	α -Eudesmol	17.300
14	2.46	Anisole	18.208
15	1.45	Neophytadiene	18.923
16	4.68	Epimanool	21.432
17	1.43	Caryophyllene	22.086
18	1.49	Prop-2-enyl cyclopenta-1,3-diene-1-carboxylate	22.932
19	1.73	Abietatriene	23.256
20	1.54	Cembrene	23.563
21	1.08	Cembrene	23.417
22	2.14	Caryophyll	23.671
23	5.77	Cycloisolongifolene	23.763
24	1.16	٢,3-Diphenyl-7-[(E)-styryl]dibenzothiophene	23.848
25	2.14	Torreferol	24.179
26	1.66	Ethenylcyclohexane	24.271
27	0.97	Antioxine	24.325
28	2.92	8,13-Epoxylabda-2,14-Diene	24.610
29	4.53	Methyl abietate	25.079
30	8.26	1-Nonadecanol	31.289
31	1.43	Vitamin E	31.851
32	14.06	(Benzyloxy)-6,11-dimethoxy-1,2,3	34.006

Weli *et al.* (2014) considered the effect of essential oil of Omani *J. excelsa* fruit on foodborne pathogenic

bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. They found 48 chemical compounds by GC–MS. The antibacterial properties of the essential oil of *J. excelsa* was inactive against studied bacteria. The results of the present study showed that the acetone extract of *C. sempervirens* and *J. excelsa* had a more appropriate inhibitory effect on controlling the growth of Gram-positive bacteria including *S. aureus* and *B. cereus* compared to its methanol extract. Antibacterial and antiparasitic properties of *C. empervirens* leaves were evaluated by Zhang *et al*. (2012) on *B. subtilis*, *E. coli*, and *Agrobacterium* sp. In this research, different methanol, ethanol, ethyl acetate, and aqueous extracts of the leaves have been investigated. The results showed that the methanol extract had the highest lethal effect on the considered bacteria. The results of present study are somewhat contradictory to the findings of Zhang' study, because of difference in the type of plant and the species of bacteria studied. In the present study, acetone and methanol extracts of *C. sempervirens* and *J. excelsa* were only effective against Gram-positive bacteria, while in the aforementioned study, methanol, ethanol, ethyl acetate, and aqueous extracts of the plant were effective against both Gram-positive and Gram-negative bacteria. This difference can be related to the type of solvent used, and even related to the type of plant and organ under investigation. Also, in that study, the most effective extract was methanol extract, while in the present study, the most effective extract was acetone extract. It is noteworthy that in numerous studies, the selection of a solvent may exert a considerable influence on the efficacy of bioactive compounds extraction. Moreover, it appears that the botanical origin's geographic location and climatic conditions may exert a discernible impact on the compounds identified through GC-MS analysis in the plants (Hosni *et al.*, 2019; Azzaz *et al.*, 2019). Different compounds such as limonene, α-pinene, Sabinene, bornyl acetate, myrsene and delta-cadinene have been detected in *C. sempervirens* (Boukheris *et al.*, 2012). These compounds showed antibacterial, antifungal, antiviral, anti-inflammatory, anti-diabetic, and larvicidal properties (Orhan *et al.*, 2015; Rawat *et al.*, 2009). Therefore, it is expected that in the future it can be used as a suitable alternative to chemical antibiotics for the treatment of infections and important food pathogenic bacteria. In previous study on antibacterial and antiparasitic properties of *C. sempervirens*, it has been concluded that the ethanol extract of the plant cones has lethal effects against *S. aureus*. According to GC analysis, diterpenes substances such as Taxodion, Ferruginol and Sugiol were present in the cone extract of this plant (Zhang *et al.*, 2012).

4. Conclusion

The present study proposes that the *C. sempervirens* and *J. excelsa* extracts were rich sources in natural phytocompounds and exhibiting noteworthy antimicrobial characteristics. The present study revealed that the acetone extract of *J. excels* exhibited a higher potency of antimicrobial compounds in comparison to the methanolic extract of the aforementioned plant. Notably, the most efficacious antimicrobial compounds, such as Benzene, 1, 2, 4, 5-tetramethyl, and cyclopropane cyclopenta, were predominantly present in the acetone extract of *J. excels*. The acetone extract of *C. sempervirens* and *J. excelsa* had a more appropriate inhibitory effect on controlling the growth of Gram-positive bacteria such as *S. aureus* and *B. cereus*. Among the tested food poisoning bacteria, *S. aureus* and *B.* cereus exhibited the pronounce sensitivity against the extract of *J. excels*. In addition, the extracts derived from both investigated plant species did not show any significant effect on the proliferation of Gram-negative bacteria

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