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Effect of habitat variations on the chemical composition, antioxidant, and antimicrobial activities of *Achillea fragrantissima* (Forssk) Sch. Bip

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ABSTRACT

Achilea fragmentisma plant is widely distributed along northern regions of Saudi Arabia with various traditional medical uses. The plant was collected from Tabuk and Arar regions to study the effect of the variation in habitat on the chemical compositions, antimicrobial and antioxidant activities of the plant. The results showed significant differences between the two studied region regarding the parameters of the weather in years from 2010–2016.The antioxidant and antimicrobial of the plant showed significant variation in two habitats. Plant collected from Arar showed high antioxidant activity with IC50 (0.21 ± 0.01 g/L) by DPPH radical scavenging methods, and good antibacterial activity with gram-positive bacteria (Staphylococcus epidermidis, *Bacillus cereus* and *Staphylococccus aureus* clinical isolate), antibacterial activity ranging between high to no activity (between 14.5 ± 0.5–6.0 ± 0.0 mm zone of inhibition), On the other side the tested plant extracts showed no effect on, all gram-negative bacteria. GC/MS data showed marked variation in chemical compositions of both phenolic and alkaloid compounds in plants collected from both regions. Phenolic compounds were accumulated with higher amounts(Ferulic acid, Eugenol and Salicylic acid ester) in Arar region, while the alkaloid fractions (Ethyl isoallocholate, Pterin 6-carboxylic-acid and kadain) showed higher concentrations in plants collected from Tabuk region. The results reflect the variation in of weather parameter, affect on chemical compositions and biological activities of the plant in two studied regions.

1. Introduction

Saudi Arabia shows a wide diversity in the plants flora, which contain 96 species belonging to 75 genera and 38 families (34 dicots and 4 monocots). Asteraceae had the highest contribution (12.5% of the total species) followed by Fabaceae (10.42%), Zygophyllaceae (6.25%) and Lamiaceae (5.21%). The life form spectrum of the recorded species showed the prevalence of theorphytes (37%) followed by chamaephytes (32%), (13%), geeophytes (13%), hemi-cryptophytes (10%) and Phanerophytes (8%). The chorological analysis of the recorded species indicated the predominance of monoregional taxa over the other elements [1].

Saudi Arabia has large area so with different habitats including mountains, valleys, sandy, and rocky deserts [2]. In addition, The climatic behavior in Saudi Arabia from year 2000–2016 are characteristic by distribution in temperature over the country, in three distinct areas: the northwest is below 10 °C, the center is 10°–15 °C, and the east and the western coastal areas are 15°–20 °C during winter season. During the spring season, the temperatures increase all over the country, ranging between 20° and 35 °C, climbing more quickly in the north, whilst in the summer season, the whole country is warm, with temperatures reaching above 35 °C. In the summer season, the highest temperatures are observed in the south-eastern region, where the mean is above 35 °C. During autumn, moderate temperatures are observed over the country, which range between 25° and 30 °C, except in the northwest [3].

The genus of Achelia is represented by 115 species in North Africa, Asia and Southeast Europe. One of most famous plant of this genus A. fragarntissima known by its Arabic name Qaysoom represented in Egypt and Saudi Arabia [4] it is strongly fragrant perennial herbs and have been used by Bedouins as stomachic and anthelmintic. A. fragarnitissima used traditionally to treat fever (viral) and patients with chronic diseases such as arthritis and diabetes with reported anti-inflammatory, antioxidant, and anti-proliferative capacity on the cancer cells [5–9].

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Tabuk is located in the Northern part of Saudi Arabia and it has an area of 117,000 km2 with wide diversity in the habitats such as mountains, oasis and coastal line. While Arar, the capital city of the northern Border principality, also has a wide diversity in habitat (Fig. 1).Generally, climate of Tabok and Arar regions are considered as arid region and plant grow in this region supposed to drought stress [10].

As the difference in the environment conditions of different habitats is expected to affect the rates of photosynthesis and metabolism of the plants with marked effects on their primary, secondary metabolite and biological activity of the plant. So the present study was aimed to investigated the effects of the geographical variation of (Arar and tabuk), two big city in Northern region of Saudi Arabia, on antioxidant, antimicrobial activities, and chemical composition of plant *Achelia fragmentissima.*

2. Materials and methods

2.1. Habitat study

The data regarding the weather parameters in both Tabuk and Wadi Arar, during the years from 2010 to 2016, were kindly obtained from General Authority for Meteorology and Environment.

2.2. Plant material

Flowering aerial parts of *A. fragmentissimaa* were collected from two region of Tabuk and wade Arar, of Northern Region in Saudi Arabia, through year 2016 in spring season. The identity of the plants has been kindly verified in Faculty of Science, Tabuke University. Voucher specimens, (A.f. TU:274-s) were deposited in the Herbarium of Faculty of Science.

The fresh aerial parts of *A. fragmentissima* (100 gm plant powder) of the samples were extracted by percolation with a mixture of methanol water (80: 20, v/v), and filtered off. Also, the marc lifted was extracted by the same way (process repeated three times). The combined methanol extracts were concentrated under reduced pressure at temperature not exceeding 40 °C till dryness. Crude methanolic plant extract (10 g) of each sample were fractionated with chloroform (200 m) and filtered. The air-dried residue was washed successively with methanol (100 mL) and filtered. To the residue, 20 mL water and 10 mL 1 N HCl were added, stirred and 100 mL methylene chloride was added to extracts, tannins, flavonoid and other compound separated in neutral layer [11]. The aqueous layer was neutralized by adding 10% Na_2co_3 (5 mL) solution and kept for one day in a freezer and filtered. All these fractions were kept in desiccators for further assays.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging capacity

The DPPH radical scavenging activity of plant was assayed as a method described [12]. Quercetin and ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted. In this study, 0.5 mL of each sample concentration was homogenized with the same volume of DPPH methanolic solution (0.04 g/L). The mixture was shaken vigorously and allowed standing for 30 min in darkness at a temperature of 25 °C; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. The experiments were performed in triplicate for reliable data. Ascorbic acid was used as a positive control. The percentage inhibition was calculated as the following formula:

$$\%\ \text{Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

IC_{50} values were calculated using non-linear regression analysis. All statistical procedures were performed using PRISM 5 (GraphPad Software Inc., San Diego, CA). Significance was considered when p-value <0.05.

2.3.2. Ferric reducing antioxidant power (FRAP)

Methanol extract of plant *A. fragrantissima* was assayed for its reduction capacity following [13]. Briefly, potassium hexacyanoferrate $K_3Fe(CN)_6$ solution and 2.5 mL of phosphate buffer (0.2 mol/L, pH 7.0) were added to prepared extracts in concentrations range from 1–1000 mg/mL and incubated at 50 °C for 30 min. After, we added 2.5 mL of trichloroacetic acid (10%) to the mixture. Then, 2.5 mL of this solution was homogenized with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). Vitamin E was used as positive controls. The absorbance was measured by (details of the machine) at 700 nm and (EC_{50}) was determined. The assay was conducted in triplicate in each extract concentration.

2.4. Antibacterial activity

2.4.1. Microorganisms

In the current study, different bacterial species from gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876 and *Staphylococccus aureus* clinical isolate), and gram-negative bacteria

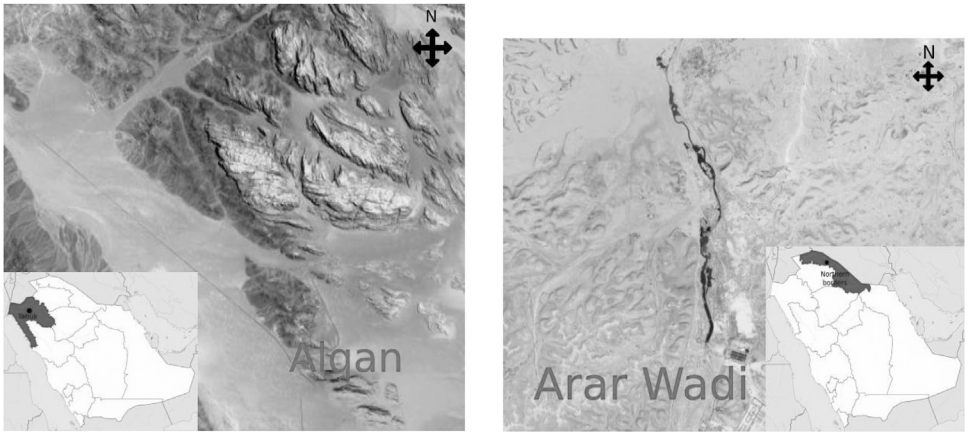

Fig. 1. The map of Saudi Arabia showing the two regoins from where the samples of the studied plants were collected. Both Arar and Tabuk area in the North-east and North-west of the Kingdom are highlighted.

Table 1
Climate parameters in both Arar and Tabouk regions during the period from January 2010 to December 2016. AR: Arar, TK: Tabouk.

Year	T		TM		Tm		T (Sum)		T (Win)	
	AR	TK	AR	TK	AR	TK	AR	TK	AR	TK
2010	24.8	24.6	33.0	32.1	17.0	16.7	37	34.16	12.6	15.04
2011	22.5	22.3	29.6	29.6	15.2	14.9	36.2	31.36	8.8	13.24
2012	23.5	23.0	30.7	30.2	16.2	15.4	37.2	33.26	9.8	12.74
2013	22.9	22.9	30.0	30.1	15.8	15.5	36.5	30.96	9.3	14.84
2014	23.5	23.2	30.7	30.4	16.4	16	38.1	31.06	8.9	15.34
2015	23.7	23.3	30.9	30.5	16.5	16.2	37.5	32.06	9.9	14.54
2016	24.0	23.6	31.3	30.8	16.7	16.3	37.2	32.9	10.8	14.3
	$P = 0.004^{**}$		$P = 0.03^{*}$		$P = 0.0012^{**}$		$P < 0.0001^{****}$		$P = 0.0002^{***}$	

Average annual temperature, TM: Annual average maximum temperature, Tm: Average annual minimum temperature, T (Sum): Average summer temperature, T (Win): average temperature in winter. V: Annual average wind speed, RA: Number of days with rain, TS: Number of days with storm, SN: Number of days with snow, PP: Rain or snow precipitation per annum, FG: Number of foggy days, GR: Number of days with hail.

(*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *Klebsiella pneumoniae* ATCC 27736 and clinical isolates of *Pseudomonas aeruginosa* and Acinito bacterbaum annii) were used to evaluate the antibacterial activities of the tested extracts.

2.4.2. Antibacterial test

Kirby-Bauer disc diffusion test NCCLS, 2002 [14,15] was used in the with minor modification to be suitable for the plant crude extract. The plant extracts of *Achillea fragrantissima* were reconstituted in 10%DMSO (Di-methyl-sulphoxide) to make a final concentration of 500 mg/mL of each extract. Microbial strains were sub-cultured in nutrient broth, samples from the broth cultures were pipette and diluted with sterile normal saline to make a suspension equivalent to the turbidity of the 0.5 McFarland standard. A sterile cotton swab was dipped in the adjusted suspension and smeared over previously prepared plates containing 20 mL Mueller Hinton agar. Then, A sterile paper discs, 6 mm in diameter were cut from No.1 Whatman filter paper and were immersed in the reconstituted extract (the paper disc is able to trap about 15 μL) and loaded over the smeared plates. Other paper discs of erythromycin (15 μg)and chloramphenicol (30 μg) were also used as positive control for ram positive and negative pathogens respectively. A disc saturated with 10% DMSO was also added (vehicle control). All plates were incubated at 35−37 °C for up to 24 h Then, the diameter of inhibition zones (in mm) were measured using a ruler including the diameter of the disc, recorded and the mean was calculated from three repetitions. Inhibition zones of less than 8 mm are considered as ineffective. The inhibition zone from 14 mm and above, is considered as high antimicrobial activity.

2.5. GC–MS analysis

The constituents of the alkaloid and phenolic fraction obtained from methanol extracts were analyzed by GC–MS., The GC–MS was analyzed using Shimadzu GC–MS-QP 2010 gas chromatography-mass spectrometer equipped with capillary column DB-5-MS Agilent (30 m × 0.25 mm) and film thickness 0.25 μm. Helium was adjusted at pressure 81.90 K Pa which is used as a carrier gas, with a flow of 1.33 mL/min. The temperature in the injector was 250 °C, while the temperature of the oven progressed from 60 to 240 °Cby 3 °C /min. The mode of ionization was the electronic impact at 70 eV. After that, each fraction was co-injected with a homologous series of linear hydrocarbons under the same experimental conditions in order to calculate the retention index (RI) of sample constituents using Van Den Dool and Kratz equation. The compounds were identified by analyzing and comparing the mass spectra with a database of Wiley 7 libraries and also by comparing the RI with those of the literature [15] Compounds were identified by comparison of their retention indices(RI), and mass spectra with those reported in the literature [9].

2.6. Statistical analysis

Student *t*-test was used to compare antibacterial inhibition zones of the tested extracts. Paired *t*-test was used to compare the different parameters of the weather between the both cities, while One-way ANOVAtest with Dunn's multiple comparisons post-test were used for comparisons of different group's data.

3. Results

The current work was conducted to study the effects of climatic changes among two different regions (Arar and Tabuk), on chemical composition, antioxidant and antimicrobial of plant *A. fragmentissimaa*.

3.1. Seasonal climatic changes

Annual climatic variation through years2010 to 2016 of Northern region (Arar and Tabouk) are shown in Tables 1 and 2 There was statistically significant differences between both

Table 2
Climate temperature in both Arar and Tabouk regions during the period from January 2010 to December 2016. AR: Arar, TK: Tabuk.

Year	V		RA		TS		SN		PP		FG		GR	
	AR	TK	AR	TK	AR	TK	AR	TK	AR	TK	AR	TK	AR	TK
2010	14.7	8.7	11	15	5	7	0	0	–	19.6	0	0	0	3
2011	14.5	9.8	8	19	8	7	0	0	–	20.8	1	0	0	1
2012	14.7	10.8	17	15	2	9	0	1	16.5	–	4	0	0	0
2013	15.1	11.0	18	30	10	4	0	0	–	93.7	6	0	0	0
2014	14.3	11.4	16	37	21	6	0	0	–	42.9	4	1	0	0
2015	14.6	12.1	12	24	17	9	0	0	–	86.1	5	2	0	0
2016	14.3	11.6	6	20	14	7	1	0	–	26.7	4	0	0	0
	P=0.0002***		P=0.01*		P=0.19									

V: Annual average wind speed, RA: Number of days with rain, TS: Number of days with storm.
SN: Number of days with snow, PP: Rain or snow precipitation per annum, FG: Number of foggy days,GR: Number of days with hail.

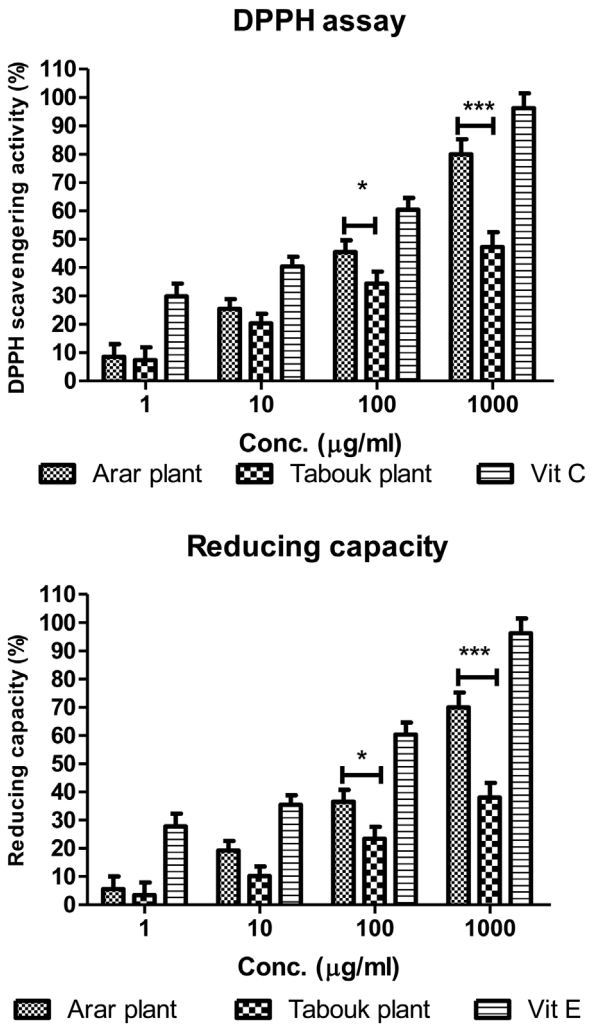

Fig. 2. DPPH scavengering activities (A) and iron reducing capacities (B) of *Achillea fragrantissima* collected from Arar and Tabuk regions.

regions in their climate parameters. Generally, the climatic are characteristic by relatively low temperatures in Winter season with cloudy sky - and decreased sunshine hours. Temperatures start rising from spring and reach a maximum (45 °C in some days of the month) during summer season. Arar shows extreme of hot and cold weathers in summer and winter month with less rates of rain falls.

3.2. Antioxidant activities

The DPPH scavenging capacity Method was used to measure the antioxidant capacity of *A. fragmentissma* methanol extract in two habitats, by DPPH scavengering activity assay. As shown in Fig. 2A, *A. fragmentissma* from Arar region has significantly higher antioxidant activity than plants collected from Tabuk in concentrations 100 and 1000 µg/mL with estimated IC50 s of 0.21 ± 0.01 and 1.12 ± 0.03 mg/mL respectively.

FRAP method was used to test the reducing capacity of methanol extracts by reducing the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). The results (Fig. 2B) showed that plant from wade Arar habitat present the significantly higher reduction capacity in concentrations 100 and 1000 µg/mL with estimated IC_{50} of 0.23 ± 0.02 g/L, than plant habitat in Tabuk with IC_{50} of 1.91 ± 0.05 g/L.

3.3. The antibacterial activity

Firstly, the experiment showed that the vehicle control (10% DMSO) hasno effect on the growth of bacterial cells. The antibacterial activity of tested plants *A. fragmentissima* in two different habitats, showed good to weak or no activity at all on the examined bacteria. In general, the gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 49461, *Bacillus cereus* ATCC 10876 and *Staphylococccus aureus* clinical isolate) exhibited values ranged from high, good, moderate to weak susceptibility towards the plant extracts. Accordingly, *Staphylococccus aureus* showed high susceptibility to the plant extract collected from Arar region (14.0 ± 0.5), while, almost all other gram-positive bacteria are ranging between 11.5 ± 0.5 mm to 7.5 ± 0.5 mm. The gram-negative bacteria did not show susceptibility toward the plant extracts as showed in Table 3.

3.4. Effect of habitat variation on chemical composition

It is evident from the GC–MS analyses results that *A. fragmentissima* plant, collected in two different habitat exhibited diverse pattern of accumulation of chemicals in both neutral and alkaloid fractions of the plant methanol extracts (Tables 4 and 5). The shared compounds showed significant difference in their concentrations in extracts of both habitats. In addition, some compounds were only found in one plant extract and not found in the plant collected from the other habitat.

4. Discussion

For many years, plants show a reputation among herb experts in Saudi Arabia as traditional medicinal herbs. The current study was conducted to study the effect of the two different habitats, Arar and Tabuk on antioxidants, antibacterial activities as well as their

Table 3
The antibacterial activity of alcoholic extract of *Achillea fragrantissima* in two different habitats.

Tested compounds	Mean zone of inhibition (mm)*								
	Sa1	Sa2	Se	Bc	Ec	Pa	Ab	Kp1	Kp2
Extract Arar habitat	11.5 ±0..5	14.0 ±0.5	11.5 ±0.5	10.0 ±0.01	6.0 ±0.0	6.0 ±0.1	6.0 ±0.2	6.0 ±0.3	6.0 ±0.02
Extract Tabuk habitat	9.75 ±0.25	11.5 ±0.5	11.5 ±0.5	10.5 ±0.5	9.5 ±0.1	6.0 ±0.1	6.0 ±0.2	6.0 ±0.2	6.0 ±0.01
P-value	0.0056**	0.0018**	1	0.158					
Positive control Antibiotics	18.5 ±0.5	23.5 ±0.5	30.5 ±0.5	28.0 ±2.0	18.2 ±0.2	12.6 ±0.2	18.2 ±0.3	19.2 ±0.3	21.3 ±0.2

* 6.0 mm zone of inhibition = no activity, Sa1=*Staphylococccus aureus* clinical isolate, *Sa2*=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 49461, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Pa=*Pseudomonas aeruginosa* clinical isolate, Ab=*Acinitobacterbaumanni*iclinical isolate, Kp1=*Klebsiellapneumoniae* ATCC 27736, Kp2=*Klebsiellapneumoniae* ATCC 700603.

Table 4
Phytochemical compound in neutral fraction of methanol extract of Achilea fragmmentissima in two habitats.

Compounds	Biological activities	SI	RSI	Rt	Arar Pl	Tabuk Pl
Eugenol	Antimicrobial [21]	700	757	14.94	2.06	**1.02**
Quinic acid	Antioxidant [22]	756	750	15.9	1.58	**1.45**
Ascorbic acid	Antioxidant [24]	661	737	19.7	0.82	**0.72**
Pyrocatechol	Lowering the eye pressure [25]	780	789	19.8	1.96	**0.23**
Butyl citrate	———	677	720	37.2	0.65	**0**
Dihydroxanthin	———	674	716	33.32	1.09	**0**
Ferulic acid methyl ester	Antioxidant antimicrobial [22]	920	925	40.25	6.4	**2.05**
Fumaric acid	Treatment of psoriasis [23]	559	605	9.93	0	**0.03**
Methyl jasmonate	Antioxidant & antimicrobial [22]	568	620	22.6	1.13	**0**
Methyl stearate	Antioxidant & antimicrobial [22]	872	872	34.02	0	**8.65**
Phytol acetate	Antioxidant & antimicrobial [22,24]	812	823	33.72	1.12	**0.5**
Salicylic acid ester	Antioxidant & antimicrobial [24]	890	882	38.2	2.3	**0.5**
Santolina alcohol	Antioxidant & antimicrobial [22,26]	753	759	5.6	5.31	**0**

Table 5
Chemical Composition of Alkaloid fraction of Achileafragmmentissima in two habitats.

Compounds	Biological activities	SI	RSI	RT	Arar Pl	Tabuk Pl
Dasycarpidan1methanol, acetate	Antibacterial antifungal [27]	501	567	31.44	1.14	1.15
Digitoxin	Cardiac glycoside [28]	515	560	39.86	1.03	1.15
Eseroline	Anticholinestrase metabolite [29]	444	492	27.98	0	1.15
Ethyl isoallocholate	Antimicrobial (EC) [30]	712	720	34.36	1.74	5.51
kadain	opioid [31]	632	661	22.9	1.01	2.16
Pseudosolasodine diacetate	Promote neurogenesis [32]	722	777	33.5	1.25	1.07
Pterin 6-carboxylic-acid	Folic acid photoproduct [33]	669	720	40.52	1.18	2.32
Methylinosine	Immunostimulant [34]	730	758	20.12	1.96	0

chemical composition of their neutral and alkaloid fractions *of A. fragmentissima* alcoholic extracts. Firstly, atmospheric conditions showed significant different in climate parameters of both regions during the years from 2010 to 2016 preceding the study. The antioxidant activities were significantly higher in Arar collected plants. Also Arar plant showed significantly higher antibacterial activities against the studied staph aureus strains. Finally, GC–MS studies showed significant difference in the plant collected from both habitats in both the neutral and alkaloid extracts of the plants (Fig. 3).

Arar region is characterized by highly variable environmental conditions from extremely low in winter to high temperatures in summer months, while Tabuk region showed different conditions with a significant decrease in temperature and more rainfall rats. These differences are expected to affect *A. fragmentissima* plant metabolism, as adaptive mechanism, with changes chemical composition of the plants collected from both areas. This dryness causes some compounds to be accumulated in higher amount or found in ester forms. The current study showed higher accumulation of polyphenolic compounds as ferulic acid methyl ester, Salicylic acid ester, ascorbic acid, and methyl jasmonate in Arar collected plant as a result of water stress due to low rainfall and higher temperature. This is in agreement with the data published by cheongjong (2003) [16], who reported higher level of jasmonic acid and methyl jasmonate in plants as a response to a biotic stresses.

Polyphenols are reported to have antiallergic, anti-inflammatory, anticancer, antibacterial and anticancer activities [6]. Plants contain different phenolic compounds as Procatchol, Santolina alcohol, Egenol, Artenimol and Salicylic acid ester, these compounds are strongly influenced by different factors [17]. Their levels are enhanced by both biotic and a biotic stress. The current data showed higher level of polyphenols compounds in Arar collected plants. Ferulic acid, one of the most phenolic compound, which present in plant cell walls of seeds and leaves was reported to be 4 times higher in extracts of plants collected from Arar in comparison to Tabuk plant [18].

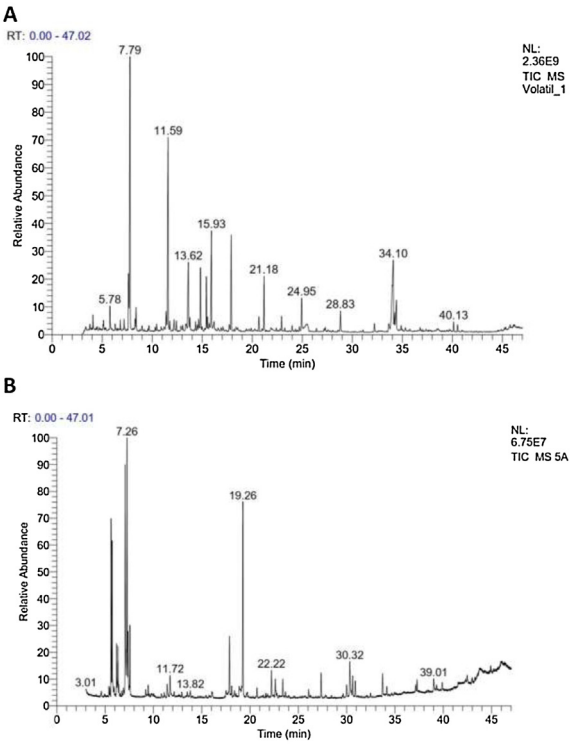

Fig. 3. Chromatogram of GC–MS of extract of Alkaloid fraction of i *Achillea fragrantissima* n two regions.

The current study showed that more significant antioxidant and antibacterial activities against gram positive pathogens of Arar collected *A. fragmentissima*. This higher levels of antibacterial and antioxidant activities of Arar plant can be explained mainly by the higher level of polyphenolic metabolites. That is in accordance with the previously published data. The antioxidant activity of

phenolic compounds was explained due to their scavenging capacity against the free radicals by donation of hydrogen atoms and electrons as well as chelation of the metal cations [19]. In addition, polyphenols were reported to have bactericidal and bacteriostatic action via inhibition of the bacterial enzymes and toxins and alter their cytoplasmic membrane as well as they can exert synergistic effect with the commonly used antibiotics and increase their effect on the bacterial pathogens [20–23]

5. Conclusion

Plant *Achilea fragmentisma* which distributed along northern region were collected from Tabuk and Arar. The change in habitats between Arar and Tabuk were found to significantly affect the chemical compositions, antimicrobial and antioxidant activities among plants collected from both habitats. This difference is mainly attributed to higher level of polyphenolic compounds in Arar region plants due to lower levels of rainfalls and plants adaptation to dryness. This reflects the role of geographical location on the variation in chemical compositions and biological activity of plant.

Author contribution

Dr. Eman Elsharkawy: Practical work for extraction, GC–MS analysis [1], Anttioxidant, writing paper – editing.

Dr Suliman Alghanem: Collecting plant sample- identify the plant- writing paper.

Ekramy Elmorsy: Do practical work (antimicrobial), writing paper.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00581.

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