Changes in chemical composition of Zilla spinosa Forssk. medicinal plants grown in Saudi Arabia in response to spatial and seasonal variations

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Extracts of different medicinal plants had been used to control several diseases in both traditional medicine and modern drugs. In the current study, we aimed to examine the changes in chemical composition of Zilla spinosa Forssk. plants collected from different habitats in Saudi Arabia in response to spatial and seasonal variations. Z. spinosa samples were collected from two different sites in Riyadh and Eastern regions in Saudi Arabia to examine the spatial variations effects on the studied parameters. Samples were collected from both sites at two different times (3:00 PM and 3:00 AM) to examine the effect of light on the chemical content and composition of these plants. Samples was, also, collected from the same sites at two different seasons (on start of January 2018 "winter season" and end of May 2018 "summer season") to examine the effect of temperature changes (seasonal variations) on the chemical content and composition of the different studied plants. In Z. spinosa plants collected from Riyadh region, squalene was found to be the major constitute of 3 samples; however, surprisingly, the sample collected in Winter at 3:00 AM showed the presence of mome inositol and (Z)-5-(formylmethylene)-4-methoxy-2(5 h)-furanone as the dominant components. Similarly, chemical compositions of essential oils extracted from Z. spinosa samples collected from Eastern region in the Summer season was dominated by squalene. Z. spinosa plants showed that all collected samples had high carbohydrate and protein contents with very low content of fats.

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1. Introduction

Different medicinal plants had been used to control several diseases in both traditional medicine and modern drugs. Many studies indicate the effective usage of medicinal plants different extracts as antimicrobial/antifungal agents (Ibrahim et al., 2017). Moreover, using such extracts as additives for food and drinks and their use in cosmetics industries are becoming more important nowadays. This is resulted from the increased interest of consumers for such extracts and that they have no side effects comparing to synthetic agents. The essential oil extracted from Z. spinosa could be used as a hepatoprotective agent. The essential oil extracted from Z. spinosa contains different flavonoids, triterpenes and sterols (El-Toumy et al., 2011).

Zilla spinosa Forssk. is a member of the Brassicaceae family (WCP). Z. spinosa grows in different parts in Saudi Arabia including Riyadh and Asir (Heneidy and Bidak, 2001). This plant is considered one of the most important medicinal plants because of its use in traditional medicine as a treatment for stones of kidney and gall bladder (Heneidy and Bidak, 2001). SA El-Toumy, FS El-Sharabasy, HZ Ghannem, MU El-Kady and AF Kassem (El-Toumy et al., 2011) indicated that the ethanolic extract of Z. spinosa could be used as a hepatoprotective agent. The essential oil extracted from Z. spinosa contains different flavonoids, triterpenes and sterols (El-Toumy et al., 2011).

Z. spinosa is intricate, rounded, glabrous and nearly leafless spiny-branched shrublet reaches up to 75 cm high. Basal leaves are usually pinnately lobed while upper ones grow oblong-linear. Older growth turns into leafless plant with stems hardening to tapering spines. Flowers’ petals are pink or violet (sometimes white). Fruits are ovoid-globose measure 8–10 mm in diameter with a broad-conical of 3–4 mm-long beaks at apex. The plant is found in wadis and silt basins. In Saudi Arabia, it could be found widely distributed along different parts of the kingdom (Mandaville, 1990).
genera most of them shows medicinal importance and biological activities e.g. antiviral, antibacterial, antifungal, antidiabetic, antirheumatic, anticancer and insecticidal activities (Beilstein et al., 2006; El-Sharabasy and Mohamed, 2013). In traditional medicine, Z. spinosa is primarily used for the treatment of gall bladder and kidney stones and/or ailments (Karawya and Wassel, 1974). Some studies indicated the antibacterial, antiviral, antifungal, antioxidant and hepatoprotective effects of Z. spinosa different fractions because of their contents of flavonoids, sterols and triterpenoids (El-Toumy et al., 2011; Karawya and Wassel, 1974). It was proved that aqueous extract of Z. spinosa aerial parts significantly stabilized the plasma membrane of animal hepatocytes exposed to carbon tetrachloride (CCL4) via decreasing levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) (El-Toumy et al., 2011; Ploa and Hewitt, 1989).

S Alghanem, O Al-hadithy and M Milad (Alghanem et al., 2018) examined the potential hepatoprotective and antioxidant activity of various Z. spinosa extracts (petroleum ether, methylene chloride, ethyl acetate, acetone and methyl alcohol) against different bacterial strains (B. subtilis, K. pneumonia, S. aureus and Streptococcus pyogenes) and fungi (A. niger, A. fumigatus, C. albicans and Mucor spp.). The obtained results showed that S. aureus and S pyogenes were significantly affected by petroleum ether extract the inhibition zone varied according to type of examined bacteria. On the other hand, E. coli and K. pneumoniae was not affected by methylene chloride and acetone extracts while other bacterial species were affected. The ethyl acetate extract inhibits B. subtilis, S. aureus and S. pyogenes. All bacterial strains except B. subtilis were significantly inhibited by methyl alcohol extract. With regard to antifungal activity, methylene chloride extract significantly has inhibited the growth of A. fumigatus. All tested fungi species were inhibited by ethyl acetate and methyl alcohol extracts except A. niger. Furthermore, acetone extract showed a significant fungicidal effect against C. albicans and Mucor spp.

Riaz Ullah, S Alsaid Mansour, A Shahat Abdelaaya, A Naser Almoqbil, A Al-Mishari Abdulla, M Adnan and A Tarq (Ullah et al., 2018) examined the potential hepatoprotective and antioxidant activities of whole plant methanolic and n-hexane extracts of Z. spinosa on CCl4-induced hepatotoxicity in rats. Different applied concentrations of the two extracts didn’t show any toxicity and/or mortality against cells. The obtained results indicated poor to moderate antioxidant and hepatoprotective activity of Z. spinosa extracts. Similarly, biological activities of chloroform extract of Z. spinosa aerial parts were investigated (El-Sharabasy and Mohamed, 2013). In their study, they isolated different structures of the extract and examined their anti-inflammatory, analgesic and antimicrobial activities. The obtained results showed a significant anti-inflammatory effect of Z. spinosa extract (approved by decreased edema rates) as a result of the presence of phenolics and/or triterpenes. Moreover, the presence of phenolic compounds in the extract led to a great analgesic activity. Nevertheless, Z. spinosa extract showed weak antibacterial effect against gram-positive and gram-negative bacteria and showed no antifungal effect. Interestingly, the chloroform extract of Z. spinosa aerial parts showed anti-cancer potential against human liver (HEPG2) and colon (HCT116) cancer cell lines with IC50 values of 15.7 and 14.5 μg mL⁻¹, respectively.

To examine the hepatoprotective potential of ethanol extract of Z. spinosa aerial parts, SA El-Toumy, FS El-Sharabasy, HZ Ghanem, MU El-Kady and AF Kassem (El-Toumy et al., 2011) investigated the levels of ALT, AST, gamaglutamyl (γ-GT) and alkaline phosphatase (ALP) in blood serum of CCl4-induced hepatitis rats, in addition to histological examination of liver tissues. The obtained results showed that Z. spinosa extract significantly alleviated the elevation of ALT, AST, γ-GT and ALP in blood serum resulting from CCl4 application. They attributed the observed hepatoprotective effects to the combined action of flavonoids found in the extract. Furthermore, anti-cancer potential of ethanolic extract was examined against liver, breast and colon human cancer cell lines (HEPG2, MCF7 and HCT116, respectively). The ethanolic extract showed a great activity against MCF7 breast cancer cell lines with IC50 value of 7.5 μg mL⁻¹.

However, to the best of our knowledge, there are no available comparable studies regarding effect of soil, light and temperature on the content and composition of bioactive compounds in P. undulata. Therefore, in the current study, we aimed to examine the changes in chemical composition of Zilla spinosa Forssk. plants collected from different habitats in Saudi Arabia in response to spatial and seasonal variations.

2. Materials and methods

2.1. Study site

Surveying field trip were conducted at the start of the study on 2017 in March and April to examine several sites in Riyadh region

Table 1

| Site          | Riyadh Winter | Riyadh Summer | Eastern Winter | Eastern Summer |
|---------------|---------------|---------------|----------------|----------------|
| pH            | 7.97          | 7.86          | 7.88           | 7.81           |
| EC (μS m⁻¹)   | 0.33          | 0.2           | 0.16           | 0.15           |
| Moisture (%)  | 0.63          | 1.18          | 0.38           | 0.39           |
| OM (%)        | 2             | 3.27          | 1.4            | 1.58           |
| Soil texture  |               |               |                |                |
| Sand          | 77            | 75            | 81             | 80             |
| Silt          | 9             | 10            | 9              | 11             |
| Clay          | 14            | 15            | 10             | 9              |
| Texture       | Sandy Loam    | Sandy Loam    | Sandy Loam     | Sandy Loam     |
| P (mg L⁻¹)    | 71.23         | 384.97        | 482.23         | 322.92         |
| K (μg L⁻¹)    | 7653.04       | 2641.68       | 4023.67        | 3442.33        |
| Mg (μg L⁻¹)   | 2698.94       | 1336.98       | 1404.05        | 1148.14        |
| Ca (μg L⁻¹)   | 5484.25       | 4708.86       | 4436.49        | 3364.93        |
| Na (μg L⁻¹)   | 3785.27       | 3079.24       | 3204.78        | 2594.95        |
| Fe (μg L⁻¹)   | 135.45        | 150.06        | 238.14         | 478.3          |
| Cu (μg L⁻¹)   | 6.08          | 4.18          | 4.05           | 5.14           |
| Zn (μg L⁻¹)   | 0.5           | 0.63          | 0.79           | 1.48           |
| Mn (μg L⁻¹)   | 0.29          | 0.71          | 2.3            | 2.45           |
| Cd (μg L⁻¹)   | 0.1           | 0.09          | 0.1            | 0.08           |
| Pb (μg L⁻¹)   | 0.1           | 0.2           | 0.42           | 0.59           |
| Cr (μg L⁻¹)   | 1.95          | 2.46          | 2.59           | 3.15           |

* EC: electrical conductivity, OM: organic matter content.
and Eastern region with the aim to identify the locations where the studied plant abundant. Based on the field trips survey, Jilah located 110 km western Riyadh (24° 24’ 47.4” N; 45° 43’ 01.2’ E) and a place located 40 km away from southern of Hafr Al Batin (28° 00’ 33.2” N; 45° 46’ 43.6’ E) were chosen to collect soil and plant samples for mineral and chemical analysis. Data of climactic characteristics of the studied sites were obtained from the nearest meteorological station (Supplementary table 1).

2.2. Soil samples collection and analysis

Soil samples were collected from the rhizosphere of each collected plants. Samples were packed in sealable plastic bags. Sample number and collection time were recorded for each sample. Soil samples were, then transferred to the labs of Botany and Microbiology Dept., College of Sciences, King Saud University to perform soil analysis. Samples were prepared for analysis by air-drying in the oven at 60 °C for 48 h. Dried soil samples were sieved through a 2-mm-diameter sieve. Fine soil particles were collected and used for further physical and chemical analysis. Soil physical characteristics including pH, electrical conductivity (EC), moisture content, organic matter (OM) content and soil texture as percentages of sand, silt and clay in the soil were estimated. Chemical analysis of the soil included the contents of P, K, Mg, Ca, Na, Fe, Cu, Zn, Mn, Cd, Pb and Cr. Table (1) shows physical and chemical characteristics of soil samples collected from different sites in different seasons.

2.3. Plant samples collection and processing

The samples of Pulicaria undulata was collected from two different sites in Riyadh and Eastern regions in Saudi Arabia to examine the spatial variations effects on the studied parameters. Samples were collected from both sites at two different times (3:00 PM and 3:00 AM) to examine the effect of light on the chemical content.

| #  | Name                                      | RT  | Area      | Area %  |
|----|-------------------------------------------|-----|-----------|---------|
| 1  | 4-ETHENYL-2-METHOXY-PHENOL                | 13.05 | 30152     | 1.040   |
| 2  | 3-TERT-BUTYL-4-METHOXY-PHENOL             | 17.26 | 103632    | 3.580   |
| 3  | MOME INOSITOL                             | 20.26 | 419069    | 14.490  |
| 4  | 2-HEXADECEN-1-OL                          | 23.61 | 80573     | 2.790   |
| 5  | (E)-4-METHYL-1-P-NITROPHENYL PENTA-1,3-DIENE | 23.89 | 27269     | 0.940   |
| 6  | DEHYDROASPIDOSPERMIDINE                   | 25.08 | 228879    | 7.910   |
| 7  | 3,3-BIS(3-INDOLYL)INDOLINE                | 27.13 | 78576     | 2.720   |
| 8  | QUEBRACHAMINE                             | 27.38 | 67647     | 2.340   |
| 9  | (Z)-5-(FORMYLMETHYLENE)-4-METHOXY-2(SH)-FURANONE | 27.76 | 424947    | 14.690  |
| 10 | PLEIOCARPAMINE                            | 28.71 | 60096     | 2.080   |
| 11 | SQUALENE                                  | 29.15 | 259088    | 8.960   |
| 12 | PROSTAGLANDIN F1A TMS ESTER TR            | 29.91 | 39211     | 1.360   |
| 13 | FUSCOSIDE B                               | 30.88 | 72732     | 2.510   |
| 14 | VITAMIN E                                 | 31.52 | 87650     | 3.030   |

Fig. 1. Chemical composition of Z. spinosa sample collected from Riyadh in Winter at 3:00 AM.
and composition of these plants. Samples was, also, collected from the same sites at two different seasons (on start of January 2018 "winter season" and end of May 2018 "summer season") to examine the effect of temperature changes (seasonal variations) on the chemical content and composition of the different studied plants.

After transferring plants collected from Riyadh region to the lab, samples were cleaned thoroughly from soil and other plant materials so that such materials will not affect the validity of the obtained results. Cleaning process were performed for plants collected from Eastern region in the collection sites before transferring to the lab. In lab, air drying was conducted by leaving plant materials on filter papers in shaded and well-aerated area with mild temperature. Dried plant materials were grinded into fine powder using an electrical grinder machine obtained for this purpose.

2.4. Plant mineral analysis

About 0.5 g of plant leaves dry powder were transferred to a glass test tube and 10 ml of HNO$_3$ were added. Test tube with plant material and nitric acid was heated on a hotplate until reaching full digestion. Milli-Q water was then used to thoroughly transfer the digested mixture from the tube to a volumetric flask via filtering through filter paper (110 mm diameter) with pore diameter of 20–25 μm and the total volume was completed to 100 ml using Milli-Q water. A dilution of 1.2 ml of the digested mixture in 10 ml of Milli-Q water were used for analysis in Perkin Elmer NexION 300D ICP Mass Spectrometer (Perkin Elmer, USA). Each sample was replicated three times and the average were reported. Plant contents of P, K, Mg, Ca, Na, Fe, Cu, Zn, Mn, Cd, Pb and Cr were measured.

2.5. Plant proximate analysis

Plant samples were air-dried in oven at 60 °C for 48 h and then grounded into fine powder. After sieving with 100-mesh sieve, the resulted powder was used to estimate the contents of carbohydrate, crude fat, crude protein, ash and moisture. Contents of these materials were reported as percentages representing the average of three replicates for each sample.

2.5.1. Carbohydrates

Anthrone method described by CS James (James, 2013) was followed to estimate the carbohydrate content in plant samples. A dilution of 45 ml of the plant sample extract in 450 ml of Milli-Q water were used for analysis. In a test tube, 1 ml of the extract were added and 5 ml of freshly prepared Anthrone 0.1% reagent were added. Milli-Q water was used as blank; while glucose

| # | Name                                    | RT  | Area  | Area % |
|---|-----------------------------------------|-----|-------|--------|
| 1 | 3',4'-DIMETHOXY ACETOPHENONE             | 17.28 | 1843  | 4.610  |
| 2 | 1-METHYL-BUTYL-DOCOSANOIC ACID           | 18.47 | 4500  | 11.270 |
| 3 | MOME INOSITOL                            | 20.17 | 450   | 1.130  |
| 4 | PHYTOL                                  | 20.75 | 6327  | 15.840 |
| 5 | 2-METHYLTRYPTAMINE                      | 20.93 | 9101  | 22.790 |
| 6 | 4H-PYRROLO[3,2,1-IJ]QUINOLINE            | 21.52 | 1634  | 4.090  |
| 8 | QUEBRACHAMINE                           | 27.33 | 150   | 0.380  |
| 11| SQUALENE                                | 29.15 | 9862  | 24.690 |
| 13| FUSCOSIDE B                             | 30.85 | 3696  | 9.250  |
| 14| VITAMIN E                               | 31.51 | 950   | 2.380  |

Fig. 2. Chemical composition of Z. spinosa sample collected from Riyadh in Winter at 3:00 PM.
solution was used as standard. After adding Anthrone, the mixture was mixed thoroughly via shaking. All the tubes were left in a heating water bath at 30 °C for 12 min. Tubes were, then, removed and left to cool down to room temperature. Light absorbance of the samples was read at 630 nm using DR 6000™ UV VIS Spectrophotometer (Thomas Scientific, USA). Total carbohydrate was calculated as the percentage of glucose present in the sample extract using the following equation:

\[ \text{Carbohydrate}(\%) = \frac{25 \times A_1}{W \times A_2} \times 100 \]

where:
- \( A_1 \): absorbance of the sample at 630 nm
- \( A_2 \): absorbance of the standard at 630 nm
- \( W \): weight of the sample extracted (g)

2.5.2. Crude fat

Solvent extraction gravimetric method (Kirk and Sawyer, 1991) was used to determine the crude fat content in plant samples. In details, 5 g of the powdered plant sample were wrapped in filter paper and put inside a thimble. The thimble was, then, put inside a Soxhlet reflux flask. The flask was mounted in a known-weight extraction flask containing 200 ml of petroleum ether as an organic solvent. A water condenser was connected to the upper side of the Soxhlet reflux flask. Extraction process via petroleum ether was performed by heating it until evaporation and then condensing it into the Soxhlet reflux flask. The solvent covers the sample and then siphoned over carrying extracted oil down to the boiling flask. Extraction process were continued for 18 h before removing the sample. The flask containing the solvent and extracted fats were oven dried at 135 °C for 2 h, cooled down in a desiccator and weighed. The percentage of crude fats in the plant sample were calculated using the following equation:

\[ \text{Crude fat}(\%) = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \]

where:
- \( W_1 \): weight of empty extraction flask (g)
- \( W_2 \): weight of extraction flask with oil after drying (g)

2.5.3. Crude protein

Crude protein content was measured based on the total N content in the sample. Nitrogen content was estimated following the Kjeldahl method (AOAC, 1990). A conversion factor (6.25) was

![SAMPLE-27](image_url)
multiplied by the total N content to obtain crude protein content. This conversion factor depends upon the fact that the average N content of proteins was found to be about 16%. Nitrogen extracted via mixing 0.5 g of dried samples with 10 ml of concentrated H₂SO₄ in a digestion flask. Digestion mixture containing CuSO₄ and K₂SO₄ were added with proportion of 1:4 until the mixture was clear (the digest). Afterwards, 10 ml of the digest were thoroughly mixed with 10 ml of 50% NaOH in Kjeldahl distillation apparatus. The mixture was distilled into 10 ml of 40% boric acid containing 3 drops of mixed indicator (bromo cresol green). 50 ml of the mixture were titrated with 0.02 N EDTA (Ethylenediaminetetraacetic acid) and the reaction end point was conversion from green to dark red color. A reagent blank was also digested, distilled and titrated. The crude protein content was determined using the following equations:

\[ N_2 = \frac{100}{W} \times \frac{20}{1000} \times \frac{V_t}{V_a} \times T \times B \]

\[ \text{Protein\%} = N_2(\%) \times 6.25 \]

where:

- W weight of sample (0.5 g)
- N normality of titrant (0.02 N)
- V_t total digest volume (100 ml)
- V_a analyzed digest volume (10 ml)
- T volume of EDTA used in the sample
- B volume of EDTA used in the blank

### 2.5.4. Ash

Gravimetric method via furnaces incineration described by CS James (James, 2013) was followed to determine the total ash content in plant samples. A known-weight (W₁) porcelain crucible was used to burn 5 g of the dried powdered plant sample in a muffle furnace at 550 °C until reaching complete ash. The crucible containing the sample was left to cool down in a desiccator and then weighed (W₂). The following equation was used to calculate the total ash content in samples:

\[ \text{Ash\%} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \]

### 2.5.5. Moisture

Moisture content in plant samples were measured using the gravimetric method (AOAC, 1990). In details, 5 g of the sample were transferred to a moisture can with a known weight (W₁). The can containing the sample were weighed (W₂), oven-dried for 4 h at 105 °C and then let to cool down in a desiccator. After

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**Fig. 4.** Chemical composition of *Z. spinosa* sample collected from Riyadh in Summer at 3:00 PM.
cooling, the can containing the dried sample were weighed \( W_3 \). Drying process as described above were repeated until no change in the weight is observed. Moisture content was measured using the following equation based on the weight differences:

\[
\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100
\]

2.6. Preparation of methanolic extract

Plant material was extracted using methanol. In details, 50 g of the dried-plant material powder were extracted in 200 ml of methanol. Plant material was left in the methanol for 24 h with shaking at 50 rpm and then filtered. The extraction was repeated three times for the same plant material. All the collected filtrates were combined and dried in rotary evaporator machine. Collected powder was re-dissolved in distilled water and used for further analysis.

2.7. GC–MS analysis

The GC–MS analysis was performed on a gas chromatograph HP 5890 (II) interfaced with a HP 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). A HP-5MS capillary column (60 m × 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 μm film thickness, Hewlett–Packard, Palo Alto, CA, USA) was used. The column temperature was programmed to rise from 40 to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 ml/min. Scan time and mass range were 1 s and 50–550 m/z, respectively. Different chemical compounds were identified by comparing their retention times with those of authentic standards. The identification of essential oil constituents was based on the comparison of their retention indices relative to (C8–C22) n-alkanes with those of literature or with those of authentic compounds. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and other published mass spectra (Adams, 2001).

2.8. Statistical analysis

Obtained data was analyzed using SPSS 18.0 software. Analysis of variance (ANOVA) was used to examine the effects of temperature and/or light on the chemical composition of the studied plants (Khan et al., 2014; Khan et al., 2015). Means were calculated for each sample based on three replicates and were separated using the Duncan’s Multiple Range Test at significance level \( P \leq 0.05 \) (Khan et al., 2019).

3. Results

3.1. Chemical composition of Zilla spinosa Forssk

Eight Z. spinosa plant samples were collected from two different locations (Riyadh and the Eastern regions) in two different seasons

| # | Name                                | RT   | Area | Area % |
|---|-------------------------------------|------|------|--------|
| 1 | 3',4'-DIMETHOXYACETOPHENONE          | 17.28| 4914 | 8.520  |
| 2 | 1-METHYL-BUTYL-DOCOSANOIC ACID       | 18.52| 926  | 1.610  |
| 3 | MOME INOSITOL                        | 20.19| 1484 | 2.570  |
| 4 | PHYTOL                              | 20.75| 3585 | 6.210  |
| 5 | 2-METHYLTRYPTAMINE                  | 20.93| 4351 | 7.540  |
| 12| 9,12-OCTADECADIENOIC ACID           | 30.32| 1245 | 2.160  |
| 13| FUSCOSIDE B                         | 30.84| 8610 | 14.920 |
| 14| VITAMIN E                           | 31.52| 4512 | 7.820  |

Fig. 5. Chemical composition of Z. spinosa sample collected from Eastern region in Winter at 3:00 AM.
(winter and summer) at two different times (3:00 PM and 3:00 AM). The chemical composition of each plant sample was examined using GC–MS.

Z. spinosa samples collected from Riyadh region in Winter at 3:00 AM showed the presence of 14 different compounds with different concentration (Fig. 1). The most dominated compounds found were (Z)-5-(FORMYL METHYLENE)-4-METHOXY-2(5H)-FURANONE (RT 27.76) and MOME INOSITOL (RT 20.26) with concentrations reached 14.69 and 14.49%, respectively. Fig. 2 showed that only 10 compounds were identified in samples collected at 3:00 PM in Winter. The most dominated compounds in these samples were SQUALENE (RT 29.15), 2-METHYLTRYPATMINE (RT 20.93) and PHYTOL (RT 20.75) with concentrations reached 24.69, 22.79 and 15.84%, respectively. Similar composition and number of compounds were observed in Z. spinosa samples collected from Riyadh region in Summer. Samples collected at 3:00 AM had 14 different compounds in their extract (Fig. 3). However, the chemical composition of these samples was dominated by SQUALENE (RT 29.15), 2-METHYLTRYPATMINE (RT 20.93) and PHYTOL (RT 20.75) with concentrations of 27.54, 18.98 and 12.73%, respectively. Similar to Winter samples, samples collected in Summer at 3:00 PM showed the presence of 10 compounds only (Fig. 4). The most present compounds in these samples were SQUALENE (RT 29.15), 2-METHYLTRYPATMINE (RT 20.93) and PHYTOL (RT 20.75) with concentrations of 35.15, 17.12 and 11.30%, respectively.

Regarding Z. spinosa samples collected from the Eastern region, only 8 compounds were identified in the extracts of samples collected in Winter at 3:00 AM (Fig. 5). The most represented compounds were FUSCOSIDE B (RT 30.84) with concentration of 14.92%, 3',4'-DIMETHOXYACETOPHENONE (RT 17.28) with concentration of 8.52%, VITAMIN E (RT 31.52) with concentration of 7.82% and 2-METHYLTRYPATMINE (RT 20.93) with concentration of 7.54%. On the other hand, 10 compounds were identified in the samples collected in Winter at 3:00 PM (Fig. 6). The dominated compounds in these samples were FUSCOSIDE B (RT 30.84) with concentration of 33.27%, SQUALENE (RT 29.16) with concentration of 27.17%, VITAMIN E (RT 31.52) with concentration of 12.01% and PHYTOL (RT 20.73) with concentration of 10.64%. Z. spinosa samples collected from the Eastern region in Summer season had different chemical composition as it was dominated mainly by squalene in both samples collected at morning or evening. Fig. 7 shows that 10 different compounds were identified in samples collected at 3:00 AM. The dominated compound was SQUALENE (RT 29.15) as the concentration reached 79.01%. Similarly, 9

| #  | Name                                | RT  | Area | Area % |
|----|-------------------------------------|-----|------|--------|
| 1  | 3',4'-DIMETHOXYACETOPHENONE          | 17.24 | 1406 | 4.110  |
| 2  | 1-METHYL-BUTYL-DOCOSANOIC ACID       | 18.57 | 196  | 0.570  |
| 3  | MOME INOSITOL                        | 20.08 | 770  | 2.250  |
| 4  | PHYTOL                              | 20.73 | 3638 | 10.640 |
| 5  | DEHYDROASPIDOSPERMIDINE              | 25.07 | 735  | 2.150  |
| 6  | GUANETHIDINE                         | 26.36 | 650  | 1.900  |
| 7  | QUEBRACHAMINE                        | 27.40 | 625  | 1.830  |
| 11 | SQUALENE                            | 29.16 | 9288 | 27.170 |
| 12 | FUSCOSIDE B                          | 30.80 | 11373 | 33.270 |
| 13 | VITAMIN E ACETATE                    | 31.57 | 4104 | 12.010 |

Fig. 6. Chemical composition of Z. spinosa sample collected from Eastern region in Winter at 3:00 PM.
compounds identified in the samples collected at 3:00 PM (Fig. 8). The SQUALENE (RT 29.15) was the dominated compound again with concentration of 78.99% in these samples.

3.2. Proximate analysis of Pulicaria undulata gaertn

Contents of carbohydrates, fats, crude protein, ash and moisture in the tissues of Z. spinosa plants were examined for all samples collected from the two studied regions (Riyadh and Eastern regions) both at Winter and Summer whether at morning (3:00 AM) or evening (3:00 PM). ANOVA revealed that the overall (3-way) interaction between region, season and time of collection significantly affected plants’ contents of carbohydrates, fats, crude protein and ash, but didn’t affect contents of moisture in these plants. Furthermore, contents of carbohydrates were affected by all the studied factors and their interactions except the 2-way interaction between season and time of collection. Moreover, interaction between region and season of collection didn’t significantly affect content of fats in Z. spinosa plants. Crude protein contents were affected by time of collection, interaction between season and time of collection and the overall interaction between the studied factors only. Whilst, moisture contents were affected by all the studied factors and their interactions except region of collection.

The highest content of carbohydrates was observed in plants collected from Riyadh in Summer at 3:00 PM (Table 2). While, the highest facts content was found in plants collected from the same region but in Winter at 3:00 AM. It is worth noting that there were no significant differences by all the other plants collected from different regions at different season and times in terms of contents of fats. Plants collected from Riyadh in Summer at 3:00 AM was characterized by the highest protein contents among all other plants; however, surprisingly, the lowest crude protein content was found in plants collected from Riyadh in summer at 3:00 PM with roughly 13.8% reduction in protein contents of these plants. The highest moisture content (12.57%) was observed in plants collected from Riyadh in Winter at 3:00 PM, while the lowest content (1.83%) was observed in plants collected from the Eastern region in Summer at 3:00 AM with more than 80% reduction in moisture contents of Z. spinosa plants.

3.3. Mineral content of Pulicaria undulata gaertn

Eight different samples of Z. spinosa were collected from two different regions in two different seasons at two different times

| #  | Name                                          | RT  | Area | Area % |
|----|-----------------------------------------------|-----|------|--------|
| 1  | 3',4'-DIMETHOXACETOPHENONE                    | 17.27 | 514  | 0.600  |
| 2  | 1-METHYL-BUTYL-DOCOSANOIC ACID                | 18.58 | 1877 | 2.200  |
| 3  | MOME INOSITOL                                 | 20.21 | 1295 | 1.520  |
| 4  | PHYTOL                                        | 20.75 | 3316 | 3.890  |
| 5  | 2-METHYLTRYPTAMINE                            | 20.93 | 4337 | 5.080  |
| 6  | 4H-PYRROLO[3,2,1-IJ]QUINOLINE                 | 21.52 | 668  | 0.780  |
| 11 | SQUALENE                                      | 29.15 | 67410| 79.010 |
| 12 | 9,12-OCTADECADIENOIC ACID                    | 30.31 | 249  | 0.290  |
| 13 | FUSCOSIDE B                                   | 30.89 | 720  | 0.840  |
| 14 | VITAMIN E                                     | 31.52 | 4397 | 5.150  |

*Fig. 7. Chemical composition of Z. spinosa sample collected from Eastern region in Summer at 3:00 AM.*
and were analyzed for their contents of macronutrients (i.e. P, K, Mg and Ca) using ANOVA. The obtained results showed that all contents of macronutrients were significantly affected by the studied factors and their interactions except phosphorus that wasn’t affected by season of collection and calcium which wasn’t affected by the interaction between season and time of collection.

Plants collected from Riyadh in Summer at 3:00 AM showed the highest contents of P, K and Ca with average values of 5183.76 mg L\(^{-1}\), 23611.93 and 7178.25 mg L\(^{-1}\), respectively (Table 3). Regarding Ca contents, there was no significant differences (\(P \leq 0.05\)) between plants collected from Riyadh in Summer at 3:00 AM or at 3:00 PM. The highest content of Mg was observed in plants collected from the Eastern region in Winter at 3:00 PM (3061.46 mg L\(^{-1}\)) and in Summer at 3:00 PM (2840.76 mg L\(^{-1}\)). On the other hand, the lowest contents of P were found in plants collected from the Eastern region at 3:00 AM in both Winter and summer seasons. Similarly, plants collected from the Eastern region in Winter at 3:00 AM had the lowest content of K (7906.27 mg L\(^{-1}\)). The lowest contents of Mg and Ca were observed in plants collected from Riyadh in Winter at 3:00 PM with average values of 862.64 and 3613.61 mg L\(^{-1}\). (See Table 4.)

Furthermore, ANOVA revealed Na contents were affected by all the studied factors and their interactions except time of collection and the interaction between region and time of collection. However, Zn contents were affected by all factors and their interactions.

Table (4) shows the effect of different factors and their different levels of interactions on \(Z.\ spinosa\) contents of micronutrients and heavy metals. There wasn’t too much variation in Na contents of different \(Z.\ spinosa\) samples. The highest Na content was found in plants collected from the Eastern region in Summer at 3:00 AM and 3:00 PM with values of 2784.25 and 2782.94 mg L\(^{-1}\). However, the lowest content was found in plants collected from Riyadh in Winter at 3:00 AM with an average of 2178.55 mg L\(^{-1}\). Similarly, there wasn’t great variation in Fe contents in the collected plants as they ranged from 240.21 mg L\(^{-1}\) in plants collected from the Eastern region in Winter at 3:00 AM to 294.79 mg L\(^{-1}\) in plants collected from the same region in Summer at 3:00 AM. The highest content of Cu (36.07 mg L\(^{-1}\)) was observed in the plants collected from Riyadh in Summer at 3:00 PM, while the lowest content (11.87 mg L\(^{-1}\)) was observed in the plants collected from Riyadh in Summer at 3:00 PM, while the lowest content (11.87 mg L\(^{-1}\)) was observed in plants collected from Riyadh in Winter at 3:00 AM. The lowest contents of Zn and Mn was observed in plants collected from the Eastern region in Winter at 3:00 AM with average values of 28.45 and 38.57 mg L\(^{-1}\), respectively. On the other hand, the highest content of Zn (88.32 mg L\(^{-1}\)) was observed in plants collected from Riyadh in Summer at 3:00 AM, and the highest content of Mn (72.25 mg L\(^{-1}\)) was observed in the samples collected from the Eastern region in Winter at 3:00 AM.

The contents of heavy metals (Cd, Pb and Cr) in \(Z.\ spinosa\) plants were significantly affected by all the studied factors and their different levels of interactions; however, Pb contents weren’t affected

|    | Name                           | RT  | Area | Area % |
|----|--------------------------------|-----|------|--------|
| 1  | 3',4'-DIMETHOXYACETOPHENONE     | 17.28 | 1903 | 1.660  |
| 3  | MOME INOSITOL                   | 20.24 | 660  | 0.580  |
| 4  | PHYTOL                         | 20.76 | 3636 | 3.180  |
| 5  | 2-METHYLTRYPTAMINE              | 20.93 | 3914 | 3.420  |
| 11 | SQUALENE                       | 29.15 | 90377| 78.990 |
| 13 | FUSCOSIDE B                    | 30.89 | 3821 | 3.340  |
| 14 | VITAMIN E                      | 31.52 | 6880 | 6.010  |
by time of collection. Plants collected from Riyadh in Winter at 3:00 AM showed the lowest contents of all heavy metals (Cd: 0.45 μg L⁻¹, Pb: 4.98 μg L⁻¹ and Cr: 6.69 μg L⁻¹) as compared to all other collected plants. Contrarily, the highest contents of Pb and Cr (8.72 and 17.28 μg L⁻¹, respectively) were observed in plant samples collected from the Eastern region in Winter at 3:00 PM. Furthermore, plants collected from Eastern region in Winter at 3:00 AM was characterized by the highest content of Cd among all the collected plants.

4. Discussion

In the current study, the effects of spatial and seasonal variation on the chemical composition of the essential oil of Zilla spinosa Forssk. were examined. In this regards, samples of the studied plant were collected from two different region (Riyadh and Eastern regions) at two different seasons (Winter and Summer) both at morning (3:00 AM) and evening (3:00 PM). The results obtained showed the spatial and seasonal variation led to significant changes in essential oil composition of these plants.

In Z. spinosa plants collected from Riyadh region, squalene was found to be the major constitute of 3 samples; however, surprisingly, the sample collected in Winter at 3:00 AM showed the presence of mome inositol and (Z)-5-(formylmethylene)-4-methoxy-2-(5 h)-furanone as the dominant components. Similarly, chemical compositions of essential oils extracted from Z. spinosa samples collected from Eastern region in the Summer season was dominated by squalene. On the other hand, the samples collected from the same region in Winter was dominated, in addition to squalene, by fuscoside B. The essential oil extracted from aerial parts of Z. spinosa plants collected from Suez-Cairo desert road in Egypt was found to be dominated by squalene (El-Sharabasy and Mohamed, 2013).

Significant variations were observed in the chemical compositions of the studied plants that was collected from different regions or from the same region at different seasons. Furthermore, in some cases, variations were observed between plants collected from the same region and in the same season but at different collection times (3:00 AM and 3:00 PM). Such variations could be attributed to the environmental differences due to seasonal and spatial variations. According to the climatic data obtained in this study, Riyadh and Eastern region showed great variation in the temperature, rainfall and humidity levels along the year. Furthermore, soil physical and chemical analysis showed significant differences between soil samples collected from the different studied site in different seasons. TP Chavez, CP Santana, G Véras, DO Brandão, DC Felismino, ACD Medeiros and DMdB Trovão (Chaves et al., 2013) examined the seasonal variations in chemical composition of two plants used in Brazilian traditional medicine, namely Guapira graciliflora and Pseudobombax marginatum. Their results showed a significant seasonal variation in the chemical compositions of the extracts of both plants as some the concentrations of total flavonoids and total polyphenols varied significantly between the studied seasons. Furthermore, variations in the production of polyphenols in Tulbaghia violacea, Hypoxis hemerocallidea, Merwilla plumbea and Drimia robusta plants collected in different seasons were reported (Ncube et al., 2011), and were attributed mainly to the climate differences, biotic and environmental conditions in addition to genetic differences. Several studies reported seasonal

### Table 2

Proximate analysis of *Z. spinosa* plants as affected by the studied parameters.

| Factors                     | Carbohydrates (%) | Fats (%) | Protein (%) | Ash (%) | Moisture (%) |
|-----------------------------|-------------------|----------|-------------|---------|--------------|
| **Single factors**          |                   |          |             |         |              |
| 1. Collection region        |                   |          |             |         |              |
| Riyadh                      | 57.21 a b          | 2.10 a   | 27.86 a     | 5.96 a  | 6.93 a       |
| Eastern region              | 56.04 b            | 2.28 a   | 27.82 a     | 5.73 a  | 8.08 a       |
| 2. Collection season        |                   |          |             |         |              |
| Winter                      | 54.62 b            | 2.34 a   | 28.12 a     | 5.83 a  | 9.10 a       |
| Summer                      | 58.63 a b          | 2.04 b   | 27.56 a     | 5.86 a  | 5.91 b       |
| 3. Collection time          |                   |          |             |         |              |
| 3:00 AM                     | 54.43 b            | 2.31 a   | 28.32 a     | 5.82 a  | 5.88 b       |
| 3:00 PM                     | 58.83 a            | 2.08 b   | 27.36 b     | 5.87 a  | 9.13 a       |
| **Two-way interaction**     |                   |          |             |         |              |
| 1. Region × season          |                   |          |             |         |              |
| Riyadh–Winter               | 56.47 ab           | 2.48 a   | 28.00 a     | 5.65 a  | 7.40 b       |
| Riyadh–Summer               | 57.95 ab           | 2.08 a   | 27.72 a     | 5.80 a  | 6.45 b       |
| Eastern–Winter              | 52.77 b            | 2.20 a   | 28.23 a     | 6.00 a  | 10.80 a      |
| Eastern–Summer              | 59.32 a b          | 2.00 a   | 27.40 a     | 5.92 a  | 5.36 c       |
| 2. Region × time            |                   |          |             |         |              |
| Riyadh–3:00 AM              | 52.07 c            | 2.50 a   | 28.23 a     | 5.75 a  | 2.40 c       |
| Riyadh–3:00 PM              | 62.35 a            | 2.07 b   | 27.48 a     | 5.70 a  | 11.45 a      |
| Eastern–3:00 AM             | 56.78 b            | 2.12 b   | 28.40 a     | 5.88 a  | 6.82 b       |
| Eastern–3:00 PM             | 55.30 bc           | 2.08 b   | 27.23 a     | 6.03 a  | 9.35 a       |
| 3. Season × time            |                   |          |             |         |              |
| Winter–3:00 AM              | 51.98 a b          | 2.62 a   | 27.30 b     | 5.92 a  | 6.02 b       |
| Winter–3:00 PM              | 57.25 a            | 2.07 b   | 28.93 a     | 5.73 a  | 12.18 a      |
| Summer–3:00 AM              | 56.87 a            | 2.00 b   | 29.33 a     | 5.72 a  | 5.73 b       |
| Summer–3:00 PM              | 60.40 a            | 2.08 b   | 25.78 c     | 6.00 a  | 6.08 b       |
| **Overall interaction**     |                   |          |             |         |              |
| Riyadh–Winter–3:00 AM       | 51.90 d            | 2.97 a   | 26.70 cd    | 5.87 a  | 2.23 d       |
| Riyadh–Winter–3:00 PM       | 61.03 b            | 2.00 b   | 29.30 ab    | 5.43 a  | 12.57 a      |
| Riyadh–Summer–3:00 AM       | 52.23 d            | 2.03 b   | 29.77 a     | 5.63 a  | 2.57 d       |
| Riyadh–Summer–3:00 PM       | 63.67 a            | 2.13 b   | 25.67 d     | 5.97 a  | 10.33 abc    |
| Riyadh–Winter–3:00 PM       | 52.07 d            | 2.27 b   | 27.90 bc    | 5.97 a  | 9.80 bc      |
| Eastern–Winter–3:00 PM      | 53.47 d            | 2.13 b   | 28.57 ab    | 6.03 a  | 11.80 ab     |
| Eastern–Summer–3:00 AM      | 61.50 b            | 1.97 b   | 28.90 ab    | 5.80 a  | 1.83 d       |
| Eastern–Summer–3:00 PM      | 57.13 c            | 2.03 b   | 25.90 d     | 6.03 a  | 8.90 c       |

* Values are the mean of 3 replicates. Means followed by the same letter are not significantly different (P ≤ 0.05).
Macronutrient contents in Z. spinosa plants as affected by the studied parameters.

| Factors          | P mg L⁻¹ | K µg L⁻¹ | Mg µg L⁻¹ | Ca µg L⁻¹ |
|------------------|----------|----------|-----------|-----------|
| **Single factors** |          |          |           |           |
| 1. Collection region |          |          |           |           |
| Riyadh           | 3956.28 a| 18096.34 a| 2461.56 a | 5619.48 a |
| Eastern region   | 1891.44 b| 5165.63 b | 1759.74 b | 5450.51 b |
| 2. Collection season |          |          |           |           |
| Winter            | 2909.89 a| 13445.19 b| 1863.84 b | 4470.59 b |
| Summer            | 2937.83 a| 13816.50 a| 2357.46 a | 6599.40 a |
| **Two-way interaction** |          |          |           |           |
| 1. Region × time  |          |          |           |           |
| Riyadh–Winter     | 3514.38 b| 14617.37 b| 1251.80 b | 4098.60 d |
| Riyadh–Summer     | 4398.19 a| 21575.31 a| 2267.67 a | 7140.35 a |
| Eastern–Winter    | 2305.41 c| 12273.61 b| 2475.88 a | 4842.58 c |
| Eastern–Summer    | 1477.47 d| 6057.68 c | 2447.24 a | 6058.45 b |
| 2. Region × time  |          |          |           |           |
| Riyadh–3:00 AM    | 4671.18 a| 21167.10 a| 1820.00 b | 5843.02 a |
| Riyadh–3:00 PM    | 3241.39 b| 15025.58 b| 1699.48 b | 5395.93 b |
| Eastern–3:00 AM   | 1977.05 c| 7648.70 c | 1972.02 b | 5457.49 b |
| Eastern–3:00 PM   | 1805.83 c| 10682.59 bc| 2951.11 a | 5443.54 b |
| 3. Season × time  |          |          |           |           |
| Winter–3:00 AM    | 3068.46 b| 13314.27 b| 1765.64 b | 4613.95 a |
| Winter–3:00 PM    | 2751.33 b| 13576.71 b| 1962.05 ab| 4327.23 a |
| Summer–3:00 AM    | 3579.77 a| 15501.54 a| 2026.38 ab| 6868.56 a |
| Summer–3:00 PM    | 2295.89 c| 12131.46 c| 2688.54 a | 6512.24 a |
| **Overall interaction** |          |          |           |           |
| Riyadh–Winter–3:00 AM | 4158.60 b| 18722.27 c| 1640.97 c | 4583.59 e |
| Riyadh–Summer–3:00 PM | 2870.16 d| 10512.48 e| 862.64 d | 3613.61 f |
| Riyadh–Summer–3:00 AM | 5183.76 a| 23611.93 b| 1999.03 c | 7102.46 a |
| Riyadh–Summer–3:00 PM | 3612.62 c| 19538.69 b| 2536.32 b | 5040.85 d |
| Eastern–Winter–3:00 AM | 1978.32 f| 7906.27 f| 1890.30 c | 4644.31 e |
| Eastern–Winter–3:00 PM | 2632.50 b| 16640.95 d| 3061.46 a | 5004.83 f |
| Eastern–Summer–3:00 AM | 1975.77 f| 7391.14 g| 2053.73 a | 6270.67 b |
| Eastern–Summer–3:00 PM | 979.16 g | 4724.23 h | 2840.76 ab| 5419.71 b |

* Values are the mean of 3 replicates. Means followed by the same letter are not significantly different (P ≤ 0.05).

The results obtained in the current study showed significant variations in the contents of carbohydrates, fats, crude protein, ash and moisture in the tissues of the studied plants collected from different regions in different seasons. Proximate analysis of Z. spinosa plants in the current study showed that all collected samples had high carbohydrate and protein contents with very low content of fats. Our study is the first study to examine the proximate analysis of Z. spinosa plants. The high proteins and carbohydrate contents of these plants could be considered as an advantage for further studies examining their potential uses and benefits e.g. using as fodder.

Proximate composition analysis of the studied plants was further supported by estimating contents of macro and micronutrients in the tissues of these plants. In general, all the studied plants showed high levels of macronutrients (P, K, Mg and Ca) and micronutrients (Fe, Cu, Zn, Mn). Furthermore, relatively high levels of Na were observed in the tissues of the studied plants and this could be attributed to the fact that they are growing in arid regions in Riyadh and Eastern regions of Saudi Arabia that characterized by dry weather with relatively low rainfall levels. This dry weather usually leads to increase in soil salinity which reflects on the plants’ content of Na⁺ ions.

Furthermore, relatively high accumulation levels of heavy metals (Cd, Pb and Cr) was observed in all the studied plants with Cr showed the highest levels among all the three heavy metals especially in A. sieberi and R. stricta plants. Some studies indicated the ability of different species belonging to Artemisia genus to accumulate heavy metals e.g. (Alizayeva et al., 2006; Rebhi et al., 2019; Kumari et al., 2018; Branković et al., 2019). Similarly, some studies indicated the ability of R. stricta plants to accumulate high amounts of different heavy metals e.g. (Alsibany et al., 2019; Lanjwani et al., 2018; Al-Farraj et al., 2010). Furthermore, the role of R. stricta in phytoremediation of heavy metal-contaminated soil is evident and widely accepted (Alsheikh and Kirkham, 2018). Amounts of these heavy metals were examined in the soil samples collected...
from the rhizosphere of each plant. Nevertheless, most of the samples showed normal levels of heavy metals which could provide an explanation for the high amounts of heavy metals in plant tissues as they accumulate these metals from the soil.

5. Conclusion

It could be concluded that spatial and seasonal variation has affected the chemical and proximate compositions of *Z. spinosa* plants. Furthermore, mineral contents were changed in response to seasonal and spatial variation. Further studies are needed to verify the effect of such variations on other medicinal plants chemical composition and activity. Moreover, various biological activities of *P. undulata* plants need to be studied.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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*Values are the mean of 3 replicates. Means followed by the same letter are not significantly different (P ≤ 0.05).*
