



Original article

Soil structure influences proteins, phenols, and flavonoids of varied medicinal plants in Al Jubail, KSA

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ABSTRACT

In Al Jubail, Saudi Arabia, 29 medicinal plants have been collected from 15 diverse sites. The goal of this study was to determine how soil texture affected the protein, phenol, and flavonoid contents, and their relationship with the degree of genetic similarity. Most soil samples were loamy sand, except for sites 6 and 10, which were sandy loams. A total of 13 protein bands were shown where four were polymorphic and nine were monomorphic, with hereditary similarities ranging from 1 to 0.86. The results indicated that the protein content ranged from (9.32 $\mu\text{g/gm}$) in *Anabasis setifera* to (0.92 $\mu\text{g/gm}$) in *Juncus rigidus*. The highest phenol content was found in *Halopeplis perfoliata* (21.45 mg/gm), whereas the lowest was found in *Zygophyllum qatarense* 7 (2.133 mg/gm). *Salsola imbricate* 2 showed the highest flavonoid content (74.97 mg/gm), whereas *Juncus rigidus* had the lowest (1.43 mg/gm). The concentration varied based on the accession and species. In comparison to the other soils tested, the soil at site 7 had the highest concentrations of calcium (132.5 mEq/L), magnesium (47.5 mEq/L), sodium (52.83 mEq/L), potassium (26.96 mEq/L), chloride (63.00 mEq/L), and electric conductivity (25.9 ds/m). The surveyed accessions were classified into two groups using cluster analysis, principal component analysis, and multivariate heatmap. These findings imply that variations in active compounds that are important for plant tolerance to wild habitats are associated with different soil structures, allowing plants to be used in the pharmaceutical and biomedical industries, as well as selective breeding of accessions with high antioxidant properties.

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1. Introduction

Traditional medicine uses medicinal plants for human health care where plant materials were collected from the natural environment. Secondary metabolites are abundantly found in medicinal plants. Variations in secondary metabolite composition and content can be found in a variety of medicinal plant species (Ahn, 2017). Morphological, metabolic, and DNA-based polymorphs can be used to identify genetic variation among people

or populations (Alotaibi and Abd-Elgawad, 2022; Goswami et al., 2021).

Plants contain high levels of bioactive compounds, such as phenols and flavonoids, which not only control plant improvement but also have substantial health benefits for humans (Chen et al., 2018; Singh et al., 2017). Phenols have a variety of functions in plants, including protection against plant eaters, grasses, and microbes, as well as mechanical stability (Otálora et al., 2018). They are also associated with improving plant adoption to stressful situations caused by environmental changes (Šamec et al., 2021). The type of plant habitat, the dominance of specific abiotic variables inside the habitat, season, and existence or lack of adverse circumstances are all variables that affect the substance, value, and quantity of bioactive components (Karahan et al., 2016; Stawarczyk et al., 2021). Flavonoids are found in all plant tissues, although their quantity relies mainly on the species as well as ecological and genetic factors (Pérez-Gregorio et al., 2014; Stanković et al., 2015). The phenolic components of *Olea europaea* L. in the wild are ecologically diverse and farmed environments show ecological diversity (Stanković et al., 2017). Impact of soil type on polyphenol

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Table 1

Soil analysis shows the variation between 29 species distributed in 15 various accessions at Al Jubail illustrated by physical and chemical analysis.

NO.	Species	Sites	Texture	CaCO ₃ %	pH	EC ds/m	Soluble cations and anions, milliequivalent/litre					
							Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Cl ⁻	HCO ₃ ⁻
1	<i>Zygophyllum qatarense</i> 1	Site-1	Loamy Sand	2.54	7.88	0.97	5.00	1.00	2.31	4.8	2.00	4.00
2	<i>Zygophyllum qatarense</i> 2											
3	<i>Cyperus conglomeratus</i> 1	Site-2	Loamy Sand	4.24	7.98	2.93	30.00	4.00	2.75	4.63	2.40	4.00
4	<i>Zygophyllum qatarense</i> 3											
5	<i>Zygophyllum qatarense</i> 4	Site-3	Loamy Sand	1.02	8.11	1.10	4.00	3.00	2.25	3.82	3.00	6.00
6	<i>Zygophyllum qatarense</i> 5											
7	<i>Cyperus conglomeratus</i> 2	Site-4	Loamy Sand	3.27	8.36	1.10	5.00	3.00	2.30	2.43	5.00	2.00
8	<i>Zygophyllum qatarense</i> 6											
9	<i>Zygophyllum qatarense</i> 7	Site-5	Loamy Sand	5.12	8.02	1.90	10.00	2.00	2.37	7.00	2.00	6.00
10	<i>Salsola imbricate</i> 1											
11	<i>Fagonia bruguieri</i>	Site-6	Sandy Loam	8.54	8.21	2.00	4.00	2.00	2.66	12.41	3.00	4.00
12	<i>Salsola imbricate</i> 2											
13	<i>Salsola imbricate</i> 3	Site-7	Loamy Sand	7.32	8.34	25.9	132.5	47.5	52.83	26.96	63.00	6.00
14	<i>Halopeplis perfoliata</i>											
15	<i>Seidlitzia rosmarinus</i>	Site-8	Loamy Sand	3.17	8.11	2.61	30.0	3.00	1.09	1.74	7.00	6.00
16	<i>Anabasis setifera</i>											
17	<i>Salsola imbricate</i> 4	Site-9	Loamy Sand	5.61	8.24	7.19	37.0	15.0	5.74	11.80	59.00	10.00
18	<i>Salsola imbricate</i> 5											
19	<i>Zygophyllum qatarense</i> 8	Site-10	Sandy Loam	5.80	8.82	1.70	5.00	3.00	3.51	3.96	3.00	8.00
20	<i>Heliotropium bacciferum</i>	Site-11	Loamy Sand	4.15	8.64	1.30	3.00	2.00	2.78	4.05	3.00	8.00
21	<i>Zygophyllum qatarense</i> 9											
22	<i>Zygophyllum qatarense</i> 10	Site-12	Loamy Sand	2.15	8.26	2.40	7.00	4.00	3.72	3.86	2.60	6.00
23	<i>Juncus rigidus</i>											
24	<i>Haloxylon salicornicum</i>	Site-13	Loamy Sand	5.39	8.24	2.10	5.00	4.00	3.39	5.48	3.00	4.00
25	<i>Zygophyllum qatarense</i> 11											
26	<i>Cyperus conglomeratus</i> 3	Site-14	Loamy Sand	3.22	8.47	1.50	4.00	2.00	3.57	3.30	2.00	4.00
27	<i>Calotropus procera</i>											
28	<i>Zygophyllum qatarense</i> 12	Site-15	Loamy Sand	2.15	8.43	1.90	4.00	3.00	2.84	3.38	3.00	6.00
29	<i>Zygophyllum qatarense</i> 13											

Notes: CO⁻³ not detected

characteristics and quantity in *Capsicum chinense* Jacq (Oney-Montalvo et al., 2020). The taste, pharmacological, and industrial quantities of species are all influenced by phenolic chemicals (Ferreira-Santos et al., 2020; Otálora et al., 2018).

Flavonoids have vital biological and environmental effects on species, acting as supplemental oxidativestress in tissues exposed to a wide range of environmental stressors (Hectors et al., 2014; Shi et al., 2021). Species from various communities subjected to various abiotic stresses typically exhibit differential flavonoid concentrations (Böttner et al., 2021). Because abiotic factors in communities change with latitude, longitude, and altitude, flavonoid concentrations may exhibit regional trends (Arista et al., 2013; Prendeville et al., 2013). Differential patterns of flavonoid exhibit accumulation in various plant sections between individuals and communities of *Silene littorea* (Del Valle et al., 2015). Flavonoids are Produced from a variety of edible plants and are considered vital human nutritional elements (Patil and Masand, 2018; Pinedo-Espinoza et al., 2020). These chemicals exhibit various biological effects.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate polypeptides (Kakaei and Kahrizi, 2011) which is a viable method for examining plant phylogenetic relationships (Rayan and Osman, 2019) and the genetic diversity of plants (Abd-Elgawad and Alotaibi, 2017; Shaban et al., 2022). Protein content levels represent inherited relationships within a genus and species, as well as between separate biological systems (Anwar et al., 2011).

Few attempts have been made to investigate chemical and genetic variation among wild medicinal plant species using different molecular markers (Alotaibi and Abd-Elgawad, 2022; Abd-Elgawad and Alotaibi, 2017). Accordingly, the goal of the current study was to determine the impact of soil structure on the protein patterns, phenol, and flavonoid levels, as well as their correlation with the genetic similarity of Saudi Arabian plant species in Al Jubail.

2. Methods

2.1. Plant material and collection sites

In this study, 29 herbal plants were collected from 15 distinct ecosystems in Saudi Arabia's Al Jubail area in December 2019. The geographical area of the collection sites is cited in Fig. 1 (Alotaibi and Abd-Elgawad, 2022). The median temperature ranged from 16 °C to 27 °C in day and night, respectively. The plant leaves were used for total protein, phenolic, and flavonoid contents. The plant soil was used for soil analysis.

2.2. Soil structure

The physical and chemical analyses of the specimens were performed. The hydrometer procedure was used to estimate the physical analysis, including particle size (Gee and Or, 2002). Chemical analysis, which included calcium carbonate, was determined by a calorimeter, pH using a pH meter, and electric conductivity (EC) using an EC meter. Soluble anions of soil extracts were estimated by titration (1:1) using silver nitrate for chloride, and sulfuric acid for bicarbonate. Soluble cations of extracts were estimated by using a flame photometer (1:1) for sodium and potassium, and by titration (1:1) using EDTA for calcium and magnesium (Helmke and Sparks, 1996).

2.3. Protein extraction

The leaves were ground to a fine powder. Briefly, one gm of leaf tissue was cut from each sample. It was grinded using sand in a mortar with 1 mL of cold QB solution (100 mM Tris-Cl (pH 6.8), 4 % SDS (sodium dodecyl sulfate), and 200 mM DTT (dithiothreitol)) including a protease. One mL of extract was transferred into a 1.5 mL Eppendorf which was kept on ice. The specimens were



Fig. 1. A map illustrated the collection sites in Al Jubail by using GPS data (Alotaibi and Abd-Elgawad, 2022).

centrifuged for 15 min at 15,000 rpm and 4 °C. The liquid supernatant should be put into a new microfuge tube. When the Bradford protein survey is finished, keep the specimens at 4 °C. The extracted proteins were used for Quantification and sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE).

2.4. Total protein content

Those specimens have been split to use 50 µl of protein mixture and 2.5 mL of Bradford reagent. To begin, 5 µl of the specimen was dissolved in 45 µl of water. The benchmark was a 1 mg/ml bovine

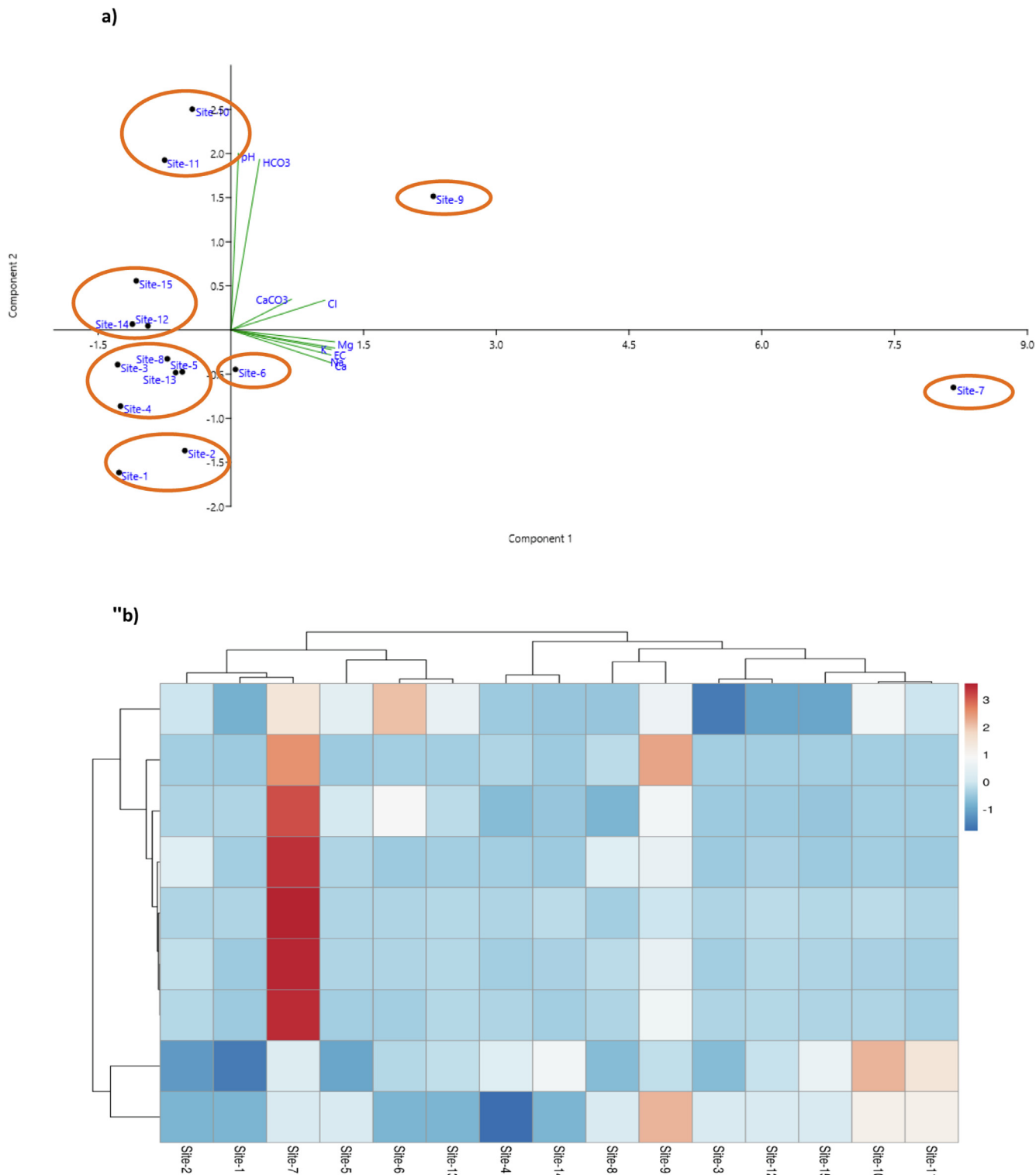


Fig. 2. The soil analysis illustrated the variation between 15 various ecosystems is shown by a) PCA BiPlot with PC1 (65.2%) and PC2 (15.2%), and b) multivariate heatmap.

serum albumin (BSA) method. The samples were assayed the absorption at 595 nm wavelength and then compared with the standards.

2.5. Total phenolic content

The phenolic quantity of samples was assessed using the Folin-Ciocalteu process (Kaur and Kapoor, 2002). A total of 200 µl of 70 % methanolic separate (1 mg/mL) was diluted to 3 mL of water, completely shuffled of 0.5 mL of Folin-Ciocalteu reagent for 3 min, and then 2 mL of 20 % (w/v) sodium carbonate was added. After another 60 min in the dark, the absorbance at 650 nm was assessed. The standard curve was used to compute the phenolic amount, which was indicated as mg of gallic acid comparable for each g dry weight.

2.6. Total flavonoid content

Different aliquots of the solution of quercetin comparable to 5–300 µg were placed in different tubes and dried in a water bath (40–50 °C). Two grams of each defatted powder were extracted with petroleum ether, the extract was then adjusted to 50 mL by adding (95 %) ethanol. Five ml of the extract were transmitted to a tube, followed by 5 mL aliquots of 0.1 M AlCl3 solvent. The solution was evaporated to dryness in the water bath. The transmittance of each evolved color was assessed using UV at 266 nm for kaempferol and at 445 nm for quercetin (Karawya and Aboutabl, 1982).

2.7. Data analysis

Protein profile examinations revealed banding designs that were evaluated as ‘1’ for group proximity and ‘0’ for group absence. Distinguishability was the groups with the same compactness. The Dice coefficient was used to calculate the coefficient of genetic similarity (GS) between varieties (Sneath and Sokal, 1973).

Clustering for the Unweighted Pair-Group plan with Arithmetic Average (UPGMA) (Sneath and Sokal, 1973) yielded a framework of similarity. PAST 3.2 program was used to assess molecular variation and relationships among samples using cluster analysis and PCA (Hammer et al., 2001; Havill et al., 2007; Muthusamy et al., 2008). Multivariate analysis was performed using the application to create a heatmap network (Metsalu and Vilo, 2015).

3. Results

3.1. Soil structure

The soils from where the specimens were collected were loamy sand, except for two sites where the soil samples were sandy loam (site-6 and site-10) (Table 1, Fig. 2a and b). Soil samples had pH values ranging from 7.88 on site-1 to 8.82 on site-10. On the other hand, the EC value ranged from 0.97 ds/m (site-1) to 25.9 ds/m (site-7). Anions such as chloride ranged from 2 mEq/L (site-1, site-2, site-5, site-12 and site-14) to 63 mEq/L (site-7), bicarbonate from 2 mEq/L (site-4) to 10 mEq/L (site-9), and no traces of carbonates were detected. Soluble cations such as calcium ranged from 3 mEq/L (site-11) to 132.5 mEq/L (site-7), magnesium ranged from 1 mEq/L (site-1) to 47.5 mEq/L (site-7), sodium and potassium ranged from 1.09 mEq/L and 1.74 mEq/L (site-8) to 52.83 mEq/L and 26.96 mEq/L (site-7), respectively. The calcium carbonate percentage in soil samples ranged from 1.02 % (site-3) to 8.54 % (site-6).

The PCA was arranged based on a correlation between soil parameters (Fig. 2a). The first PCA accounted for roughly 65.2 %, while the 2nd PCA accounted for 15.2 %. Heatmap was clustered using correlation distance and average linkage between species and soil parameters into two main clusters (Fig. 2b). The first cluster was divided into two clusters. Sites 1 and 7 were clustered together, with site 2 as an isolated branch, while sites 6 and 13 were clustered together, with site 5 as an isolated branch. The 2nd cluster was categorized into four clusters. Sites 4 and 14, sites 8 and 9, sites 3 and 12, and sites 10 and 11 were clustered together, with site 15 acting as an isolated branch.

3.2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

We detected 13 peptides of different molecular weight groups in our collected species: 221, 197, 180, 165, 149, 133, 112, 101, 64, 35, 30, 25, and 15 K_D (Table 2). All 13 protein bands were observed in several species of *Zygophyllum* (1, 2, 4, 5, 7, 8, 12, 13), *Fagonia*, *Salsola* 3, *Haloxylon*, *Cyperus* 3, and *Calotropus*. Four protein patterns disappeared in different species the 1st molecular weight was 112 K_D for *Anabasis*, *Salsola* 5, *Heliotropium*, *Zygophyllum* 10, and 11, and the 2nd was 101 K_D for *Cyperus* 2, *Zygophyllum* 6, *Salsola* (1, 2), and *Seidlitzia*, the 3rd was 64 K_D for *Cyperus* (1, 2), *Zygophyllum* (3, 6), *Salsola* (1, 2, 4, 5), *Halopeplis*, *Seidlitzia*, *Heliotropium*, *Zygophyllum* 9, and *Juncus*, and the 4th 35 K_D for *Salsola* 4, 5, *Heliotropium*, *Zygophyllum* 9, and *Juncus* (Table 2).

Table 2 Electrophoretic banding analysis of proteins of 29 genotypes collected from 15 various habitats.

MW	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Frequency
221	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
197	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
180	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
165	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
149	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
133	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
112	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	1	0	1	1	0	1	1	1	1	0.8
101	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.8
64	1	1	0	0	1	1	0	0	1	0	1	0	1	0	0	1	0	1	0	1	0	1	0	1	1	1	1	1	1	0.6
35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	0	1	1	1	1	1	1	0.8
30	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1.0
Total Bands	13	13	12	12	13	13	11	11	13	11	13	11	13	12	11	12	11	10	13	10	11	12	11	13	12	13	13	13	13	

The UPGMA and Dice coefficients (Fig. 3a) were used to illustrate the inheritance correlations and classification arrangement of the protein fingerprint polymorphism between species. The maximum hereditary similarities were (1) appeared between Zygophyllum (1, 2, 3, 4, 5, 7, 8, 12, 13), *Fagonia*, *Salsola* 3, *Haloxylon*, *Cyperus* (1, 3), *Calotropus*, and *Halopeplis*. On the other hand, the minimum was (0.86) observed between *Cyperus* 2, *Zygophyllum* 6, *Salsola* (1, 2, 5), *Seidlitzia*, and *Heliotropium*. The tree of protein

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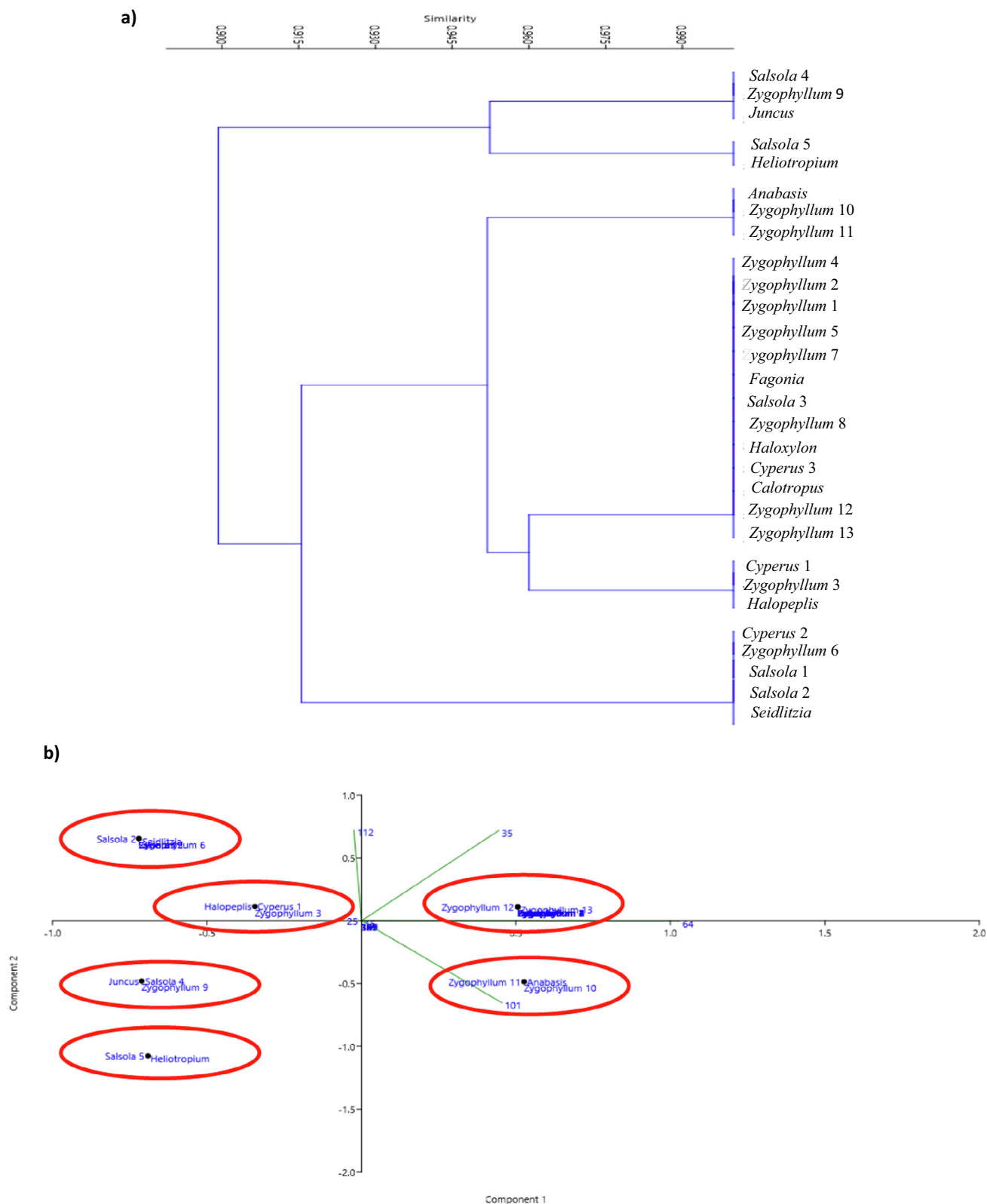


Fig. 3. Electrophoretic banding profiles of protein illustrated the variation between 29 genotypes collected from 15 various habitats is shown by a) clustering analysis, and b); PCA BioPlot analysis with PC1 (49%) and PC2 (30.9%).

Table 3

The diversity in total protein, phenol, and flavonoid content amongst 29 genotypes obtained from 15 various ecosystems is shown.

Sample	Protein ($\mu\text{g/gm}$)	Phenol (mg/gm)	Flavonoid (mg/gm)
<i>Zygophyllum</i> 1	6.76 \pm 0.081	10.07 \pm 0.002	58.97 \pm 0.007
<i>Zygophyllum</i> 2	5.84 \pm 0.089	6.13 \pm 0.002	60.5 \pm 0.018
<i>Cyperus</i> 1	2.08 \pm 0.065	3.37 \pm 0.006	22.37 \pm 0.015
<i>Zygophyllum</i> 3	3.84 \pm 0.016	6.95 \pm 0.001	30.7 \pm 0.038
<i>Zygophyllum</i> 4	7.52 \pm 0.033	12.62 \pm 0.003	53.17 \pm 0.004
<i>Zygophyllum</i> 5	5.64 \pm 0.033	8.2 \pm 0.004	12.1 \pm 0.009
<i>Cyperus</i> 2	7.28 \pm 0.049	4.95 \pm 0.003	45.17 \pm 0.013
<i>Zygophyllum</i> 6	6.16 \pm 0.049	12.45 \pm 0.004	33.9 \pm 0.058
<i>Zygophyllum</i> 7	8.88 \pm 0.041	2.133 \pm 0.003	52.23 \pm 0.021
<i>Salsola</i> 1	6.48 \pm 0.049	8.367 \pm 0.003	26.57 \pm 0.012
<i>Fagonia</i>	6.6 \pm 0.033	21.1 \pm 0.03	15.03 \pm 0.003
<i>Salsola</i> 2	3.68 \pm 0.033	7.83 \pm 0.004	74.97 \pm 0.021
<i>Salsola</i> 3	8.24 \pm 0.041	5.067 \pm 0.003	33.03 \pm 0.021
<i>Halopeplis</i>	2.56 \pm 0.033	21.45 \pm 0.004	23.97 \pm 0.018
<i>Seidlitzia</i>	5.68 \pm 0.024	9.083 \pm 0.003	69.7 \pm 0.013
<i>Anabasis</i>	9.32 \pm 0.033	4.45 \pm 0.003	3.9 \pm 0.005
<i>Salsola</i> 4	3.12 \pm 0.041	7.15 \pm 0.001	8.23 \pm 0.008
<i>Salsola</i> 5	4.4 \pm 0.041	16.95 \pm 0.003	57.43 \pm 0.008
<i>Zygophyllum</i> 8	3.28 \pm 0.033	5.883 \pm 0.002	54.43 \pm 0.023
<i>Heliotropium</i>	3.32 \pm 0.024	5.5 \pm 0.003	7.77 \pm 0.007
<i>Zygophyllum</i> 9	6.56 \pm 0.041	8.117 \pm 0.004	49.3 \pm 0.017
<i>Zygophyllum</i> 10	2.68 \pm 0.033	9.067 \pm 0.003	37.77 \pm 0.017
<i>Juncus</i>	0.92 \pm 0.033	4.65 \pm 0.005	1.43 \pm 0.007
<i>Haloxylon</i>	8.24 \pm 0.024	10.517 \pm 0.004	9.167 \pm 0.018
<i>Zygophyllum</i> 11	5.2 \pm 0.024	4.417 \pm 0.007	8.1 \pm 0.015
<i>Cyperus</i> 3	5.92 \pm 0.016	6.117 \pm 0.006	35.367 \pm 0.038
<i>Calotropus</i>	6.36 \pm 0.016	5.883 \pm 0.004	9.43 \pm 0.004
<i>Zygophyllum</i> 12	6.84 \pm 0.041	8.933 \pm 0.011	12.37 \pm 0.009
<i>Zygophyllum</i> 13	5.2 \pm 0.041	9.133 \pm 0.006	12.233 \pm 0.013

profiles was distinguished into two main clusters. The first main cluster had been encircled *Salsola* 4, *Zygophyllum* 9, *Juncus*, *Salsola* 5, and *Heliotropium*. The 2nd main cluster was split into two distinct branches and one cluster (Fig. 3a). The 1st isolated branch contained *Cyperus* 2, *Zygophyllum* 6, *Salsola* (1, 2), and *Halopeplis*, while *Anabasis*, and *Zygophyllum* (10, 11) were found in the 2nd branch. *Zygophyllum* (1, 2, 3, 4, 5, 7, 8, 12, 13), *Fagonia*, *Salsola* 3, *Haloxylon*, *Cyperus* (1, 3), *Calotropus*, and *Halopeplis* were clustered together. The PCA was designed using Dice's matrix (Fig. 3b). The first PCA contributes to approximately 49 % of all varieties, while the 2nd PCA contributed to 30.9 %. The PCA model equaled the arrangement of the clustering assessment.

3.3. Total protein, phenolic and flavonoid contents

The total protein content ranged from 0.92 $\mu\text{g/gm}$ in *Juncus* (site-10) to 9.32 $\mu\text{g/gm}$ in *Anabasis* (site-8), as shown in Table 3. The total phenol content in various species was depicted in Table 3. The results showed that *Halopeplis* of site-7 was the richest source of phenol, (21.45 mg/gm), while the lowest was *Zygophyllum* 7 of site-5 (2.133 mg/gm). The total flavonoid content was reached from (74.97 mg/gm) in *Salsola* 2 of site-6 to (1.43 mg/gm) in *Juncus* of site-12 (Table 3).

The PCA was arranged based on a correlation between contents (Fig. 4a). The first PCA is responsible for approximately 36.3 % of all varieties, while the 2nd PCA accounted for 35.3 %. The PCA framework matched the bunching examination structure. Heatmap was clustered using correlation distance and average linkage between species and protein, phenol, and flavonoid content into two trees (Fig. 4b). The two trees were divided into two clusters once more. The first major cluster was divided into two loops, the initial of which was divided into four groups of *Zygophyllum* (1, 2, 3, 8, 10), *Juncus*, *Salsola* (2, 5), *Cyperus* 1, and *Seidlitzia*. The 2nd loop was split into two groups of *Zygophyllum* (4, 7, 9), and *Cyperus* (2, 3) with *Salsola* 3 as a branch. On the other hand, the

2nd major cluster was separated into two loops; the first was split into two groups of *Salsola* 4, *Halopeplis*, *Fagonia*, *Zygophyllum* 6, and *Heliotropium*, while *Zygophyllum* 13 was a separate branch. The 2nd loop was made up of two groups of *Zygophyllum* (5, 11, 12), *Haloxylon*, *Anabasis*, *Calotropus*, and *Salsola* 1.

4. Discussion

The majority of the sites in this study were alkaline, with loamy sand. The soil structure may be critical, particularly for its ability to keep intact the plant nutrients. Many species grow more in loam soil (Gatiboni, 2018). Based on PCA, there is a close relationship between site-5 (*Zygophyllum* 7 & *Salsola* 1), site-13 (*Haloxylon* & *Zygophyllum* 11), and site-8 (*Seidlitzia* & *Anabasis*), as well as between site-1 (*Zygophyllum* 1 & 2), site-7 (*Salsola* 3 & *Halopeplis*) and site-2 (*Cyperus* 1 & *Zygophyllum* 3) based on the multivariate heatmap. The findings suggested that the soil texture impacts the water content of plant life (Gatiboni, 2018; Shekunyenge, 2015). This variability was regarded as a positive adaptation that led to tolerance. Other researchers found that 69 % of the urban soils under the old linden trees in Poznań Park were alkaline (Golcz et al., 2014), and 46.7 % of specimens from soil adjoining the road in Poznan were alkaline (Bosiacki et al., 2014), Poland. Other countries' urban areas (including Greece and China) had alkaline soil (Argyaki et al., 2018; Mao et al., 2014). All soils in six different locations in Saudi Arabia's *Peganum* and *Rhazya* had slightly alkaline pH (Abd-Elgawad and Alotaibi, 2017).

All soil samples in this investigation showed some saltiness (EC), except for site-7 (*Salsola* 3 & *Halopeplis*) which had the highest EC, chloride, calcium, magnesium, sodium, and potassium values. The EC of a solvent is a measure of salt content as well as an indicator of electrolyte concentration. The quantity of ions obtainable to plants in the root system is related to the nutritional solution's EC (Abd-Elgawad and Alotaibi, 2017; Fan et al., 2019). The ideal EC varies by plant and is influenced by environmental factors (Abd-Elgawad and Alotaibi, 2017; Fan et al., 2019). EC was low salinity in six regions under *Peganum* and *Zygophyllum* in Egypt and under *Peganum* and *Rhazya* in Saudi Arabia for tolerance (Abd-Elgawad and Alotaibi, 2017; Anwar et al., 2011).

SDS-PAGE of protein patterns was recorded in 13 bands, these proteins were distinguished into two classes of monomorphic and polymorphic bands in various species. SDS-PAGE analysis established a solid foundation for genotype discrimination based on particular polypeptide fragments. The cluster analysis, which was supported by clustering and PCA analysis, classified 29 medicinal plants into six distinct groups. Heritability ranged between (1) and (0.86), indicating a high level of similarity. *Zygophyllum* (1, 2, 3, 4, 5, 7, 8, 12, 13), *Fagonia*, *Salsola* 3, *Haloxylon*, *Cyperus* (1, 3), *Calotropus*, and *Halopeplis* had the highest genetic similarity. The correlation between wild relatives from the same admission and the same genus from different admissions is distinguished, with the majority of characters shared by a few plants. Numerous environmental conditions may lead to the segregation of the evaluated species of various accessions, which may contribute to the evolution of hereditary variability between species. These variations were viewed as positive adaptations that led to tolerance (Abd-Elgawad and Alotaibi, 2017; Alafari and Abd-Elgawad, 2021). Protein profile changes were also influenced by the plant tissues evaluated and the plant species (Abd-Elgawad and Alotaibi, 2017; Anwar et al., 2011). These findings matched up with the findings of (Abd-Elgawad and Alotaibi, 2017), who reported that protein profiles revealed only minor differences between the two genera of harmal, indicating inherited connectedness between communities of these plants in diverse settings. These findings were consistent with those of other researchers who used SDS-PAGE to investigate

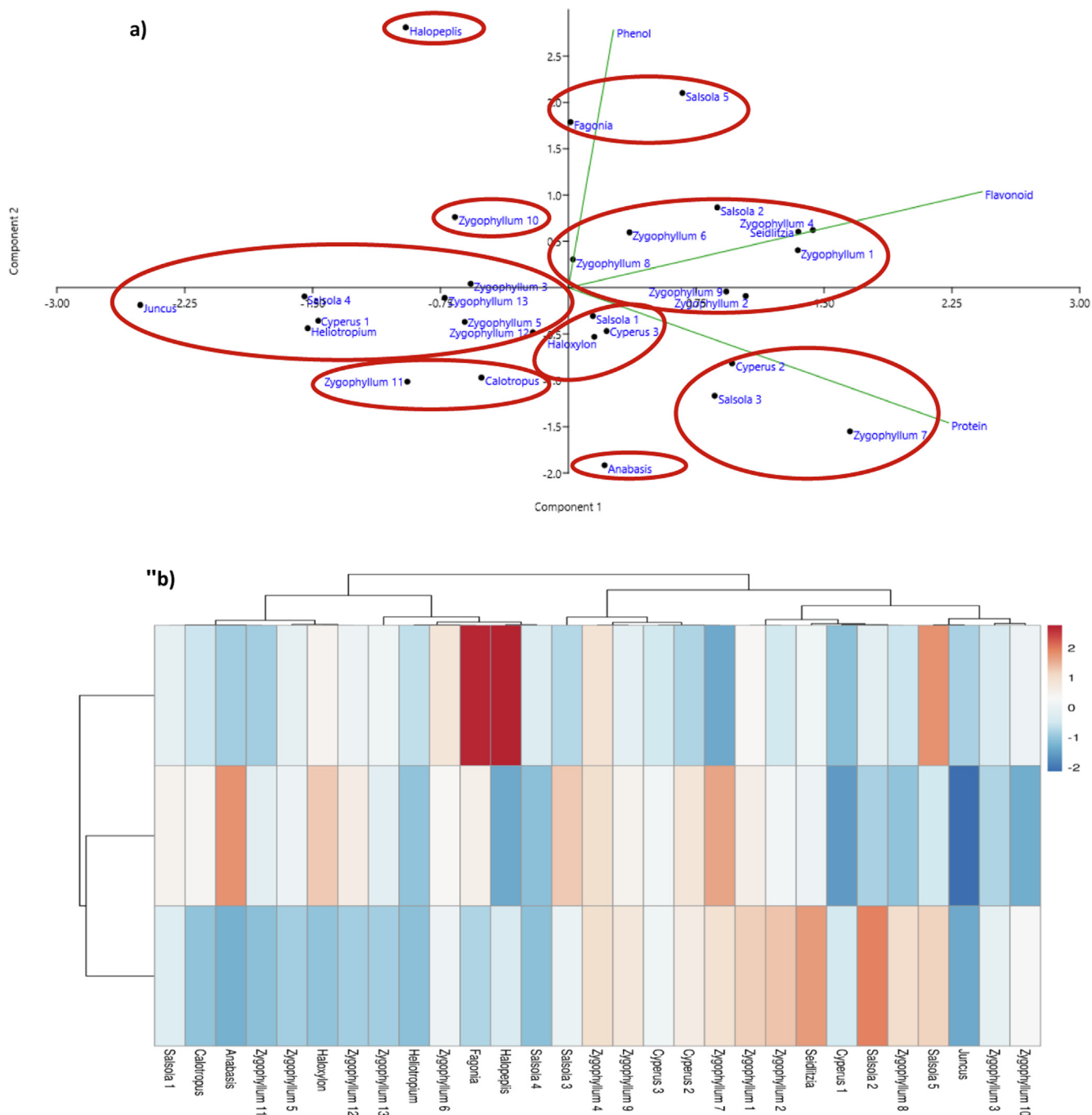


Fig. 4. The diversity in protein, phenolic, and flavonoid contents was illustrated between 29 genotypes obtained from 15 various ecosystems as shown by a) PCA biplot with PC1 (36.3%) and PC2 (35.3%), and b) multivariate heatmap.

the genetic variability and genetic relationships among different varieties gathered from various innate ecosystems (Hassan et al., 2016; Shaban et al., 2022). These results of protein profile disagreed with the results obtained from ISSR and SCoT analysis for 29 wild medicinal plants (Alotaibi and Abd-Elgawad, 2022). The EC and protein patterns have a favorable relationship.

The largest protein quantity was 9.32 µg/gm detected in *Anabasis* in site-8. The relationship between EC and protein content was adverse in 29 genotypes, suggesting that protein content was consumed to adapt to varied habitats. Protein content levels represent inherited relationships within a genus and species, as well as

between separate biological systems (Alafari and Abd-Elgawad, 2021; Anwar et al., 2011). The closeness correlation of EC and soil salinity of numerous variants can be used to describe the genetic similarities of harmful genotypes (Abd-Elgawad and Alotaibi, 2017). Genera with high genetic linkage and low saline soils were linked to more intense protein styles than variants with high saline soils. Total soluble protein increased or decreased in various communities of *Tetraena* in relation to heat stress (Alafari and Abd-Elgawad, 2021). It is well known that the overall protein content of leaves changes as they age (Abdoli Nejad and Shekafandeh, 2014; Kandyliis et al., 2009).

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