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Anatomical and chemical characteristics of wild plant species east of Al-Salman

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Summary

The present investigation entails a discovery of indigenous flora from the timeframe spanning from the 1st of December, 2021 to the 1st of May, 2023, in Al-Muthanna Governorate, situated in the south-western sector of Iraq within the confines of the Western Plateau, to the east of Al-Salman. The study included the chemical content of the leaves, which was characterized by their abundance in some of the species under study, and the chemical compounds were identified using gas chromatography-mass spectrometry (GC-MS) technology. Research has shown that these species are rich in secondary metabolic compounds, ranging from terpenes, phenols, steroids, fatty acids, vitamins, alkanes, alkaloids and esters. The compounds were distinguished by the different times of their appearance, and some of them were repeated at different times**.** The anatomical study dealt with some of the characteristics of the stomata and the guard cells of the leaf epidermis of some of the species studied. The stomatal complexes were studied and the stomata were distinguished as being of the Anisocytic and Anomotetracytic type of the *Zilla spinosa* type and similar to the surface stomata of the *Arnebia decumbens* plant of the Anomocytic and Anomotetracytic type, while the stomatal complex of the lower surface of the plant *Erodium lebelii* was of the type Anomocytic type

Keywords: wild plant, stomata , GC-MS, Al-Salman

introduction

It is necessary to distinguish one plant from another based on the possibility of distinguishing boundaries between different taxonomic classes, establish criteria for classifying and naming plants, and study the evolutionary links between taxonomic hierarchies in various branches of the biological sciences related to taxonomy, including anatomy, genetics, and biochemistry. These branches include plant biochemistry, pollen science, physiology, and other related sciences. (Akbarlou and Nodehi, 2016).

Wild plants play an important role as reservoirs of bioactive components, in particular compounds that possess antioxidant and anti-inflammatory properties (Al Bahadly, 2015). Within these plants, one can identify phytochemicals such as alkaloids, phenols, flavonoids, saponins, and terpenoids as major components found in the plant. (Yu, *et al* 2021). Plants contain important vital nutrients including B complex vitamins and vitamin C, which play crucial roles in various physiological functions of the body and contribute significantly to maintaining redox balance Kennedy, (2016).

Plants are a large and wide source of therapeutic uses, as they generate a variety of organic compounds classified into primary metabolic compounds essential for plant functions (Mahmoud *et al*., 2023). These compounds play a direct role in processes such as normal growth, development and reproduction in plants, including nucleic acids, amino acids, proteins, carbohydrates and lipids, Kennedy (2016). And secondary metabolic compounds with complex chemical structures. The latter are derived from primary metabolic compounds in trace amounts, and show differences across plant organs, developmental stages, and species.(Vermerris *et al*, 2006)

Whether growing wild within natural ecosystems or cultivated under human influence (e.g., selection or breeding) requiring management for survival (Calixto, 2000), medicinal plants have served as an essential modality in complementary medicine systems for a long time, with historical roots dating back to ancient times, the widespread use of herbal remedies underscores their importance in the use of medicinal plants and their associated therapeutic

advantages in the production of pharmaceutical preparations (de Souse *et al*., 2016), due to the diverse medicinal applications of different plant parts.

Materials and method

Anatomical study

One sample was extracted from the Middle of the leaf *Zilla spinosa* to obtain epidermal cells from the upper and lower surfaces. The physiological analysis in the current research was based on Fresh samples from the study sites, after fixation with Formalin acetic acid alcohol solution for 24 hours at room temperature. The samples were rinsed with 70% ethanol to remove any remaining fixative solution, then stored in alcohol of the same concentration in the refrigerator until needed to prepare Epidermis slides, According to the methodology outlined by Johansen,(1940) and modified by Al-Barki, (2024), the peeling technique was performed using forceps with two sharp ends and a dissecting blade. The treated samples were then transferred to a sterile glass Petri dish filled with 5.1% Sodium Hypochloride solution for ten minutes. To eliminate tissue residues adhering to the surface as well as chlorophyll pigment. The removed cuticle samples were placed on a glass slide, a drop of glycerin was placed on it, brushed, and then gently covered with a cover slide to prevent bubbles from forming in the tissue.(Dilcher,1974)

The study Ocular a lens with a magnification of .045 and converted to mm1 stoma guide, stoma length (mμ), stoma width (mμ), stoma aperture length (mμ), stoma aperture width (mμ) (the line measurement in the lens inserted at 2.4 mμ was converted to 1 mμ) using Ocular Micrometer, and the models were photographed under the Omax camera installed on the microscope according to the Vividia able scope program.

Chemical study,

Weigh 20 g of leaf powder *Andrachne telephioides***,** *Astragalus dactylocarpus* and mix with 100 ml of 96% ethanol in a 250 ml glass beaker. Next, the beaker was closed with a piece of cotton and aluminium foil and then placed in a water bath at 45 °C for 24 h. Next, filter the extract using Whatman No. With a pore size of 0.22 μm.(Durgawale *et al*., 2019) The filtrate is then collected and transferred to a glass dish, which is then placed in an electric oven at 40°C for 48 hours, allowing the sediment to adhere to the glass dish. The sediment is then removed, collected in a sealed glass bottle, and stored in the

refrigerator until needed. This entire procedure was repeated several times to obtain an appropriate amount of extract suitable for use in the GC-MS technique.

Qualitative and quantitative analysis of chemical compounds in some plant samples using the GC–MASS technique

The content of active substances in the leaves was estimated using a gas chromatograph coupled to a Mass Spectrometer Agilent 5977 A MSD, USA. Mass Hunter GC/MS Acquisition software, and Mass Hunter qualitative program of American origin in the Nahran Omar field laboratories of the Basra Oil Company.

The device was set to the ion source temperature: of 230°C, the quadrupole temperature: of 150°C, and the interface temperature of the MSD transfer line: of 290°C. The retention time is between 4.00 minutes and 35.00 - 40. minutes. The active compounds were identified using a database. National Institute of Standards and Technology by comparing the resulting spectrum of the unknown component with components stored in the NIST library.

Results and discussion

1 - Chemical compounds in the alcoholic extract of *Andrachne telephioides* L. leaves using GC-MS technology

The results of Table (1) and Figure (1) showed that the chemical compounds present in the alcoholic extract of the leaves of the *Andrachne telephioides* species were detected using the GC-MS technique, gas chromatography equipped with a mass spectrometer. The detection showed the presence of 41 active compounds of the *Andrachne telephioides* plant and that the highest peak area of the extract It was 14.7009% per minute 32.413 for the compound Hexacosene-1, and the lowest peak area was 0.4729% per minute 26.985 for the compound 4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative, which indicates that the compounds have a variation in the appearance time

Table (1) Effective compounds of the leaf extract of *Andrachne telephioides*

Peak	R.T.	Area Pct%	Library/ID
1	8.007	1.7568	2-Mercaptoethanol, TMS derivative
2	8.211	1.8358	1-Propanol, TMS derivative
3	8.8	1.7015	Butanoic acid, 3-methyl-

4	8.91	3.3071	Glycine
5	9.075	3.0793	Silane, triethylfluoro-
6	10.772	5.9423	Tetraethyl silicate
7	11.589	0.7666	Cyclohexanol, 3,3,5-trimethyl-
8	14.04	0.6535	Cyclododecane
9	16.875	1.829	1-Tetradecene
10	17.567	1.3652	(R*,R*)-5-Hydroxy-4-methyl-3-heptanone
11	17.645	0.9124	2,4-Di-tert-butylphenol
12	17.889	0.5619	Benzeneacetonitrile, 4-hydroxy-
13	18.093	0.7388	Benzeneacetonitrile, 4-hydroxy-
14	18.203	0.6705	Manganese, bis[(2-dimethylamino)ethyl-.eta.-5-cyclopentadienyl]-
15	18.313	0.6816	1-Dodecanamine, N,N-dimethyl-
16	19.365	2.1079	2-Tetradecene, (E)-
17	20.646	1.7136	1-Tetradecanamine, N,N-dimethyl-
18	21.282	0.6266	Pyrrolidine,1-cyclohexyl-
19	21.588	2.0364	1-Octadecene
20	22.091	2.0127	Neophytadiene
21	22.531	0.7775	11-Tetradecyn-1-ol acetate
22	23.309	0.8557	n-Hexadecanoic acid
23	23.615	1.9465	Hexadecanoic acid, ethyl ester
24	24.762	1.0218	Phytol
25	25.029	3.2469	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
26	25.202	3.443	9,12-Octadecadienoic acid, ethyl ester
27	25.453	1.1182	1-Nonadecene
28	26.985	0.4729	**5,7-Dimethyl-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one**
29	27.142	0.6166	Cyclotetracosane
30	28.423	7.9804	Bis(2-ethylhexyl) phthalate
31	28.713	0.5264	Fumaric acid, pent-4-en-2-yl tridecyl ester
32	29.475	3.0924	Eicosane
33	30.889	9.2206	1-Hexacosene
34	31.086	0.685	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative
35	32.413	14.7009	**1-Hexacosene**
36	32.837	1.9532	Vitamin E
37	33.906	1.6895	2-Dodecylphenol, TMS
38	34.251	1.3643	Tris(tert-butyldimethylsilyloxy)arsane
39	34.393	5.2256	Nonacos-1-ene
40	34.919	5.1751	.gamma.-Sitosterol
41	39.051	0.588	4-tert-Octylphenol, TMS derivative

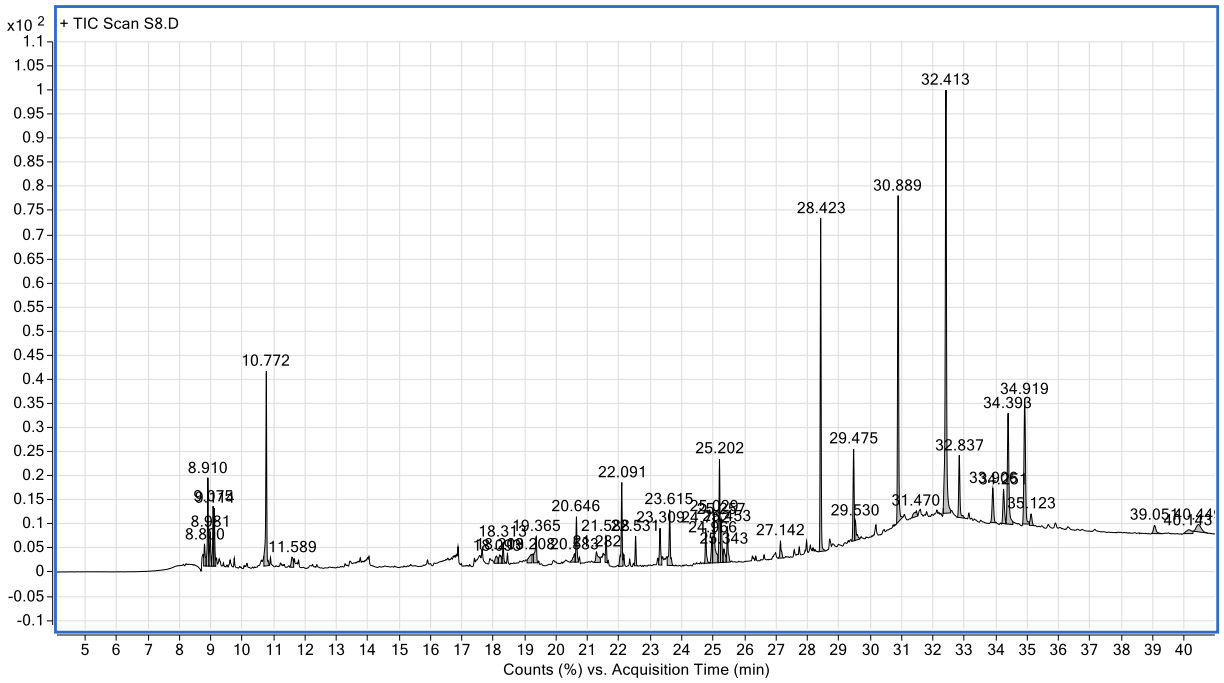


Figure (1) GC-MS analysis of *Andrachne telephioides* leaf

2 - Chemical compounds in the alcoholic extract of *Astragalus dactylocarpus* leaves using GC-MS technology

Table (2) and Figure (2) show the compounds present in the alcoholic extract of the leaves of the *Astragalus dactylocarpus* plant, which were detected using gas chromatography technology equipped with a mass spectrometer. The detection showed the presence of 25 compounds present in the *Astragalus dactylocarpus* plant. The highest peak area of the extract was 32.3562% per minute (21.581 minutes) for the compound 4-O-Methylmannose, and the lowest peak area was 1.0132% per minute (35.909 minutes) for the compound Ethoxy(phenyl) silane diol, 2TMS, which indicates that the compounds have a variation in appearance time and that there are Compounds that appear repeatedly for different periods.

Table (2) Chemical compounds in the alcoholic extract of *Astragalus dactylocarpus* leaves using GC-MS technology

Peak	R.T.	Area Pct	Library/ID
1	8.07	1.0839	2-Pyridinebutanoic acid
2	8.832	1.7368	Hydrazine, 1,1-dimethyl-
3	10.827	2.1795	Tetraethyl silicate
4	14.487	2.1821	4-Vinylphenol

5	15.76	1.4237	2-Methoxy-4-vinylphenol
6	15.901	2.2761	2-Methoxy-4-vinylphenol
7	18.203	1.0588	.beta.-D-Glucopyranose, 1,6-anhydro-
8	19.13	1.2509	Phenol, 4-ethenyl-2,6-dimethoxy-
9	19.35	1.4427	2(5H)-Furanone, 5,5-dimethyl-
10	20.646	5.2394	Ethanamine, N,N-dimethyl-
11	21.581	32.3562	**4-O-Methylmannose**
12	22.091	3.7573	Neophytadiene
13	22.531	1.5198	9-Octadecyne
14	23.317	1.546	n-Hexadecanoic acid
15	23.615	1.6027	3-Eicosene, (E)-
16	24.762	3.9886	Phytol
17	25.037	2.5301	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
18	28.423	3.7581	Bis(2-ethylhexyl) phthalate
19	29.538	2.6108	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-
20	30.882	4.3421	1-Hexacosene
21	32.421	14.3577	1-Hexacosene
22	34.251	1.1209	Stigmasterol
23	34.401	3.4265	Triacontyl acetate
24	34.919	2.1961	.gamma.-Sitosterol
25	35.909	1.0132	**Ethoxy(phenyl)silanediol, 2TMS**

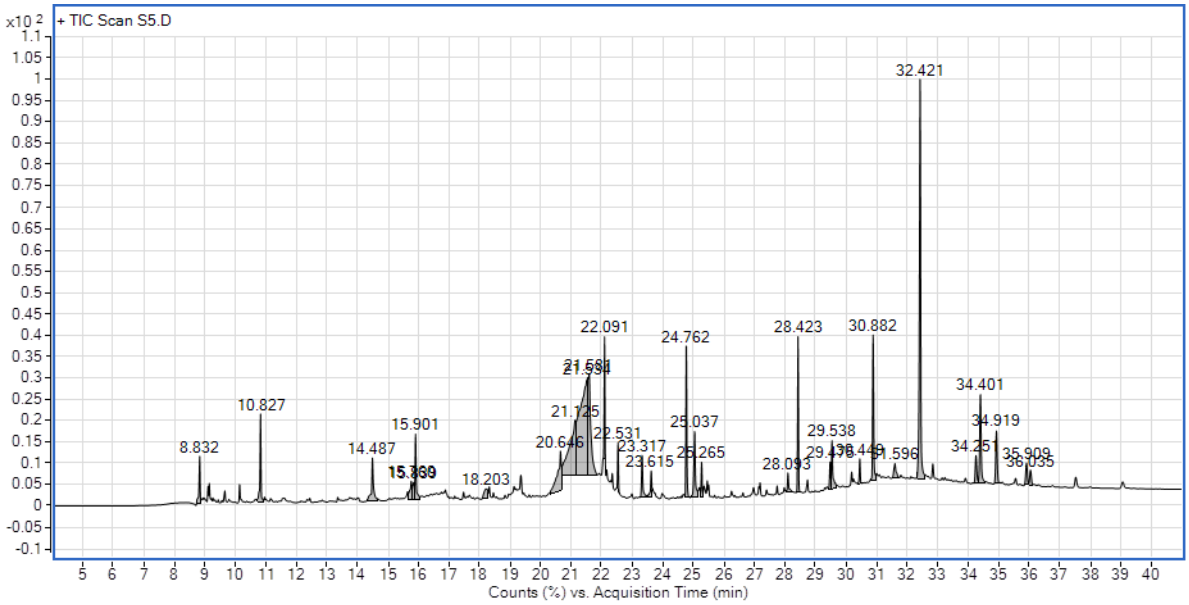


Figure (2) GC-MS analysis of *Astragalus zubairensis* leaf extract

Table (3): Characteristics of stomata on the upper and lower surfaces of leaves

Type name	Upper surfaces						Lower surfaces					
	Stoma length	Stoma width	Stoma guide	No. stomata	Stoma aperture length	Stoma aperture width	Stoma length	Stoma width	Stoma guide	No. stomata	Stoma aperture length	Stoma aperture width
Anthemis deserti	58.6-53.9 (56.1)	40.0-36.7 (37.1)	20	6	30.5-26.4 (28.4)	22.3-17.1 (20.4)	50.1-46.9 (48.5)	38.9-33.6 (34.2)	23.8	10	26.4-23.7 (24.1)	19.4-15.2 (18.6)
Zilla spinosa	62.2-58.2 (60.4)	49.1-46.5 (47.1)	21	3	30.4-25.5 (28.1)	26.3-20.5 (22.4)	58.1-55.4 (56.8)	45.4-40.5 (43.5)	25	7	28.4-23.1 (26.8)	25.5-20.1 (23.7)
Arnebia decumbens	50.2-46.4 (37.7)	41.3-38.5 (40.5)	15.9	5	27.8-22.4 (26.3)	19.1-16.4 (18.1)	43.2-39.1 (42.3)	37.5-33.5 (35.5)	24.4	10	20.4-16.3 (19.7)	18.7-12.4 (14.3)
Astragalus spinosus	47.7-42.8 (45.5)	36.3-30.5 (34.4)	33.3	12	29.6-20.8 (26.3)	19.4-15.5 (16.4)	40.4-36.5 (39.2)	30.5-27.3 (29.5)	42.8	17	19.4-16.3 (17.8)	18.3-15.9 (17.9)
Erodium lebelii	62.1-58.4 (60.5)	45.2-40.3 (43.4)	21.4	7	33.1-27.5 (30.5)	26.7-20.1 (25.5)	55.0-52.7 (53.5)	39.6-36.3 (37.6)	23	14	30.1-26.3 (28.4)	16.4-13.2 (14.5)

* The value in parentheses represents the average

micrometres for the species *Astragalus spinosus*. The lowest stomatal index on the lower surface was recorded at 23 micrometres for the species *Erodium lebelii*. The rest of the species ranged according to the stomatal index between these two species, as it is natural for the stomatal index on the upper surface to be higher than the lower surface because it is the surface exposed to heat and sunlight, and thus more occurs in the processes of transpiration and evaporation.

The results of Table (3) and figure (4) showed that the higher *Astragalus spinosus* was higher in the number of stomata in the upper surface of the leaf epidermis over the rest of the species studied, as it recorded 12 stomata, while the species *Zilla spinosa* gave the lowest number of stomata in the upper epidermis, amounting to 3 stomata, as for the lower surface of the epidermis. The *Astragalus spinosus* species recorded the highest number of stomata, with 17 stomata, while the *Zilla spinosa* species recorded the lowest number of stomata, with 7 stomata.

Regarding the character of the stomatal opening length, the data in Table (3) and figure (4) showed that the upper epidermis surface was superior as well, and the species *Erodium lebelii* was

Anatomical analysis

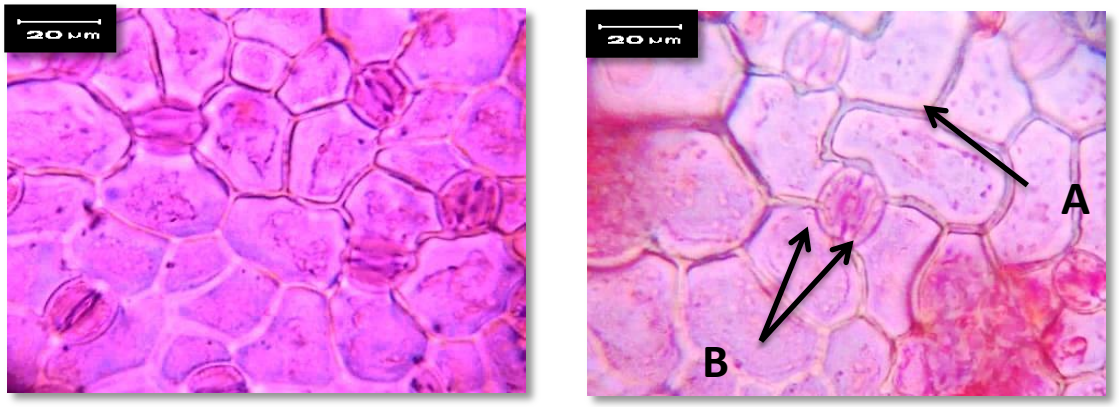
Data indicated in Table (3)and figure (3)There are large differences between different species and families in the length of the stomata, in addition to their variation between the upper and lower surfaces. It is clear that the superior appearance of the stomatal length can be distinguished mostly on the upper surface of the *Erodium lebelii* species, which reached 60.5 micrometres, while it reached about 53.5 micrometres on the lower surface. and the rest of the studied species ranged below that. As for the characteristic stoma width, the species *Zilla spinosa* recorded the highest average stoma opening at around 47.1 micrometres for the upper Epidermis surface, while the same species recorded around 56.8 micrometres on the lower surface. The rest of the studied species ranged in stoma width, and the stomatal index varied between the studied species and between families as well and differed on the upper surface from the lower surface. The highest stomatal index in general was on the upper surface of the species *Astragalus spinosus,* reaching 33.3 micrometres, and the lowest stomatal index on the upper surface was recorded. The species *Arnebia decumbens*, while the highest stomatal index on the lower surface reached 42.8

lebelii plant has the Anomotetracytic type of stomatal complexes for the two surfaces. We notice from the panels below that the majority of stomatal complexes were between closed and small in size, and in most species they were surrounded by hairs. These characteristics work to increase the plant's ability to withstand semi-desert environmental conditions in terms of high wind speeds, drought, and high temperatures, so the plants work to preserve what is present. There is water inside it, (Farooq *et al*., 2015) , (Micco and Aronne, 2012). The shape of the walls of these cells was sinuous in the species *Arnebia decumbens* and the species *Zilla spinosa*. figure (3) and figure (4) The results of this study were consistent with what was mentioned by (Al-Bahadli, 2015) and (Esmaeel, 2014), in addition to what was mentioned by (Aliwi, 2009) for some of the genera of the Compositae family. The reason for this difference is attributed to the complexes. The gap between the two surfaces in some species depends on the environmental conditions that affect the appearance, physiology and structure of the plant. (Schurgers *et al*., 2015) showed that light, heat, and Co2 gas have an effect, and humidity has an effect on the shape of cells, while (Boctsh *et al*., 1996) recorded at approximately 30.5 micrometres, while the smallest stomatal opening length was for the species *Astragalus spinosus*, which was given at 26.3 micrometres. As for the character of the stomatal opening width for the upper epidermis. The results showed that the species *Erodium lebelii* was superior and gave 25.5 micrometres in figure (4), while the species *Arnebia decumbens* recorded the smallest width of the stomatal opening on the lower epidermis surface among the species studied, which amounted to 14.3 micrometers in figure (4). Regarding the types of stomatal complexes, they were similar or different between different species or between the upper and lower surfaces of a single species or for different species of the families under study. The surfaces were of two types: Anisocytic and Anomotetracytic. The stomatal complex of the lower surface of *Zilla spinosa* was of the Anomocytic figure (3), while the stomatal complex of the lower surface of *Astragalus spinosus* was of the Anomocytic Board type (4). While the stomatal complexes differed between the two surfaces in the *Arnebia decumbens* plant, the upper surface was of the Anomotetracytic and Anisocytic type and the lower surface was of the Anomotetracytic type, while the *Erodium*

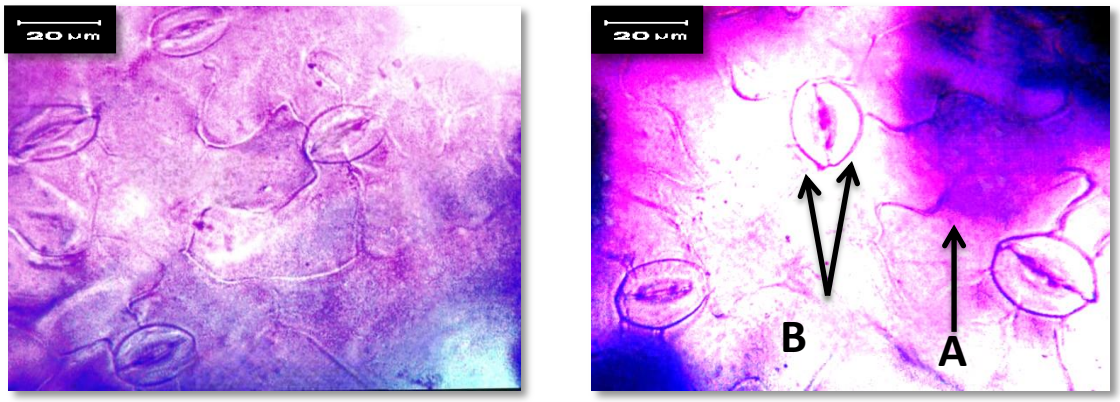
(Aliwi, 2009) showed that there is a role for genetic change due to environmental conditions, especially the long period of exposure to sunlight and drought, and this was confirmed by (Esau, 1965), (Fahn, 1974).

mentioned that Co2 gas has an effect on the pattern of the stomatal complex, which works to increase the number of subsidiary cells. And associated with the stomatal complex, in addition to the fact that normal Epidermis cells work to activate the work of subsidiary cells, as

Lower epidermis **Upper epidermis**

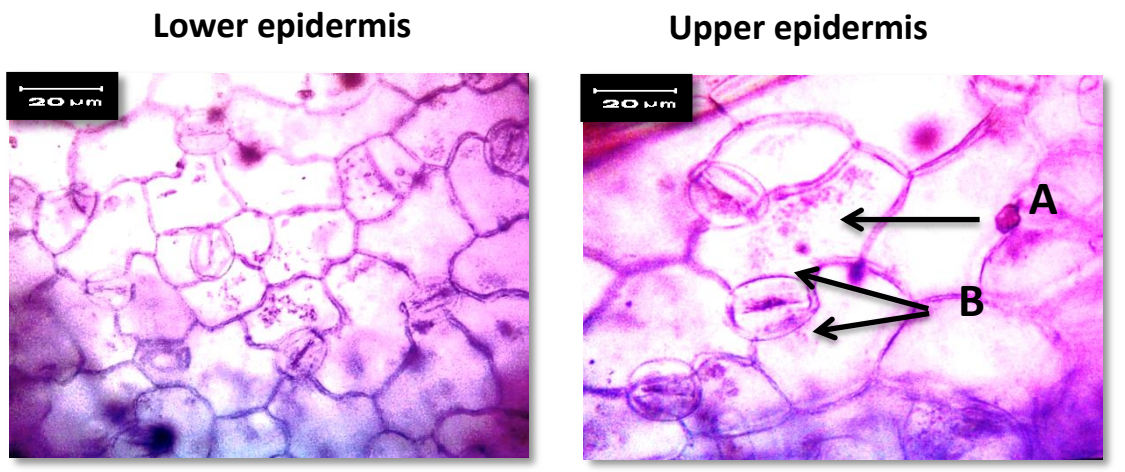


Anthemis deserti

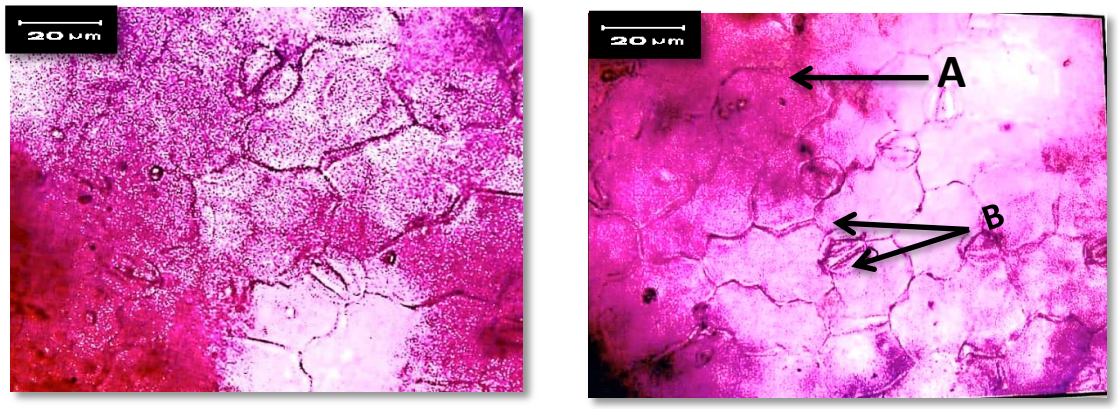


Zilla spinosa

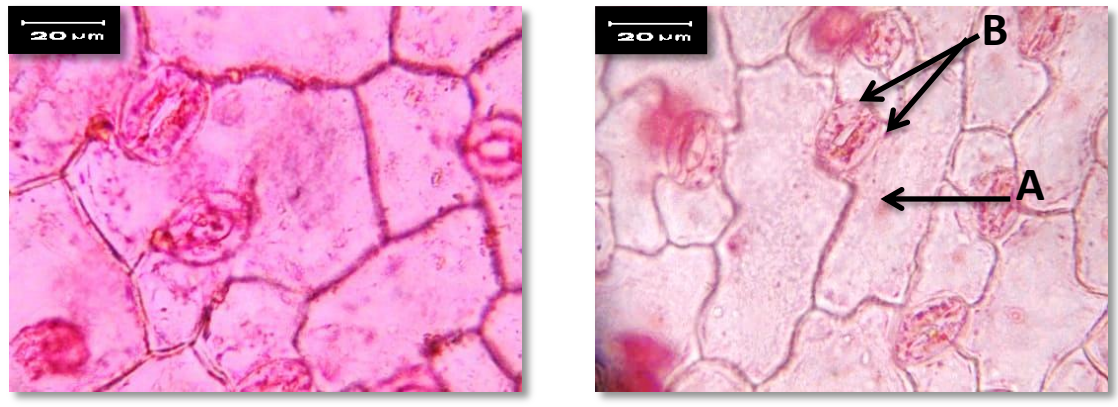
figure (3) A superficial view of the epidermis of the species *Anthemis deserti* and *Zilla spinosa*
A - Normal cells of the epidermis
B - Guardian cells



Arnebia decumbens



Astragalus spinosus



Erodium lebelii

figure (4) A superficial view of the epidermis of the species *Arnebia decumbens* ,*Astragalus spinosus* and *Erodium lebelii*
A - Normal cells of the epidermis
B - Guardian cells

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