Analysis of the Anatomical and Chemical Properties of Wild Plants in the Samawa Desert, Iraq

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Abstract
The results of this study showed the clear substance of the leaves, which was characterized by its abundance in some of the species under study, and the chemical compounds was identified using the technique of chromatography-mass spectrometry (GC-MS). It has been investigated that these species are rich in secondary metabolic compounds, ranging from terpenes, phenols, steroids, acids, vitamins, alkanes, alkaloids and esters. A compound was distinguished by the different times of its appearance, and it was repeated repeatedly at different times. The anatomical study dealt with some of the characteristics of the stomata and the guardian cells of the leaf epidermis of some of the studied species. It studied the stomata complexes and characterized the stomata as being of the Anisocytic and Anomotetracytic types of the Rumex vesicarius type and similar to the surface stomata of the plant Savignya parifere of the Anomotetracytic and Anomotetracytic plant, while the surface stomata complex was underground of the plant Convolvulus pilosellifolius.

Keywords: *Stomata, anatomical analysis, GC-MS, wild plants, Samawa desert.*

Introduction

One plant must be distinguished from another on the basis of the possibility of drawing boundaries between different taxonomic levels, finding the basis on which plants are classified and named, and searching for evolutionary relationships between the taxonomic levels of the various biological sciences related to classification such as Anatomy, Genetics, Biochemistry, Palynology, Physiology, etc. (Akbarlou and Nodehi, 2016). Which represents the southern part of the Western Plateau with vast lands and containing groundwater that flows in parts of it in the form of natural springs (Al Bahadly, 2015). Wild plants are important sources of pharmacologically important elements, such as compounds with antioxidant and anti-inflammatory properties, and phytochemicals, such as alkaloids, phenols, flavonoids, saponins, and terpenoids are some of the main substances found in these plants (Yu *et al*., 2021; Al Salman and Al-Gharawi, 2019). Plants contain essential vitamins such as vitamin B complex and vitamin C, which are pivotal compounds involved in multiple physiological processes of the organism and exert an essential function in maintaining redox balance. Kennedy (2016), and plants are also an important and large source of medicinal products, which produce a very wide range of organic compounds, which are divided into primary metabolic compounds, which are essential in plants (Mahmoud *et al*., 2023), and secondary metabolic compounds are of a complex chemical nature and are produced from primary metabolic compounds in small proportions, which vary from one plant organ to another and from one growth stage to another, as well as from one plant species to another. These are the reasons for which medicinal plants were used and their important benefits in the production of medicines. (de Sousa *et al*., 2016), as different parts of the plant can be used medicinally.

Materials and method

Anatomical study: One sample was taken from the middle of the leaf for epidermal cells from the upper and lower surfaces. The physiological analysis for the current study was based on collecting fresh samples collected directly from the study areas. After fixing with Formalin acetic acid alcohol solution for 24 hours at room temperature, it was washed with 70% ethanol. In order to remove traces of the fixative solution, it was then stored in alcohol at the same concentration in the refrigerator until used in preparing anatomical sections of plant parts according to the steps described by (Johansen, 1940): Peeling method was used using forceps with two sharp ends and a dissecting blade. The prepared samples were transferred to a clean glass petridish dish containing a 5.1% sodium hypochloride solution for ten minutes to remove Textile residues stuck to the skin and chlorophyll pigment. The removed skin 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. A lens with a magnification of x40 and a graduated lens with a magnification of mm0.045 and converted to mm1), stoma guide, stoma length (mμ), stoma width (mμ), stoma aperture length (mμ), stoma aperture width (mμ) (line measurement in the inserted lens 2.4 mμ converted to 1 mμ Using an ocular micrometer, the models were photographed under the Omax camera installed on the microscope according to the Vividia ablescope program.

Chemical analysis: 20 g of leaf powder was weighed and mixed with 100 ml of 96% ethanol in a 250 ml glass beaker. It was closed with cotton and aluminum foil and placed in a shaking water bath at a temperature of 45 °C for 24 hours. The extract was then filtered using Whatman filter papers. 0.22 millimeter microns, and the filtrate was collected and placed in a glass dish in an electric oven at a temperature of 40°C for 48 hours until the sediment from the filtrate became stuck to the glass dish. Then the sediment was scraped off and the sediment was collected in a tightly closed glass container, and kept in the refrigerator until use. The process was repeated. Several times to obtain an appropriate amount of extract for use in the GC-MS 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: 230°C, the quadrupole temperature: 150°C, and the interface temperature: MSD transmission line: 290°C. The start time was: 4.00 minutes and the end time was: 35.00-40 minutes. The active compounds were diagnosed 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

Chemical compounds in the alcoholic extract of *Calendula arvensis* leaves using GC-MS technology:

Table (1) and Figure (1) show the chemical compounds present in the alcoholic extract and analysis of the leaves of the *Calendula arvensis* plant, which were detected using gas chromatography combined with a mass spectrometer (GC-MS) technique. After separating the compounds, the chemical analysis showed the presence of 25 of the plant's active compounds, and that the highest The peak area of the extract was 57.84% per minute 30.92 for the compound Nonacosane and the lowest peak area was 0.65% per minute 7.99 for the compound 2-Mercaptoethanol, TMS derivative

Table (1): Chemical compounds in the alcoholic extract of *Calendula arvensis* leaves using GC-MS technology.

Peak	R.T.	Area Pct%	Library/ID
1	7.99	0.65	**2-Mercaptoethanol, TMS derivative**
2	8.82	1.01	Butanoic acid, 3-methyl-
3	8.93	2.39	Glycine
4	9.09	1.56	Silane, triethylfluoro-
5	10.78	3.75	Tetraethyl silicate
6	14.50	0.66	4-Vinylphenol
7	15.90	0.74	2-Methoxy-4-vinylphenol
8	17.72	2.99	2-Ethyl-oxetane
9	18.0	6.1	Hexa-2,4-diyn-1-ylbenzene

10	18.21	0.77	3-Methoxy-4-methyl-(2-aminobutyl)benzene
11	19.13	2.25	Phenol, 4-ethenyl-2,6-dimethoxy-
12	20.65	0.96	1-Tetradecanamine, N,N-dimethyl-
13	21.60	1.10	1-Octadecene
14	22.09	0.73	Neophytadiene
15	23.29	0.66	n-Hexadecanoic acid
16	23.62	1.22	3-Eicosene, (E)-
17	24.75	0.89	Phytol
18	25.01	1.21	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
19	28.42	4.10	Bis(2-ethylhexyl) phthalate
20	29.48	1.57	Eicosane
21	30.18	1.05	Eicosane
22	30.92	57.84	**Nonacosane**
23	32.26	6.18	15-Nonacosanone
24	32.42	5.14	Nonacosan-15-ol
25	34.92	1.51	.beta.-Sitosterol

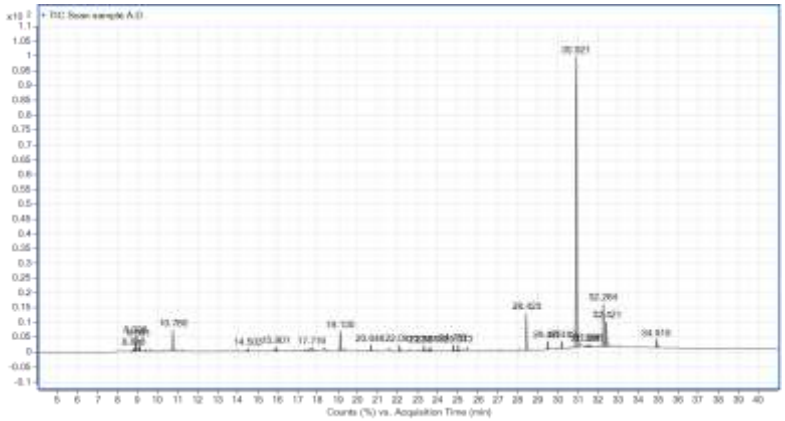

Figure (1): GC-MS analysis of the alcoholic extract of *Calendula arvensis*.

Chemical compounds in the alcoholic extract of *Hypecoum littorale*leaves using GC-MS technology

It is noted from the data in Table (2) with Figure (2) that the compounds present in the alcoholic extract of the leaves of the *Hypecoum littorale*plant that were detected using the gas chromatography technique varied in the time of appearance, and that there were compounds that appeared repeatedly for different periods of time, as the results showed the presence of 52 Of the compounds found in the *Hypecoum littorale*plant, the highest peak area of the extract was 12.24 % per minute 32.36 for the compound Tetracosane, and the lowest peak area was 0.38 % per minute 26.53 for the compound Diglycolic acid, heptyl hexyl ester. The compounds have varying times of appearance, and there are compounds that appear repeatedly for different periods of time.

Table (2): Chemical compounds in the alcoholic extract of *Hypecoum littorale*leaves using GC-MS technology

Peak	R.T.	Area Pct %	Library/ID
1	7.99	1.55	2-Mercaptoethanol, TMS derivative
2	8.83	1.19	Benserazide
3	8.93	2.36	Methylal
4	9.10	1.42	Silane, triethylfluoro-
5	9.60	0.54	Butanoic acid, 2-methyl-
6	10.16	0.52	Butane, 2-isothiocyanato-
7	10.30	1.32	Butane, 2-isothiocyanato-
8	10.77	4.53	Tetraethyl silicate
9	10.91	0.47	Silane, diethoxydimethoxy-
10	11.58	1.72	1-Amino-2,6-dimethylpiperidine
11	12.09	0.38	Ethyl 2,3-epoxybutyrate
12	13.27	0.62	Cyclododecane
13	14.77	0.38	2-Propenenitrile, 3-phenyl-, (E)-
14	14.89	1.13	2-Propenenitrile, 3-phenyl-, (E)-
15	15.49	1.72	2-Propenenitrile, 3-phenyl-, (E)-
16	15.61	3.79	2-Propenenitrile, 3-phenyl-, (E)-
17	15.91	0.58	2-Methoxy-4-vinylphenol
18	16.88	0.89	1-Tetradecene
19	17.38	0.58	2,4-Di-tert-butylphenol
20	18.17	1.63	cis-1,4-Cyclohexanediamine, N-methyl
21	18.31	0.67	1-Dodecanamine, N,N-dimethyl-
22	18.45	0.44	2,4-Di-tert-butylphenol
23	19.19	0.74	Cetene
24	19.37	1.23	2-Tetradecene, (E)-
25	20.65	1.22	1-Tetradecanamine, N,N-dimethyl-
26	21.60	1.00	1-Octadecene
27	22.09	2.61	Neophytadiene
28	22.53	0.96	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
29	23.30	1.02	n-Hexadecanoic acid
30	23.62	1.56	5-Eicosene, (E)-
31	24.76	1.30	Phytol
32	25.02	1.56	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
33	25.27	0.44	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
34	25.45	1.19	1-Nonadecene
35	26.53	0.38	Diglycolic acid, heptyl hexyl ester
36	26.87	2.20	Benzeneacetonitrile, 4-hydroxy-
37	27.02	9.24	Benzonitrile, 2,4,6-trimethyl-
38	28.09	0.58	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester

39	28.42	7.08	Bis(2-ethylhexyl) phthalate
40	28.71	0.44	1H-Indole, 5-methyl-2-phenyl-
41	29.48	3.12	Heptacosane
42	30.18	0.45	Eicosane
43	30.45	0.52	4-(7-Methyloctyl)phenol, TMS derivative
44	30.62	0.51	4-(7-Methyloctyl)phenol, TMS derivative
45	30.87	8.52	Octadecane
46	31.57	1.22	Eicosane, 9-octyl-
47	32.04	0.68	4-(4-Hydroxyphenyl)-4-methyl-2-pentanone, TMS derivative
48	32.36	12.24	Tetracosane
49	33.90	0.83	4-(7-Methyloctyl)phenol, TMS derivative
50	34.27	1.39	Methyltris(trimethylsiloxy)silane
51	34.92	6.70	.gamma.-Sitosterol
52	39.06	0.64	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-

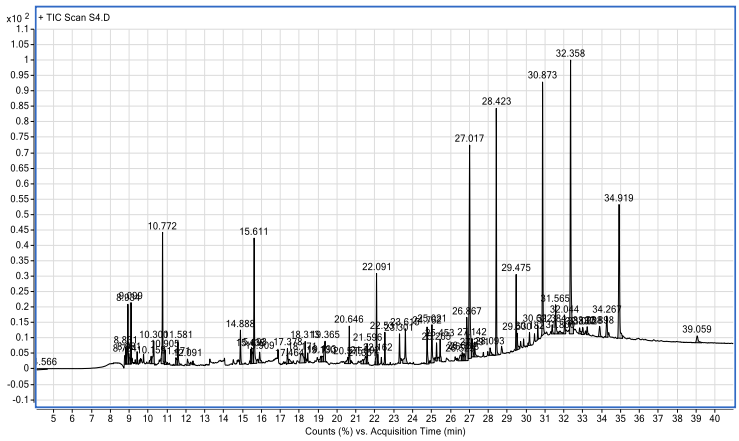

Figure (2): GC-MS analysis of *Hypecoum littorale*leaf extract.

Anatomical analysis

Data from Table No. (3) and figure (3) indicate that there are large differences between different species and families regarding the length of the stomata, in addition to their variation between the upper and lower surfaces. It is clear that the superior appearance of stomata length can be distinguished mostly on the upper surface of the species *Rumex vesicarius*. Which reached 82.45 **µm**, while on the lower surface it reached about 77.6 µm, and the rest of the studied species ranged below that. As for the stoma width characteristic, the species *Savignya parifere* recorded the highest average stoma opening at about 59.15 **µm** for the upper surface of the epidermis, while the same species recorded about 51.6 µm on the lower surface. The rest of the studied species ranged in stoma width. The stomata index varied between the studied species and between the families as well, and differed on the upper surface from the lower surface. The highest stomata index in general was on the upper surface of the species *Savignya parvifora*, as it reached 30 **µm**, and the lowest stomata index was in the lower surface. The upper surface was recorded for the *Trigoneile stellate* species and reached 20 µm, while the

highest stomataevidence for the lower surface reached 35 µm for the *Plantago amplexicaulis* species. The lowest stomata index on the lower surface was recorded at 20 micrometers for the species *Rumex vesicarius*, and the rest of the species ranged according to the stomata index between these two species, as it is natural for the stomata 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 species *Plantago amplexicaulis* was superior in the number of stomata in the upper surface of the leaf epidermis over the rest of the species studied, as it recorded 9 stomata, while the species *Rumex vesicarius* gave the lowest number of stomata in the upper epidermis, amounting to 3 stomata. As for the surface In the lower epidermis, *Plantago amplexicauli* recorded the highest number of stomata, with 15 stomata, while *Rumex vesicarius* recorded the lowest number of stomata, with 5 stomata. Regarding the character of the length of the stomata opening, data from Table (3) and figure (3) showed that the surface of the upper epidermis also excelled, and the species *Savignya parvifora* was recorded at approximately 40.2 **µm**, while the smallest length of the stomata opening was for the species *Trigoneile stellata*, which was given at 16.95 **µm**. As for the character of the width of the stomata opening for the epidermis The results showed that the species *Savignya parvifora* was superior and gave 30.65 micrometers in figure (4), while the species *Trigoneile stellata* recorded the smallest width of the stomata opening for the lower epidermal surface among the species studied, which amounted to 14.6 micrometers in figure (4). Regarding the types of stomata 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 two surface stomata of the plant *Trigoneile stellata* were similar to the Anomocytic and Anomotetracytic type, while the stomatacomplex of the lower surface of the Convolvulus pilosellifolius plant was of the Anomocytic type, while the stomata complexes differed between the two surfaces in the Hypecoum pendulum plant, as the upper surface was of the Anomotetracytic, Anisocytic type and the lower surface was of the Anomotetracytic type. *Plantago amplexicaulis* has anomotetracytic stomata complexes , while *Rumex versicarius* has anisocytic stomata complexes and In the *Savignya parvifora* plant, the stomatacomplex on the upper and lower surfaces was of the Anisocytic type, while the stomata complexes on the two surfaces of the *Trigoneile stellata* plant differed, as it was in the upper surface of the Anomotetracytic type, while in the lower surface it was of the Anomotetracytic type. The shape of the stomata was elliptical, except for the stomata on the upper surface of the *Savignya parvifora* plant, which were circular in shape. We notice from the panels below that the majority of stomata 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 them, (Ahmad *et al*., 2015) and (Micco and Aronne, 2012). The shape of the walls of these cells was sinuous in the *Savignya parvifora* species. The results of this study were consistent with what was mentioned by both (Al-Bahadli, 2015) , (Al-Hassnawi and Al-Burki, 2023) and (Esmaeel, 2014), In addition to what was mentioned by (Aliwi, 2009) for some genera of the Compositae family. Pandey (1981) also found that the stomatacomplex of the Cruciferae family is of the Anisocytic type, and this matches what was found in the studied species of the Cruciferae family, as shown by (Al-Badri, 2014) when studying the two genera, *Rumex* and *Erodium*,

showed that they have the same type of stomata complexes above, even if their types differ. The reason for this difference in the stomata complexes between the two surfaces and in some species is attributed to the environmental conditions that affect the appearance, physiology, and structure of the plant, as (Schurgers *et al*., 2015) explained that light, heat, and Co_2 gas have an effect and humidity have an effect on the shape of the cells, while (Boetsch *et al*., 1996) that Co_2 gas has an effect on the pattern of the stomata complex, which works to increase the number of subsidiary cells associated with the stomata complex, in addition to the fact that normal skin cells work to activate the work of the stomata complex, (Aliwi, 2009) also 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 both (Esau, 1965) and (Fahn, 1974).

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
Convolvulus piloselliifolius	52.0 - 55.9 53.) (95	24.3 - 29.4 26.) (85	23.5	6	24.2 - 27.1 25.) (65	14.3 - 16.4 15.) (35	46.-8 50.3 48.) (6	20.-3 25.7 22.) (5	25	13	22.1 - 29.5 25.) (8	14.4 - 18.5 16.) (4
Trigoneille stellata	39.5 - 44.6 42.) (05	25.5 - 30.4 27.) (95	20	4	15.5 - 18.4 16.) (95	13.2 - 17.9 15.) (5	37.-2 42.7 39.) (9	22.-4 26.1 24.) (4	25	8	19.2 - 24.5 21.) (8	12.5 - 16.8 14.) (6
Hypecoum pendulum	61.4 - 66.1 63.) (75	47.3 - 50.4 48.) (85	36	8	32.2 - 39.7 35.) (95	25.6 - 29.1 27.) (3	55.-3 59.7 57.) (5	40.-6 48.5 44.) (8	33	12	26.3 - 29.4 27.) (8	20.1 - 26.3 23.) (2
Plantago amplexicaulis	49.5 - 44.9 47.) (2	36.2 - 29.8 35.) (4	28.5	9	22.5 - 29.4 25.) (5	15.2 - 19.4 17.) (3	42.-6 39.1 40.) (8	28.-3 25.2 26.) (4	35	15	22.5 - 29.5 26.) (0	14.5 - 19.5 17.) (0

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
Rumex vesicarius	79.2 - 85.7 82.) (45	39.8 - 44.2 41.) (6	18.1	3	36.4 - 44.7 40.) (5	19.2 - 25.5 22.) (3	72.-6 77.9 77.) (7	36.-5 38.5 37.) (5	20	5	36.1 - 39.4 37.) (75	26.3 - 28.5 27.) (4
Savigny a parvifora	69.1 - 75.5 72.) (3	56.2 - 62.1 59.) (15	30	5	36.9 - 44.8 40.) (85	27.8 - 33.5 30.) (65	61.-6 67.5 64.) (5	49.-9 53.4 51.) (6	26	10	33.2 - 37.4 35.) (3	25.5 - 28.4 26.) (9

* The value in parentheses represents the average

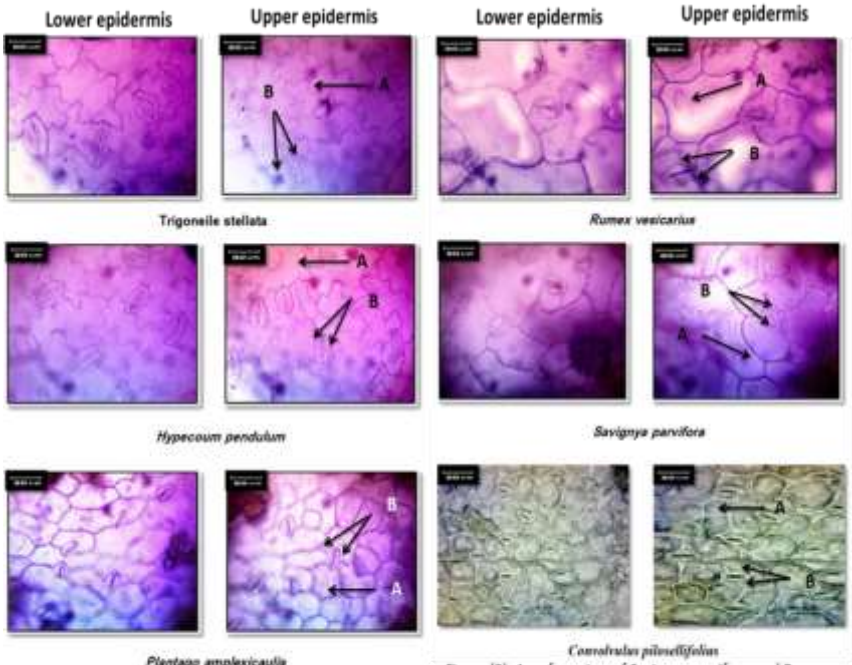

Figure (4): A surface view of Hypecoum pendulum and Trigonelle and Plantago amplexicauli species
a. Normal cells of the epiderm
b. Guard cells

Figure (3): A surface view of Savignya parvifora and Rumex and Convolvulus pilosellifoliu species
a. Normal cells of the epiderm
b. Guard cells

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