Chemotaxonomy of Citrus (Rutaceae) Based on GC-MS and Antioxidant Activities in Diyala Governorate, Iraq

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Abstrct

The Iraqi citrus is one of the plants with high medicinal values because it is used in folk medicines, and it is a source of many effective compounds. Blanco C.reticulata and (L.) Osbeck *C.limon*, *C.aurantifolia*, and Risso *C. limettioides*, the species C.reticulata contained 31 chemical compounds, and *C.limon* contained 26 chemical compounds as well as a presence in Basrah lemons *C. aurantifolia* and sweet lemon C.limettioides on 31 chemical compounds, and the antioxidant activity of the plant extracts was investigated by scavenging the free radicals of the compound (DPPH Diphenylpicrylhydrazyl) and comparing this activity with Ascorbic acid, and the results showed that the leaves of plants are no less important than their fruits and seeds, as they possess Great ability to reduce free radicals (DPPH Diphenyl picryl hydrazyl), which reached 78% in Blanco *C.reticulata*.

Key Words: Chemotaxonomy, *Citrus,* Antioxidant, deyaila, Iraq.

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Introduction

Citrus is one of the important medicinal plants, and its importance lies in its edible fruits and its magical juice, which contains a high percentage of vitamin C, and effective substances that enhance human health and support his immunity, in addition to being one of the trees with beautiful appearance and shade (Pertotos et al., 2021), was for the development of chemistry and devices The technology for qualitative and quantitative detection of active compounds has a great role in studying all plant species and has contributed to the development of chemotaxonomy and pharmacology in addition to applied devices that have a role in studying chemical compounds, including gas chromatography, which is sensitive in separating the different components of organic compounds and quantitative and qualitative analysis Her (Purcrll, 2016), which is an additional guide for classifying and isolating many taxonomic ranks and determining the best active substances within drugs and the pharmaceutical industry (Sing, 2018), and oranges and their trees occupy an important place in medical treatments (Sing et al., 2019; Al-Anbari, 2015). The gas chromatography-mass spectromete (GC-MS) device works to separate atoms or molecules of large masses. Different from each other with different masses, the mass spectrum is the result of scanning the relative abundance of ions according to the ratio of mass to their charge, and the most abundant peak in the spectrum is called the base peak. and the families of those plants (Al-Sumairi, 2016). The great progress of the life sciences has encouraged specialists in plant taxonomy to use the chemical properties and characteristics of plants in order to compare the different taxonomic ranks in addition to the phenotypic (Al-Mousawi 1987).

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Among the studies conducted to diagnose the active compounds in citrus (Olyad et al., 2020) A study of the phenolic content of peels of 4 types of citrus in Ethiopia, namely C.rticulate, C.sinensis, C.aurantifolia and C.limon, which showed that lemon peel contained the highest percentage of flavonoids and it reached 8.88 mg Quercetin equivalent. g of the extract, followed by the orange peel extract with 0.38%, and a strong correlation factor was observed between the flavonoid content of the orange peel extract.

In a study by (Habibollahi et al., 2022) conducted to investigate the orange peel of phenolic acids and flavonoids and their role in protecting the rest of the plants from pathogenic bacterial infections, and that orange peels contain a high percentage of phenolic acids p-cumaric and Ferulic in addition to their role in protecting trees from Some fungi in comparison with flavonoids, and citrus fruits are of great importance as antioxidants, and that is what they contain of active substances and secondary metabolic compounds (Ghafar, 2010).

Materials and Methods

Samples Collection and Preparation

Four species of the citrus genus were selected and the species are *C.reticulata*, *C.limon*, *C.aurantifolia*, *C.limettiodis* (Nomi Sweet), as the leaves were collected from different regions in three rounds from Diyala Governorate - Iraq (Baquba, Hoyder, Buhrz, and Mandali). Shafh, Haji Sohail, the phrase) in the month of April. The healthy and healthy leaf parts free from diseases or any ruptures were taken, then samples were taken for each of the selected species in the study to the herbarium of the College of Education for Girls, University of Anbar to be diagnosed and classified. These species belong to one genus and one family and were classified by the supervisor Prof. Dr. Ashwaq Talib Hamid.

Chemical Study

Preparation of the Plant Extract

The alcoholic extract 99% was prepared according to the method, by soaking 4 grams of vegetable powder for each of the samples selected in the study in 100 ml of ethyl alcohol with a concentration of 99% for 24 hours in a tabletop incubator. The infusion was filtered with Whattman-1-type filter papers, concentrated by evaporation for 10 minutes at a speed of 2000 rpm, by placing the extract in an incubator for 48 hours, the extract was suspended in 10 ml of distilled water and kept at 20 °C until use (Wang, 2014).

Chromatography-mass Spectrometry GC-MS Analysis

The filtered plant samples extracted from alcohol were placed in a GC-MS device of type GC-MS-QP2010 plus (Shimadzu, Japan), helium gas (purity 9) (used to carry gas at a constant flow rate of 1 ml (1m) per minute, column temperature starts from 80 °C and gradually increases every ten degrees 10°C until it reaches 280 °C °, while the temperature of the heat source of the device is 350°C, the initial temperature of the device is set to 80°C and this temperature remains for two minutes, at the end of this period the temperature of the Oven is raised to 280°C at an increase rate of 5°C per minute and remains for a period 9 minutes, Injection port temperature kept within 280°C, helium gas flow rate l mL (1m) per minute and ionization energy value ev (Electron volt 70, Separation performed at Column 5MS temperature for 30 minutes, Quadrupole mass detector used For the detection of compounds through a hole in the column, the temperature of the detector was 280 °C and the chemical compounds of the plant samples were compared with the chemical compounds stored in the computer library of the type Nist linked with the GC-MS device.

The radical scavenging activity was tested according to the method described by (Antolovich et al., 2002), using the plant root (DPPH(1,1-Diphenyl-2-picryl-hydrazyl), weighing 0.04 g of it and dissolved in 100 ml of absolute ethanol to reach a concentration of 400 µg/ ml, the tube was shaken using Vortex mixer and kept in clean test tubes and closed with aluminum foil to prevent photo-oxidation, while Ascorbic acid was prepared by dissolving as a positive control and a control sample 0.5 in 100 ml of (methanol 50 and water 50) then preparing different dilutions of each sample of the extracts The plant is (4/1, 3/1, 2/1) (volume unit) with three replicates for each dilution, as 500 microliters were taken from each dilution and 500 DPPH was added to it and 2 ml was completed by adding 1 ml of absolute ethanol and keeping it At a temperature of 37 °C for a period of half an hour, the chromatic intensity was measured at the wavelength of 517 nm compared to the control sample DPPH, the experiment was repeated three times for each sample and the average of the replicates was found.

The percentage of free radical inhibition, DPPH, is calculated using the following equation:

Inhibition= (A DPPH- A sample)/A DPPH *100sample

Results and Discussion

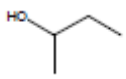
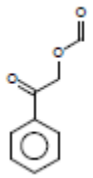
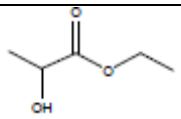
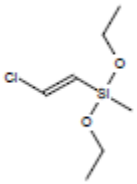
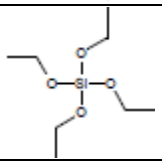
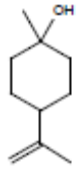
Chemical Study

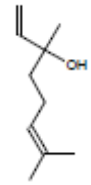
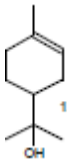
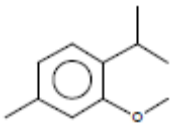
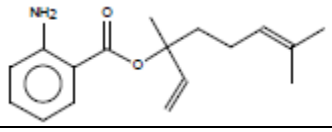
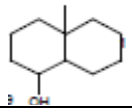
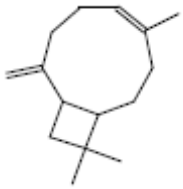
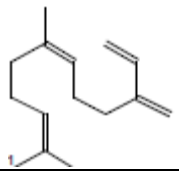
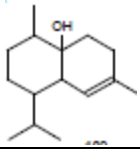
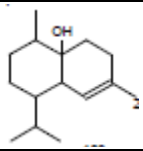
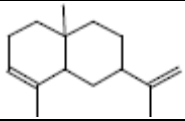
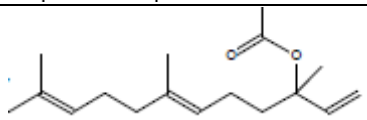
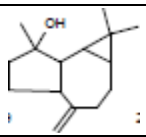
The results of the chemical study of Citrus species showed a discrepancy in the chemical content of plant chemical compounds, which was detected using GC-MS Chromatography Mass Spectrometry Gas, as a chemical classification that helps in diagnosing and isolating species, which were abundant in content in quantity and quality.

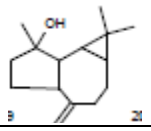
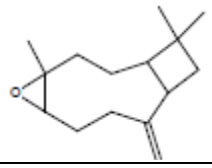
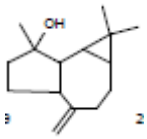
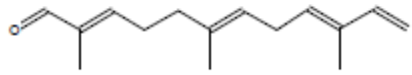
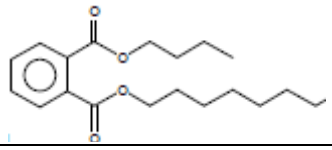
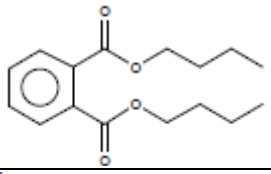
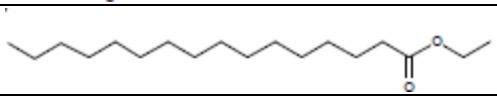
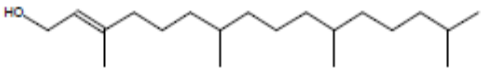
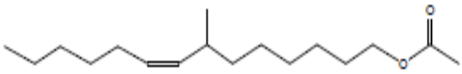
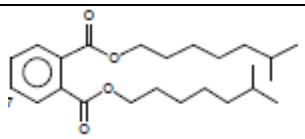
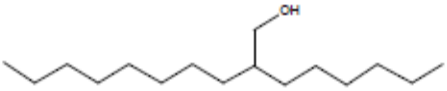
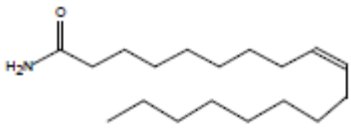
The studied species varied in terms of area, concentration, time and retention of phytochemical compounds in the alcoholic extract, and the current study led to the registration of 119 chemical compounds combined in the studied species. Citrus-reticulata contained 31 chemical compounds, citrus-limon contained 26 chemical compounds, and Basra lemon Citrus-aurantifolia contained 31 chemical compounds, while sweet nome Citrus-limettioides found 31, a variation in the chemical compounds of the sex in terms of their type. The results indicated that some of the species had shared certain compounds among themselves, and thus the species could be divided according to the presence of the following groups: in terms of their distribution and presence and the difference according to the type of chemical compounds and the time of detention. Thus, the types were divided according to the presence of compounds:

1. The species.*C. reticulata* it included 1 3 chemical compound, and the compound 1-Eicosanol was the highest in terms of time and reached 29.818, and the compound 2. Butanol was the least compound in terms of time as it reached 3.178 and as shown in the table (1).

Table 1. Chemical compounds in species *C. reticulata*

% of total	Peak height	R.T. min	ical formulaChem	Compound name	T
4.696%	921683	3.178		2-Butanol	1
13.734%	6558751	3.477		Ethanone, 2-(foryioxy)-1-phenyil-	2
0.404%	115,239	3.958		Propanoic acid	3
1,000%	430927	4.756		Trans-(2-chlorovinyl) methyldiethoxysilane	4
3.321%	1572479	5.428		Tetraethyl silicate	5
0.718 % _	237156	6.542		Cis -beta- Terpineol	6

19.117%	6170851	6.915		1,6-Octadien-3-ol, 3-ol,3,7-dimethyl-	7
1.358%	218378	8.216		p-menth-1-en-8-ol	8
0.465%	109518	8.749		Benzene,2-methoxy-4-methoy-1-(1-methlethyl)	9
5.539%	1061476	9.005		1,6-Octadien-3-ol,3,7-dimethyl-2-aminobenzoate	10
0.651%	82218	9,577		1-Naphthalienol, decahydro-4a-methyl-	11
3.574%	417912	11,385		Caryophyllene	12
1.030%	150284	11,705		1,6,10-Dodecatriene,7,11-dimethyl-3-mthylene	13
1.435%	199365	12.156		Cupenol	14
0.613%	117467	12.304		Cupenol	15th
1.0 34%	187122	12.365		Naphthaene	16
0.489%	129119	13,054		Nerolidyl acetate	17
16.4 25%	2126658	13,353		(-)- Spathulenol	18

1.018%	294633	13.435		(-)- Sapathulenol	19
3.684%	519549	13.925		Caryophyllene oxide	20
4.297%	567913	14,623		(-)- Spathulenol	21
0.876%	296940	16.418		2,6,9,11-Dodecatetraenal,2,6,10-trimethyl-	22
1.818%	574479	17,359		1,2-Benzenedicarboxylic acid,	23
0.567%	196164	17,580		Dibutyl phthalate	24
3.998%	1475145	18,646		Hexadecanoic acid,ethyl ester	25
0.413%	201610	19,747		Phintol	26
0.821%	262992	21,443		'7-Methyl-Z-tetradecen-1-ol acetate	27
1.153%	232478	22,643		1,2-Benzenedicarboxylic acid	28
2.212%	183665	23.697		1-Decanol, 2-hexl-	29
1.468%	140050	25.084		:9-Octadecenamide	30
2.072%	198914	29.818		1:1-Eicosanol	31

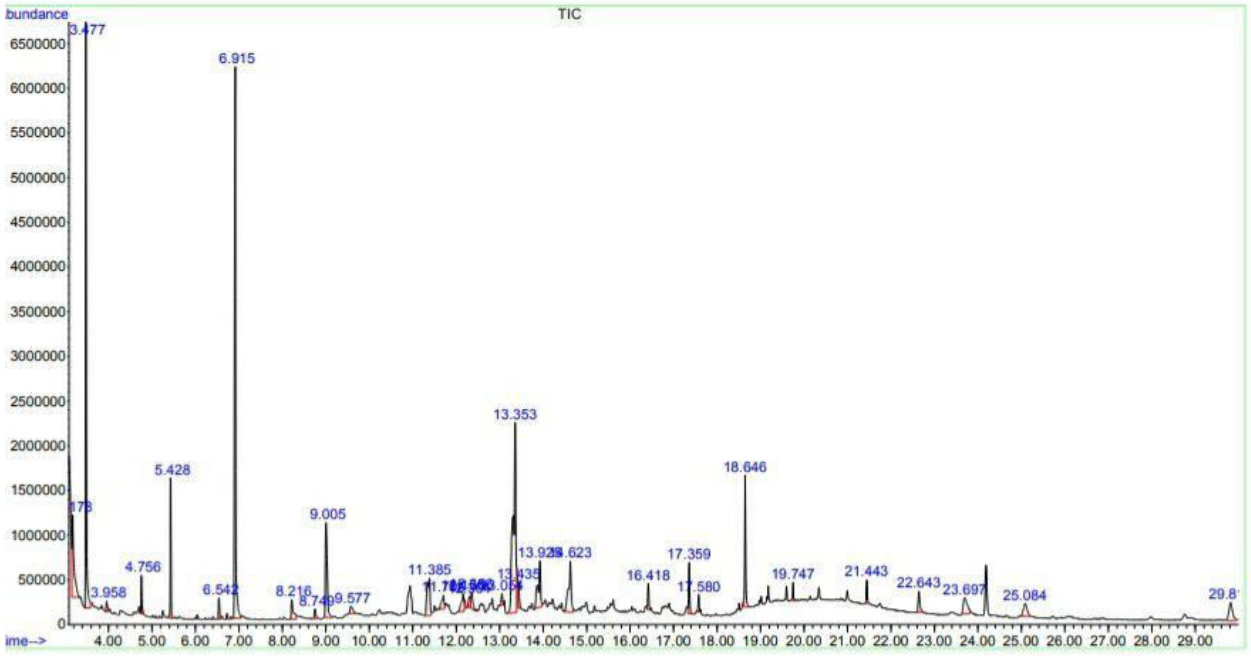
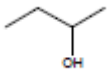
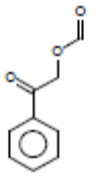
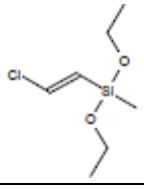
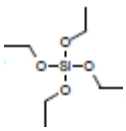
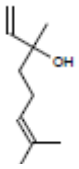
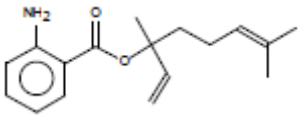
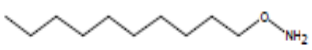
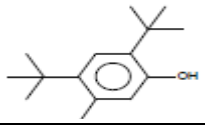
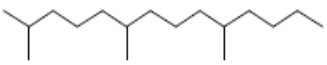
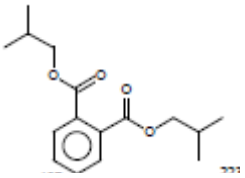
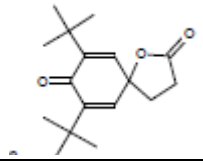
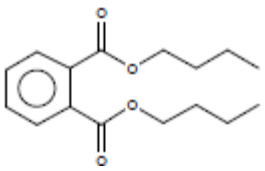
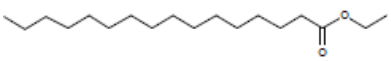
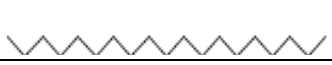
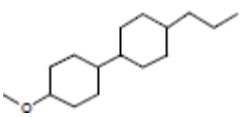
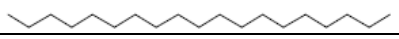
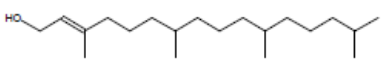


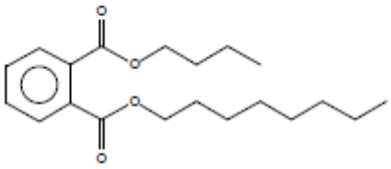
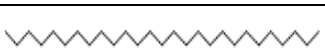
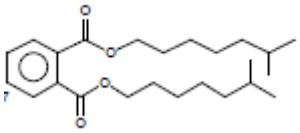
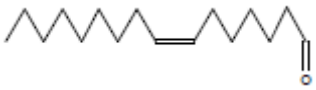
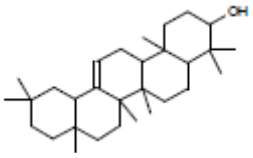
Figure 1. GC (chromatographic) mass spectrum of C-reticulata

2. Type C-limon and it included 26 chemical compounds, beta-Amyrin was the highest compound in terms of time, and the time reached 26,077, while the compound. 2-Butanol was the least compound in terms of time, as the time reached 3.130, as shown in Table (2).

Table 2. Chemical compounds in type *C- limon*

% of total	peak Height	R.T. min	hemical formulaC	The chemical compound	T
7.081%	748518	3.130		2-Butanol	1
22.650%	6201718	3.438		Ethanone,2-(formyloxy)-1-phenyl-	2
1.615%	316423	4.734		Trans-(2-Chlorovinyl) methoxysilane	3
5.128%	1303004	5.415		Tetraethyl silicate	4

1.983%	298900	6.911		1,6-Octadien-3-ol,3,7-dimethyl-,2-	5
3.495%	351180	9.013		,Octadien-3-ol,3,7-dimethyl-	6
4.343%	330914	10.938		Hydroxylamine,O-decyl -	7
1.030%	154852	12.395		Tetradecan	8
10.395%	460501	13,396		4,6-di-tent-butyl-m-cresol	9
1.819%	281357	16.418		Tetradecan,2,6,10-trimethyle-	10
0.114%	25694	16,977		1,2-Benzenedicarboxylic acid	11
3.082%	457825	17,359		7,9-Di-tert-buty-1-oxaspiro	12
0.866%	132574	17,575		Dibutyl phthalate	13
0.774%	139,277	17,619		Hexadecanoic acid	14
0.777%	104058	17,792		Nonadecane	15th
1.555%	330447	18,508		1,1-Bicyclohexyl,4-methoxy-4propyl	16
2.324%	386213	18,646		Nonadecane	17
2.532%	659992	19.175		Phytol	18
0.548%	98178	19,596		Octacosane	19

2.396%	590449	19,747		1,2-Benzenedicardicarboxylic	20
1.640%	372345	20.341		Octacosane	21
0.977%	1888517	20.996		Heptacosane	22
0.822%	138409	21,443		Nomadecane	23
0.586%	90717	21.746		1,2-Benzenedicarboxylic acid, diisooctyl ester	24
4.761%	152644	24,356		7-Hexadecenal	25
16.710%	3839844	26,077		Beta- Amyrin	26

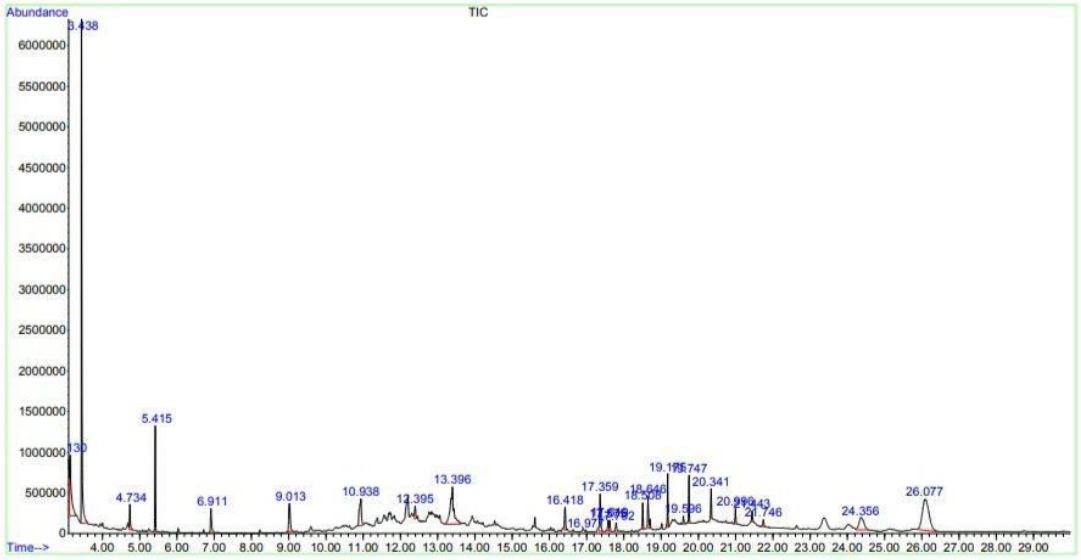
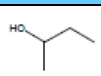
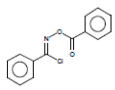
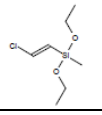
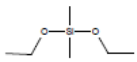
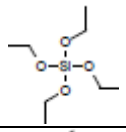
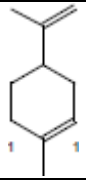
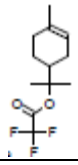
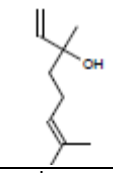
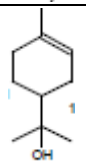
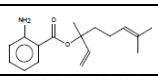
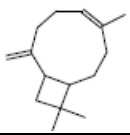
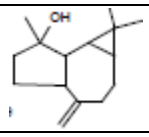
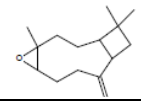
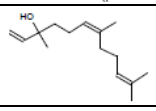
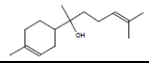
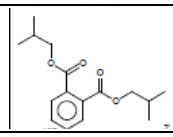
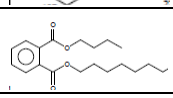
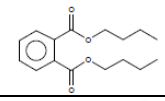
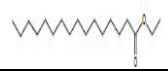
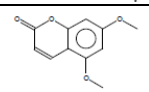
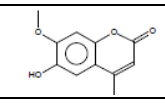
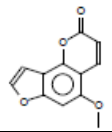
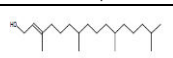
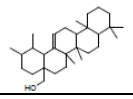
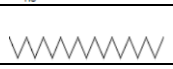
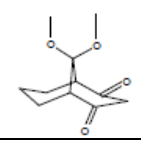
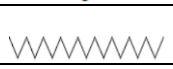
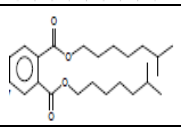


Figure 2. GC (chromatographic) mass spectrum of the species C-limon

3. The species C-aurantifoli contained 31 chemical compounds, and 2-butanol was the highest compound in terms of time, and the time reached 21.438, and the compound 1,2-Benzenedicarboxylic acid, diisooctyl ester was the least compound in terms of time, as the time reached 3.143, and as shown in Table (3).

Table 3. Chemical Compounds in species *C-aurantifoli*

% of total	peak Height	R.T. min	Chemical formula	chemical compound	T
8.817%	721518	3.143		2-Butanol	1
16.073%	3098892	3.451		O- Benzoylbenzohydroximidoyi chloride	2
0.434%	48,508	3.950		Propylene Glycol	3
0.917%	191416	4.743		Trans-(2-Chloroviny) methyldithoxyldiethoxysilane	4
0.559%	64247	5.743		Silane,diethoxydimethyl	5
5.996%	1333498	5.424		Tetraethyle silicate	6
0.312%	60597	6.035		D-Limonene	7
0.085%	19465	6.087		Trifuoroacetyle -alpha- terpineol	8
2.270%	320563	6.911		1,6-Octadien-3-ol, 3,7-dimethyl-	9
2.927%	236098	8.220		p-menth-1-en-8-ol	10
5.621%	430706	9.09		1,6-Octadien-3-ol, 3,7- dimethy-, 2-aminobenzoate	11
3.672%	239983	10.938		Tetradecane	12
5.195%	351651	11,385		Caryophyllene	13

1.882%	206799	13,357		Spathulenol	14
2.070%	314312	13.435		Caryophyllene oxide	15th
0.383%	47038	14.051		1,6,10-Dodecatrien-3-ol	16
0.524%	60984	14.515		Alpha – Bisabolol	17
0.675%	105209	15,607		Nonadecane	18
1.859%	272256	16.414		1,2-Benzenedicarboxylic acid	19
0.431%	43412	16,895		1,2-Benzenedicarboxylic acid,butyl	20
3.117%	430856	17,354		Dibutyl phthalate	21
0.843%	115847	17,575		Hexadecanoic acid	22
23.466%	1415482	17,692		2H-1-Benzopyran-2-one,5,7-dimethoxy	23
0.482%	77238	17,674		2H-1-Benzopyran-2-one,	24
3.900%	238924	18,503		2H-furo[2,3-h]-1-benzopyran-2-one	25
4.228%	682931	18.642		Phytol	26
1.305%	231278	18,698		Urs-12-en-28-ol	27
0.156%	34504	19.145		Nonadecane	28
0.693%	135885	19.175		9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	29
0.503%	105148	19,743		Nonadecane	30
0.598%	85216	21.438		1,2-Benzenedicarboxylic	31

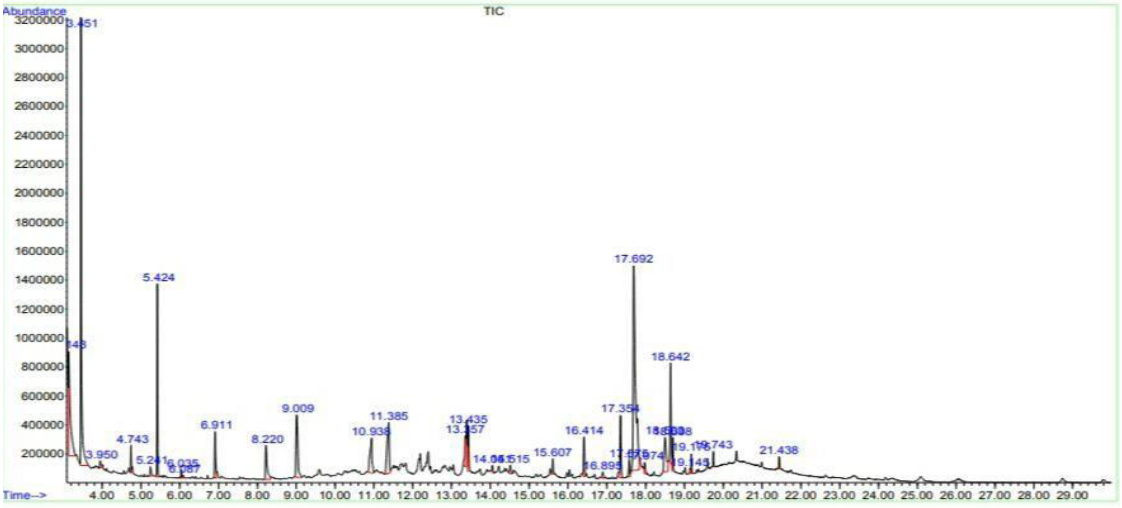
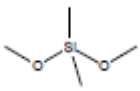
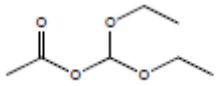
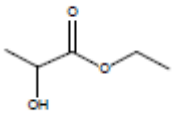
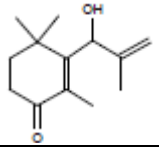
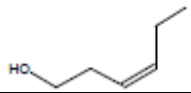
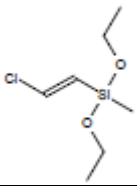


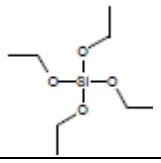
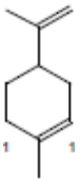
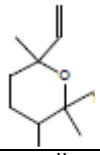
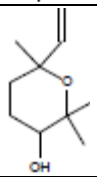
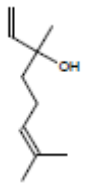
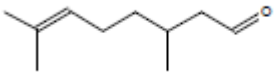
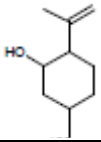
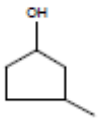
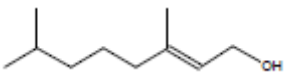
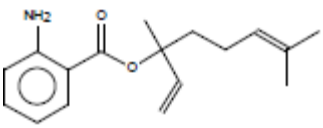
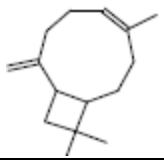
Figure 3. GC (chromatographic) mass spectrum of the species *C-aurantifoli*

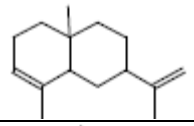
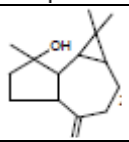
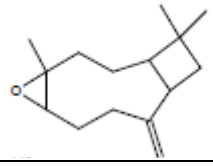
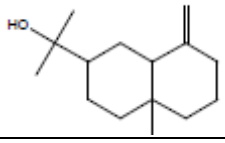
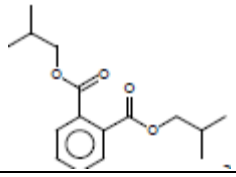
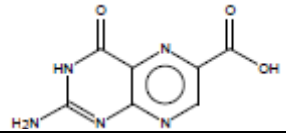
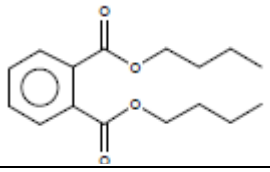
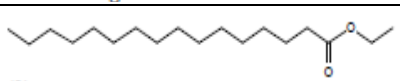
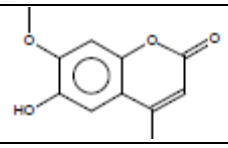
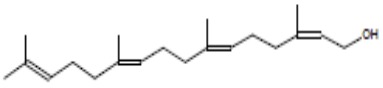
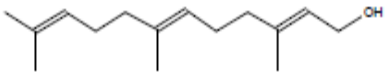
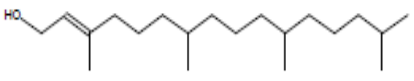
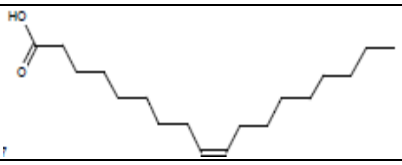
4. The type and C-limettioides, it included 31 chemical compounds, and Oleic Acid was the highest compound in terms of time, and the time reached 29.801 and the compound Ethanone, 2-(formyloxy)-1-phenyl- was the least compound in terms of time, as the time reached 3.421, and as in Table (4).

Table 4. Chemical Compounds in species *C-limettioides*

% of total	Peak height	R.T. min	Chemical formula	chemical compound	T
16.432%	4072521	3.421		Silane,dimethoxydimethyl -	1
0.912%	212511	3.837		Diethymethymethyl acetate	2
2.397%	354407	3.902		Propanoic acid	3
0.400%	100375	3.984		4-Oxo-beta— isodamascol	4
0.681%	67,761	4.223		3-Hexen-!_ ol	5
0.725%	156481	4.730		Trans-(2-Chlorovinyl)	6

2700

6.492%	1614487	5.419		Tetraethyl silicate	7
0.737%	146770	6.035		D-Limonene	8
0.287%	48822	6.607		Linalool Oxide	9
0.269%	46680	6.811		Linalool oxide(fr.1)	10
16.094%	2955232	6.911		1,6-Octadin-3-ol,3,7-dimethyl-	11
2.616%	343215	7.630		6-Octenal,3,7-dimethyl-	12
1.342%	151330	8.224		Cyclohexanol,5-methyl-2-(1-methylethenyl).	13
3.123%	166835	8.688		Cyclopentanol	14
8.754%	1194047	9.018		2-Octen-1-ol,dimethyl-	15th
3.793%	351112	10.938		1,6-Octadien-3-ol,3,7-dimethyl-	16
4.516%	399517	11,385		Nonadecane	17
3.316%	300067	12.403		Caryophyliene	18

1.800%	291662	13,353		Naphthaene	19
2.753%	478779	13.453		1H-Cyclopruop[e] azulen	20
1.602%	226461	14,207		Caryphyliene Oxide	21
2.341%	411574	16.418		2-Naphthalenemthanol	22
0.866%	92829	16,895		1,2-Benzendicarboxylic	23
3.996%	642214	17,354		Pterin-6-carboxylic acid	24
0.838%	1442575	17,575		Dibutyl phthalate	25
1.492%	38032	17,744		Hexadecanoic acid	26
4.031%	717228	17,792		2H-1-Benzopyan-2one,6-hydrxy-	27
0.389%	57977	17.979		Hexadeaca-2,6,10,14-tetrean-	28
4.784%	979335	18.642		2,6,10-Dodecatrien	29
0.393%	83206	19,310		Phytol	30
1.830%	97627	29.801		Oleic Acid	31

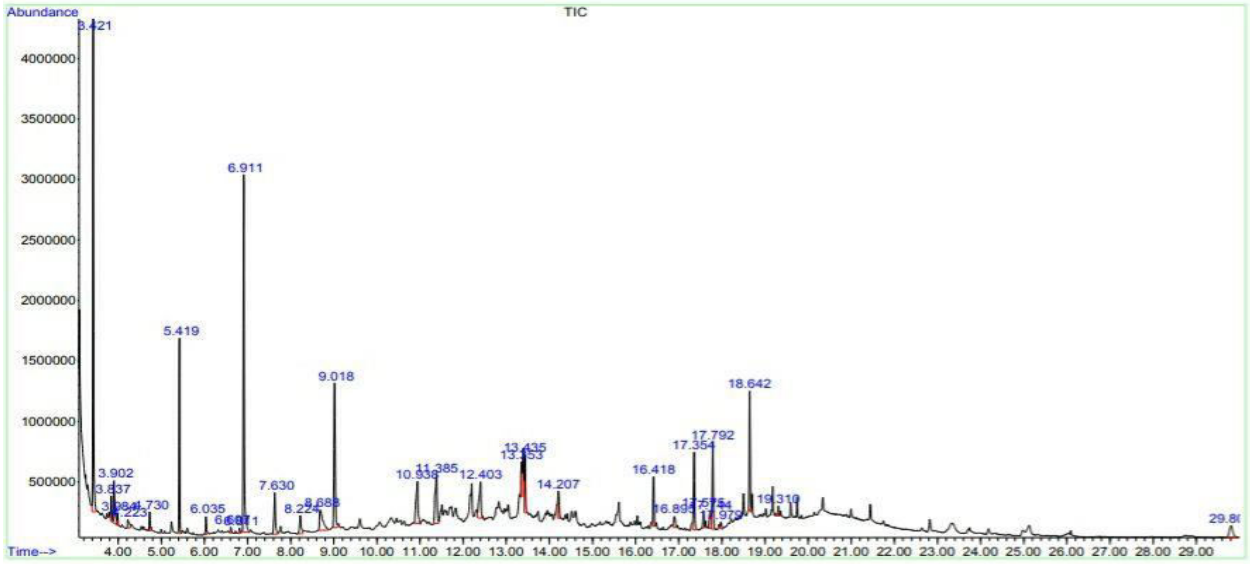


Figure 4. GC (chromatographic) mass spectrum of *C-limettioides*

Measuring the Antioxidant Activity of Plant Extracts by DPPH Method

The effectiveness of the roots was studied using the compound 2-2Diphanyl-1-picrylhydrazyl-2,2/DPPH by the effect of the plant extract of the leaves of the species of the genus Citrus, the results showed a clear discrepancy for the extracts of the studied species, as the type C.maxima was the highest percentage in scavenging free radicals and reached 84%, followed by C. aurantium, 81%, and the lowest was C. aurantifolia, which amounted to 71%. It was compared with ascorbic acid (V.C) as shown in Figure (5).

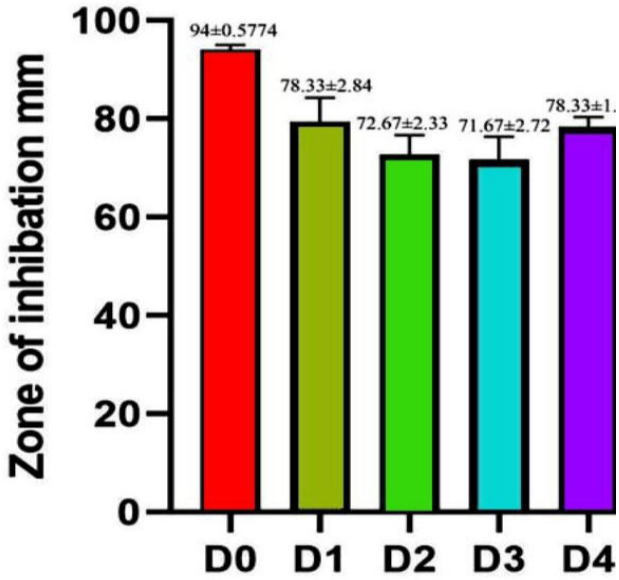


Figure 5. Shows the antioxidant activity of plant extracts at a concentration of 1/2 where. Represents**.**

D0: Ascorbic acid - D1: C.reticulata- D2:C.limon - D3:C.aurantifolia - D4: C.limattioides

The difference of the extracts in their ability to inhibit free radicals is due to the difference in the chemical content of the active substances such as phenols and flavonoids. The plant has the inhibitory activity in the field of scavenging free radicals, and thus the plant plays an important role in cellular defense against harmful radicals and (Zhang (ROS et al., 2020), results in the study of the anti-activity on the displacement of free radicals through the DPPH reaction, that the inhibitory action varies from one type to another. Another, and this is due to the nature of the active ingredients and their concentration in the studied species, as it was found (AL.Juhamimi et al., 2018) that the species had a high antioxidant capacity, reaching 78%, 72 and 71 for the species, namely Blanco *C.reticulata*, *C. limettioides*, *C.limon* and *C. aurantifolia* and, respectively, that the decrease in the presence of harmful free radicals by continuing to eat citrus fruits has a role in reducing inflammation and oxidation of fat particles.

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