ESTIMATION OF GENETIC POLYMORPHISM IN MORPHOLOGICAL TRAITS AND BIOCHEMICAL CHARACTERIZATION OF SOUR ORANGE (*CITRUS AURANTIUM* L.)

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Abstract. *Citrus aurantium* L. is grown in developed and developing countries as its fruit is edible, and it is rich in minerals, vitamins, antioxidants, and other nutrients. During the present study, a total of 18 genotypes of sour orange (*Citrus aurantium* L.) were selected from different locations of Dir Lower, KPK Pakistan for the estimation of genetic diversity through morphological and phytochemical characterization using GC-MS. For morphological characterization, a total of 19 parameters (12 qualitative and 07 quantitative) were recorded. A significant variation was found for most of the qualitative and quantitative traits. A significant level of coefficient of variance was observed for leaf lamina length and fruit volume (20%), leaf lamina width (16%), and the number of segments (14%), while a low level of coefficient of variance was recorded in fruit diameter (6.5%), fruit length (6.8%). Based on correlation analysis, a significant amount of correlation was found for leaf lamina width with leaf lamina length (0.598**), fruit length with fruit diameter (0.942**), fruit weight with fruit diameter (0.958**), and fruit length (0.883**), similarly fruit volume was found to have correlated with fruit diameter (0.998**), fruit length (0.945**) and fruit weight (0.960**). Principal component analysis with an Eigenvalue of (0.760) was found to account for 98.469% of the overall variation reported among the 18 *Citrus aurantium* L. genotypes. Through GC-MS analysis a total of 105 phytochemical compounds were identified, in the essential oil (Eos) of four *Citrus* genotypes, these genotypes were selected based on contrasting morphological traits, and the maximum number of compounds (28) were found in the EOs of sample 5 and sample 6. While the minimum (23) was found in sample 7. For antioxidant activities, the highest scavenging potential (43.63%) was observed in sample 8 and the lowest was recorded in sample 6 (21.48%).
Keywords: *qualitative and quantitative traits, PCA, cluster analysis, essential oils, antioxidant*

Introduction

Citrus aurantium L., also known as sour orange or bitter orange, is frequently used as a rootstock and offers many benefits (Nureen et al., 2023; Etebu and Nwauzoma, 2014). It is a storehouse of minerals, vitamins, antioxidants, and other nutrients (Karthikeyan et al., 2014). Citrus fruits contain vitamins C and B, minerals, essential oils, phenolic, and other bioactive compounds (González et al., 2010) that have been utilized for therapeutic

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purposes (Moraes et al., 2009). Sometimes young fruits are pickled or served as a side dish. The peel of *Citrus aurantium* L. contains the main essential oil component. Dried peel is used in bouquet garni and to flavor the Belgian beer Orange Muscat (Kiple and Ornelas, 2000). Drinks and alcoholic beverages like Curaçao, Cointreau, and Triple Sec are flavored with essential oils extracted from the dried peel of unripe *Citrus aurantium* L. fruits. The essential oil (neroli) is used in fragrances, liqueurs, and orange-flower water, which is used to flavor sweets, while the petals are used in teas (Mohagheghniapour et al., 2022). In addition to the applications, undiluted essential oils are also expensively sold in the global aromatherapy, perfume, and cosmetics markets. Additionally, numerous studies have been published on the various medical benefits of various *C. aurantium* essential oil constituents and other compounds isolated from the peel, including antioxidant, antimicrobial, antifungal, antiparasitic, anti-inflammatory, etc. (Farahmandfar et al., 2020).

The present study aimed to i) estimate the variation in *Citrus aurantium* through different morphological and genetic traits, ii) determine essential oil composition using the GC-MS technique, and iii) estimate the genetic polymorphism in antioxidants of collected genotypes of *Citrus aurantium* L.

Materials and methods

The present research study was carried out at the Department of Botany, University of Malakand KPK Pakistan. A total of eighteen different genotypes of *Citrus aurantium* L were collected from different regions of Dir L Khyber Pakhtunkhwa, Pakistan in February 2022 (*Fig. 1; Table A1*). The fruits were used for different morphological studies and biological screening using DDPH and GC-MS analysis.

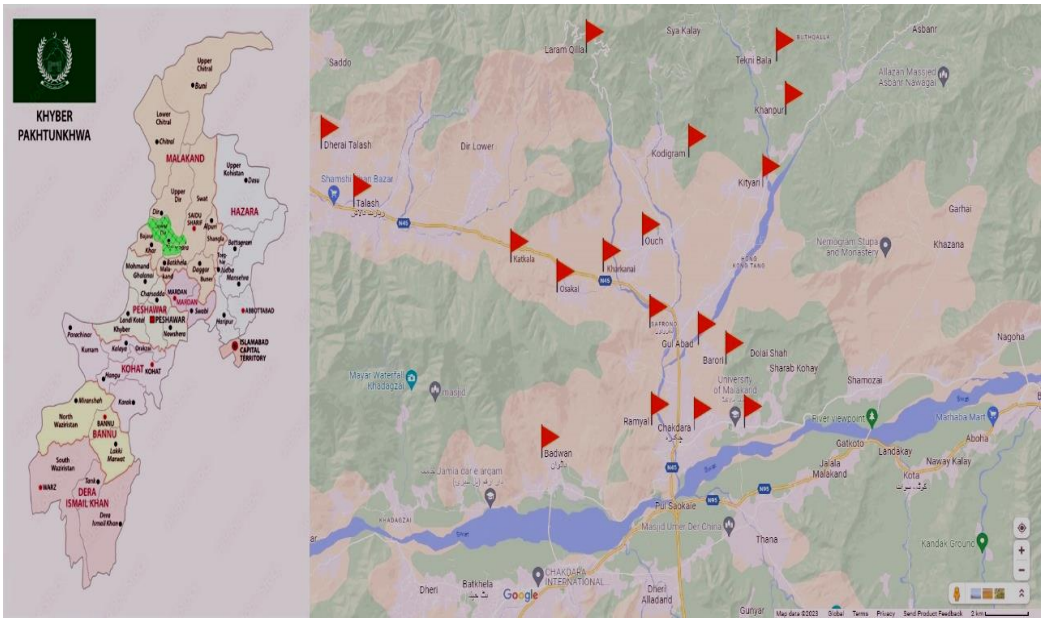

Figure 1. *Citrus genotypes collected from different ecological zones of Dir KPK Pakistan*

Morphological characterization

A total of 19 different morphological traits both qualitative and quantitative traits were recorded using basic Descriptors for Citrus IPGRI 1999 and analyzed. Twelve

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qualitative traits: Plant Morphology, Density of branches, Tree Growth Habit, Branch angle, Leaf color, Leaf Lamina Shape, Fruit shape, Fruit peel color, Fruit surface texture, Fruit attachment to stalk, Thickness of peel, Pulp color, seven quantitative traits: Leaf Lamina Length, Leaf Lamina, Width, Fruit Diameter, Fruit Weight, and Fruit Volume, Fruit length, and Number of segments.

Extraction of essential oils

The extraction of essential oils from the peels of sour orange was based on hydro distillation mainly in the soxhlet apparatus. The main components of the distillation unit include a heating mantle, a round bottom distillation flask of 250 ml, a horizontal condenser, and a collecting vessel. Firstly, the peels from the collected fresh fruits of *C. aurantium* genotypes were separated and chopped into smaller pieces. 340 gm of pre-treated citrus peels from sample number 5, 310 gm from sample number 6, 350 gm from sample number 7, and 341 gm from sample number 8 were submitted to hydro distillation separately, while one liter of distilled water was added to the distillation flask. The mixtures were boiled for 3 h, initially, the temperature was slow and then raised gradually to 100°C. After boiling, about 450 ml of distillates (water and oils mixtures) were collected in 500 ml collecting vessels. Two distillate layers were observed in each flask, one dense upper layer, and the other less dense layer. The less dense upper layer was oils. The essential oils were separated from the distillates by using a burette and clear yellow volatile oils with a fresh odor were obtained and stored in Eppendorf tubes. The essential oils obtained from sample five, sample six, sample seven, and sample number eight were 0.4 ml, 0.2 ml, 0.5 ml, and 0.4 ml respectively. The EOs were stored under refrigeration up to the tests and analysis (Sikdar et al., 2016).

Gas chromatography-mass spectrometry (GC-MS)

The chemical investigations of the extracts were carried out through gas chromatography-mass spectrometry (GC/MS) using techniques adapted by Yaşar et al. (2005). The gas chromatograph (Shimadzu) was hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) having an automatic sampler (AOC-20S) and injector (AOC-20i). Helium gas was used as a carrier medium. The chromatographic separations were carried out in a capillary column (TRB-FFAP; Technokroma) with 30 m length; 0.35 mm i.d.; 0.250 μm thickness, treated with polyethylene glycol. Other GC-MS conditions include: 250°C temperature of ion source (EI), 240°C interface temperature, 100 KPa pressure, and 1.8 min cut time for solvent. A sample and standard of 1 μl were injected into the column of GC. The injector operation was done in a split mode having a splitting ratio of 1:50 with 240°C temperature of injection. The temperature program of the column started for 1 min at 50°C and changed at a rate of 15°C/min to 150°C. The temperature was increased at the rate of 2.5°C/min up to 175°C for 5 min. The temperature was then further increased at a rate of 2.5°C/min to 220°C holding constant for 3 min. The total time for elution was noted to be 43 min. The scanning of MS was done from a mass/charge ratio of 85 to 380.

Data analysis of morphological traits

Both quantitative and qualitative traits were recorded from 18 different experimental genotypes. Five plants were randomly selected from each genotype and their mean value

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was used for data analysis. Microsoft Excel 2016 was used to analyze the frequency distribution of qualitative traits. Recorded quantitative data was averaged and mean values were used for descriptive statistics using SPSS version 22, Cluster dendrogram, and Principal Component analysis was calculated using PC-ORD version 5.

Results

Morphological diversity

During the present study, a total of 19 morphological characters were recorded in 18 *C. aurantium* genotypes. Among the traits considerable variations were recorded in the studied genotypes, the details information was discussed in their respective sections.

Diversity in qualitative traits

A total of 12 qualitative traits were, and a significant diversity was found for plant morphology. Three types of plant morphology (Spheroid, obloid, and ellipsoid) were observed among 18 *C. aurantium* genotypes in which 55.55% were spheroid, 38.88% were obloid and 5.55% were found ellipsoid type. For the density of branches, three different types of branch density were observed 50% of genotypes were medium branches, 38.88% were observed as dense, and 11.11% of the population was observed as sparse. Two types of growth habits were found 61.11% were observed as spreading and 38.88% were observed as erect. Four types of branch angles were observed, where 33.33% of genotypes were a medium angle, 22.22% of genotypes were narrow branch angles, 38.88% were genotypes were observed as wide, and 5.55 were observed as low branch angles. For the intensity of color in the leaves 3 types of color (Medium, Light, and Green) were observed among the studied genotypes of *C. aurantium* in which 44.44% of genotypes have medium color, 27.77% of genotypes have a light color and 27.77% of genotypes were found to have a green color. Among the studied genotypes, 72.22% of genotypes have ovate leaves, 11.11% have elliptical leaves, 11.11% were observed as lanceolate leaves, and 5.55% were found to have obtuse-shaped leaves. Three types of fruit shapes (Obloid, Pyriform, and Spheroid) were observed among the studied genotypes, in which 61.11% genotypes have obloid shape fruits, 22.22% were observed as pyriform shaped, and 16.66% of genotypes were found to have spheroid shaped fruits (*Table 1*).

For the trait of fruit peel color, two types of color were recorded, where 55.55% of the genotypes have light orange colored and 44.44% were found to have dark orange colored fruits. For the fruit surface texture, 3 different fruit surfaces were observed where 38.88% of genotypes have papillate fruit surface texture, 50% were observed as rough and 11.11% were genotypes were found to have pitted fruit surface texture. The character of the fruit attachment to the stalk was observed as weak and strong. 38.88% of genotypes were observed as weak attachment of fruits to stalks while 61.11% of genotypes were observed to have a strong attachment of fruits to stalks. Three types (Medium, Thick, and Thin) of peel were observed among the studied genotypes of *C. aurantium*, in which 61.11% have medium peels, 33.33% were observed as thick peels, and 5.55% were found to have thin peels. For the pulp color, it was found that 2 types of White and Yellow color were recorded where the fruits of 50% of genotypes were observed to have white pulp color, while the fruits of 50% of genotypes were found to have yellow pulp color (*Table 1*).

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Table 1. *Frequency percentage of different qualitative traits of Citrus aurantium L.*

S. No	Traits	Description	Code	Frequency percentage
1	Plant morphology	Spheroid	1	55.55
		Obloid	3	38.88
		Ellipsoid	5	5.55
2	Density of branches	Medium	1	50
		Dense	3	38.88
		Sparse	5	11.11
3	Tree growth habitat	Spreading	1	61.11
		Erect	3	38.88
4	Branch angle	Medium	1	33.33
		Narrow	3	22.22
		Wide	5	38.88
		Low	7	5.55
5	The intensity of the green color	Medium	1	44.44
		Light	3	27.77
		Green	5	27.77
6	Leaf lamina shape	Ovate	1	72.22
		Elliptical	3	11.11
		Laneceolate	5	11.11
		Obtuse	7	5.55
7	Fruit shape	Obloid	1	61.11
		Pyriform	3	22.22
		Spheroid	5	16.66
8	Fruit peel color	Light orange	1	55.55
		Dark orange	3	44.44
9	Fruit surface texture	Papillate	1	38.88
		Rough	3	50
		Pitted	5	11.11
10	Fruit attachment to stalk	Weak	1	38.88
		Strong	3	61.11
11	Thickness of peel	Medium	1	61.11
		Thick	3	33.33
		Thin	5	5.55
12	Pulp color	White	1	50
		Yellow	3	50

The data were recorded according to the basic Descriptors for Citrus IPGRI, 1999, Rome, Italy

Quantitative traits

The descriptive statistics (range, mean, maximum, minimum, and CV %) of the different quantitative traits are presented in *Table 2*. During the current study, a significant variation was found in the length of the leaf lamina. This parameter ranged from 90 to 182 mm with a mean of 138 mm, a standard deviation of 28.27, and a coefficient of variation for the trait was 20.49%. The maximum leaf length (182 mm)

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was found for Citrus-18 followed by Citrus-12 with a leaf length of 180 mm while the minimum was found for Citrus-4 with a leaf length of 90 mm followed by Citrus-1 with a leaf length of 95 mm. The leaf lamina width, ranged from 35 mm to 60 mm, with a mean value of 45.44 mm, and the coefficient of variation for the trait was 16.45%. Among the studied genotypes a significant variation was found for fruit diameter, ranging from 63 mm to 78 mm, with a mean value of 69.78 mm, and the coefficient of variation for the trait was 6.59%. Among the studied genotypes, the maximum fruit diameter (78 mm) was found in Citrus-18, while the minimum was found for Citrus-8 with a fruit diameter of 63 mm followed by Citrus-14 with a fruit diameter of 64 mm. During the current study of 18 *C. aurantium* genotypes, a considerable variation was found for the trait of fruit length, ranging from 64 mm to 80 mm, with a mean value of 71 mm, and the coefficient of variation for the trait was 6.83%. Among the studied genotypes, the maximum fruit diameter (80 mm) was found in Citrus-12 and Cit-18 followed by Citrus-1 with a fruit diameter of 76 mm while the minimum was found in Citrus-2 and Citrus-8 with a fruit diameter of 64mm followed by Citrus-4 with a fruit diameter of 65 mm. The range for the trait was 16. Among the studied genotypes, considerable variation was found for leaf fruit weight, ranging from 182.9 g to 241 g, with a mean value of 209.21 g, and the coefficient of variation for the trait was 8.3%. Among the studied genotypes, a maximum fruit weight (of 240.5 g) was found for Cit-18 followed by Citrus-12 with a fruit weight of (237.14 g) while a minimum fruit weight of (182.9 g) followed by Citrus-9 with a fruit weight of 188.84 g.

During the current study of 18 *C. aurantium* genotypes, a considerable variation was found for the trait of fruit volume, ranging from 130.53 cm^3 to 248 cm^3, with a mean value of 179.55 cm^3, and the coefficient of variation for the trait was 20.07%. Among the studied genotypes, the maximum fruit volume (248 cm^3) was found in Citrus-12 and Cit-18 followed by Citrus-1with a fruit volume of 220.22 cm^3 while the minimum was found in Citrus-8 with a fruit volume of 130.53 cm^3 followed by Citrus-14 with a fruit volume of 136.84 cm^3. The number of segments is an important character. During the current study, a significant variation was found in the number of segments among the studied genotypes of 18 *C. aurantium* genotypes. This parameter ranged from 7 to 11 segments per genotype, with a mean value of 8.94, and the coefficient of variation for the trait was 14.08%. Among the studied genotypes maximum number of segments (11) was found in Citrus-8 and Citrus-15, followed by Citrus-1, Citrus-5, Citrus-6, and Citrus-14 with 10 segments per genotype while the minimum was found for Citrus-2, Citrus-7, and Citrus-16 with 7 segments per genotype followed by Citrus-3, Citrus-10, and Citrus-12 with 8 segments per genotype.

Table 2. *Descriptive statistics of 7 quantitative traits of 18 Citrus aurantium L. genotypes*

Descriptive statistics							
Traits	**Range**	**Minimum**	**Maximum**	**Mean**	**Standard error**	**Standard deviation**	**CV%**
Leaf lamina length	92.00	90.00	182.00	138.00	6.66	28.27	20.49
Leaf lamina width	25.00	35.00	60.00	45.44	1.76	7.48	16.45
Fruit diameter	15.00	63.00	78.00	69.78	1.08	4.60	6.59
Fruit length	16.00	64.00	80.00	71.00	1.14	4.85	6.83
Fruit weight	57.60	182.90	240.50	209.21	4.09	17.37	8.30
Fruit volume	117.19	130.53	247.72	179.55	8.49	36.03	20.07
Number of segments	4.00	7.00	11.00	8.94	0.30	1.26	14.08

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Correlation analysis

Correlation is a statistical technique that shows how strongly two variables are related to each other or the degree of association between the two. Statistical tools were used for finding the correlation between the *Citrus aurantium* genotypes by using the software SPSS version 22 as shown in (*Table 3*). During the current study, a significant correlation was found for leaf lamina width with leaf lamina length with the value of (0.59^{**}), fruit length with fruit diameter (0.94^{**}), fruit weight with fruit diameter (0.95^{**}), and fruit length (0.88^{**}), similarly fruit volume was found to have correlated with fruit diameter (0.99^{**}), fruit length (0.94^{**}) and fruit weight (0.96^{**}).

Table 3. *Correlation analysis of 7 quantitative traits in Citrus aurantium L.*

Traits	Leaf lamina length	Leaf lamina width	Fruit diameter	Fruit length	Fruit weight	Fruit volume	Number of segments
Leaf lamina length	1						
Leaf lamina width	0.59^{**}	1					
Fruit diameter	0.07	0.12	1				
Fruit length	0.21	0.12	0.94^{**}	1			
Fruit weight	0.04	0.13	0.95^{**}	0.88^{**}	1		
Fruit volume	0.08	0.10	0.99^{**}	0.94^{**}	0.96^{**}	1	
Number of segments	-0.30	-0.16	-0.24	-0.13	-0.16	-0.21	1

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

Principal component analysis (PCA)

Comparative PCA (Principal component analysis) was calculated using PC-ORD version 5. Principal component analysis (PCA) was done based on 7 quantitative morphological parameters, and it was found that PC4 has an eigenvalue of (0.760) was found to account for 98.469% of the overall variation reported among the 18 *Citrus aurantium* L. genotypes (*Table 4*). The first principal component was found to have a 56.455% variation. The NS (0.145) have positive weight on PC1, while Leaf lamina length (-0.106), Leaf lamina width (-0.111), Fruit Diameter (-0.497), Fruit length (-0.480), Fruit weight (-0.481) and Fruit volume (-0.496) were found to have negative weights. In PC2 the total variation was 80.193% and it was found that Leaf lamina length (0.671), Leaf lamina width (0.622) were found positive contributions, Fruit Diameter (-0.099), Fruit length (-0.060), Fruit weight (-0.122), Fruit volume (-0.110) and the number of segments were found negative weight on PC2. The contribution of PC3 with the total variations of 92.512% and it was found that Leaf lamina length (0.129), Leaf lamina width (0.389), Fruit length (0.118), Fruit weight (0.056), Fruit volume (0.001), and Number of segments (0.903) were positively contributed on the other hand Fruit Diameter (-0.021) contributed negatively in PC3. In PC4 the total variation was 98.469% where the contribution of Leaf lamina length (0.670), Fruit length (0.270), and Number of segments (0.156) was found positive weights while Leaf lamina width (-0.651), Fruit Diameter (-0.059), Fruit weight (-0.149) and Fruit volume (-0.017) contributed negatively to the PC4.

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Table 4. *Principal component analysis of 7 quantitative traits in Citrus aurantium L.*

AXIS	PC1	PC2	PC3	PC4
Cum. % of Var.	56.455	80.193	92.512	98.469
Eigenvalue	2.593	1.593	1.093	0.760
Leaf lamina length	-0.106	0.671	0.129	0.670
Leaf lamina width	-0.111	0.622	0.389	-0.651
Fruit diameter	-0.497	-0.099	-0.021	-0.059
Fruit length	-0.480	-0.060	0.118	0.278
Fruit weight	-0.481	-0.122	0.056	-0.149
Fruit volume	-0.496	-0.110	0.001	-0.017
Number of segments	0.145	-0.350	0.903	0.156

Cluster analysis

A cluster dendrogram was constructed for 7 quantitative traits using PC-ORD version 5, and Ward's method was used for cluster analysis. All the genotypes were divided into two main groups at a distance of 25% and further subdivided into four groups at 62.5%. Each group shows less differences from one another but high variation from other groups. The first group contains 5 genotypes namely Citrus-1, Citrus-5, Citrus-17, Citrus-12, and Citrus-18. The second group comprised 5 genotypes namely Citrus-2, Citrus-6, Citrus-13, Citrus-4, and Citrus-8. The third group has 3 genotypes namely Citrus-3, Citrus-7, and Citrus-11. Similarly, group 4 is also composed of 5 genotypes namely Citrus-9, Citrus-15, Citrus-10, Citrus-16, and Citrus-14. Based on the cluster dendrogram Citrus-1 and Citrus-14 were the most diverse based on morphological characterization and are placed at the extreme periphery of the dendrogram (*Fig. 2; Table 5*).

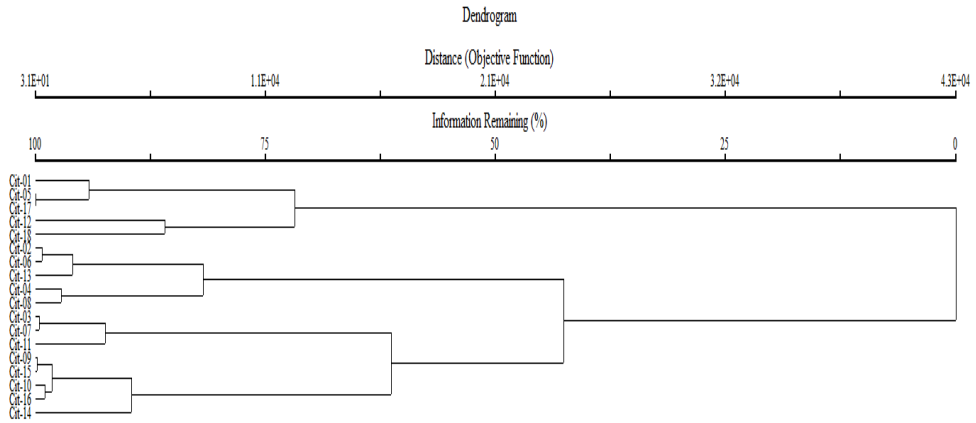

Figure 2. *Cluster dendrogram of 18 C. aurantium L. genotypes based on 7 quantitative traits*

GC-MS characterization of essential oils in selected genotypes of C. aurantium L.

Identification of phytochemicals in genotype 5 (Citrus-5)

The detailed results of the GC-MS analysis of genotype 5 (Citrus-5) are tabulated in *Table 5* in which the identified phytochemical compounds with their retention time (RT), compound name (Compound), molecular formula (Formula), molecular weight

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(Mol weight) and area (%) are shown, the GC-MS chromatogram of the phytochemicals are given in *Figure 3*. The result showed that 28 phytochemicals were identified with a percent area of 95.68% of the total compounds.

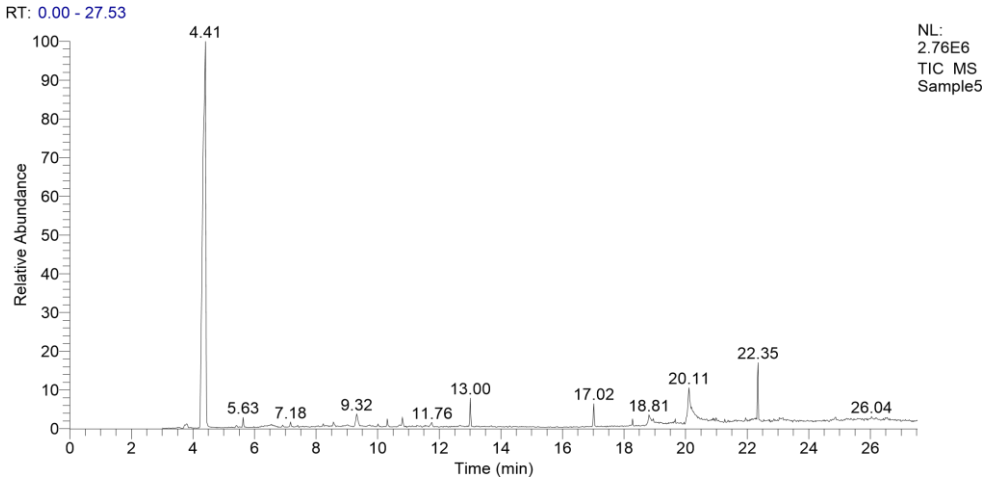

Figure 3. *GC/MS chromatogram of phytochemical compounds in genotype 5 (Cit-5)*

Table 5. *Composition of C. aurantium fruit peel essential oil in genotype 5 (Citrus-5)*

S. No	RT	Compound	Formula	Mol weight	Area %
1	3.77	á-Pinene	$C_{10}H_{16}$	136	0.73
2	4.37	Cyclohexene	$C_{10}H_{16}$	136	**64.26**
3	5.63	1,6-Octadien-3-ol,	$C_{10}H_{18}O$	154	0.76
4	6.54	2-Decenal, (E)-	$C_{10}H_{18}O$	154	0.94
5	7.18	Bicyclo heptan-3-ol, 4,7,7-trimethyl-	$C_{10}H_{18}0$	154	0.28
6	7.38	Dodecanoic acid, 3-hydroxy	$C_{12}H_{24}O_3$	216	0.1
7	8.26	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate	$C_{12}H_{20}O_2$	196	0.48
8	8.56	Cyclododecane	$C_{12}H_{24}$	168	0.54
9	9.72	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.42
10	10.31	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$C_{12}H_{20}O_2$	196	0.39
11	10.8	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	$C_{15}H_{24}$	204	0.83
12	11.31	Cholestan-3-ol, 2-methylene	$C_{28}H_{48}O$	400	0.29
13	11.74	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)	$C_{15}H_{24}$	204	0.58
14	13	1,6,10-Dodecatrien-3-ol	$C_{15}H_{26}O$	222	**1.91**
15	13.67	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	$C_{23}H_{30}N_2O_5$	414	0.06
16	14.47	1,4-Methanoazulen-3-ol	$C_{15}H_{26}O$	222	0.1
17	15	Oxiranedodecanoic acid, 3-octyl-, cis	$C_{22}H_{42}O_3$	354	0.14
18	18.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	**2.91**
19	19.64	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.66
20	20.13	Oleic Acid	$C_{18}H_{34}O_2$	282	**9.3**
21	20.93	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	0.94
22	21.64	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	0.34
23	22.33	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	**3.9**
24	23.11	Tetracosanoic acid, 3-oxo-, methyl ester	$C_{25}H_{48}O_3$	396	**1.12**
25	24.86	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502	0.42
26	25.63	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	0.63
27	26.06	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	**1.33**
28	26.51	9,19-Cyclochloestene-3,7-diol, 4,14-dimethyl-, 3-acetate	$C_{31}H_{52}O_3$	472	**1.32**
		Total			**95.68**

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Identification of phytochemicals in genotype 6 (Citrus-6)

The results of different analyzed parameters in the sample are presented in *Table 6*, whereas *Figure 4* shows the GC-MS chromatogram of genotype 6 with different phytochemicals. The result showed that 28 phytochemicals were identified with a Percent area of 99.19% of the total compounds.

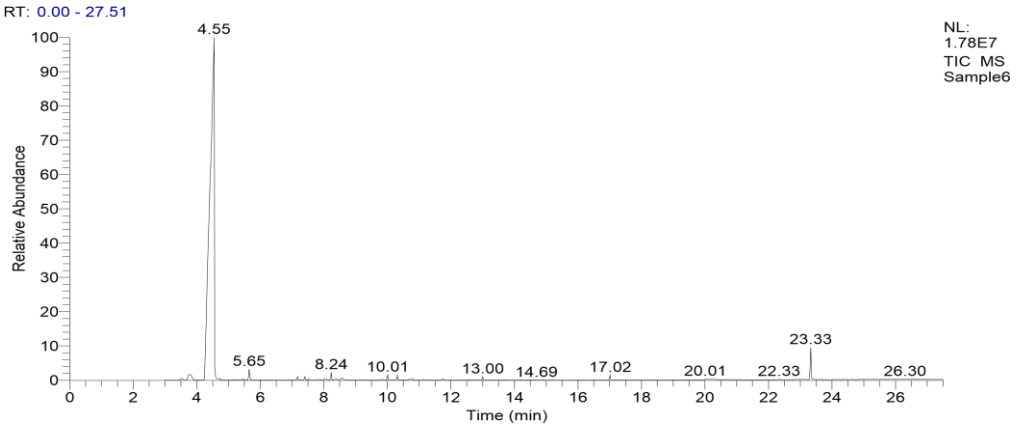

Figure 4. *GC/MS chromatogram of phytochemical compounds in genotype 6 (Citrus-6)*

Table 6. *Composition of C. aurantium fruit peel essential oil in genotype 6 (Citrus-6)*

S. No	RT	Compound	Formula	Mol weight	Area %
1	3.8	á-Pinene	$C_{10}H_{16}$	136	**1.56**
2	4.49	Limonene	$C_{10}H_{16}$	136	**91.44**
3	5.65	1,6-Octadien-3-ol, 3,7-dimethyl	$C_{10}H_{18}O$	154	0.9
4	6.24	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	$C_{10}H_{18}O_2$	170	0.01
5	7.18	3-Cyclohexene-1-methanol, à,à4-trimethyl	$C_{10}H_{18}O$	154	0.26
6	7.4	Decanal	$C_{10}H_{20}O$	156	0.27
7	8.24	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	$C_{17}H_{23}NO_2$	273	0.73
8	8.56	2-Dodecenal, (E)-	$C_{12}H_{22}O$	182	0.33
9	9.09	9,12-Tetradecadien-1-ol, acetate, (Z,E)-	$C_{16}H_{28}O_2$	252	0.02
10	10.01	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$C_{12}H_{20}O_2$	196	0.38
11	10.31	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)-	$C_{12}H_{20}O_2$	196	0.26
12	10.76	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	204	0.17
13	11.11	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, acetate, (1R-cis)-	$C_{12}H_{18}O_2$	194	0.03
14	11.31	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	$C_{16}H_{26}O_2$	250	0.01
15	12.55	12-Methyl-oxa-cyclododec-6-en-2-one	$C_{12}H_{20}O_2$	196	0.05
16	13	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	222	0.26
17	13.69	á-Himachalenoxide	$C_{15}H_{24}O$	220	0.04
18	18.24	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.04
19	18.97	3-Bromomethyl-2-(toluene-4-sulfonylamino)-pent-4-enoic acid, ethyl ester	$C_{15}H_{20}BrNO_4S$	389	0.04
20	19.48	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.01
21	19.7	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	256	0.01
22	20.09	Oleic Acid	$C_{18}H_{34}O_2$	282	0.35
23	21.07	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	0.01
24	21.92	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444	0.02
25	22.33	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	487	0.03
26	23.33	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	**1.79**
27	24.94	7,8-Epoxylanostan-11-ol, 3-acetoxy	$C_{32}H_{54}O_4$	502	0.02
28	25.69	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	496	0.15
		Total			**99.19**

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Identification of phytochemical compounds in genotype 7 (Citrus-7) through GC-MS

The detailed results of the GC-MS analysis of genotype 7 (Citrus-7) are tabulated in *Table 7* in which the identified compounds with their retention time (RT), compound name (Compound), molecular formula (Formula), molecular weight (Mol weight) and area (%), the GC-MS chromatogram of the phytochemicals are given in *Figure 5*. The result showed that 23 phytochemical compounds were identified with a Percent area of 85.69% of the total compounds.

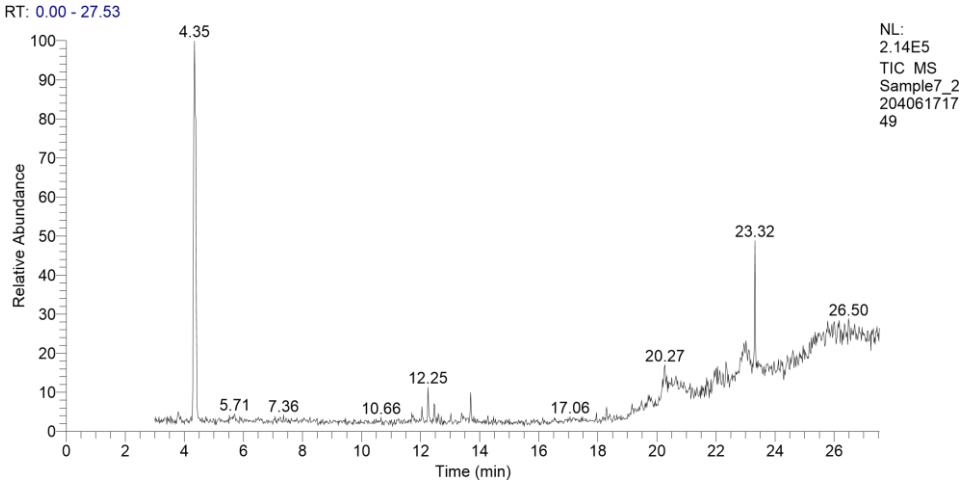

Figure 5. *GC/MS chromatogram of phytochemical compounds in genotype 7 (Citrus-7)*

Table 7. *Composition of C. aurantium fruit peel essential oil in genotype 7 (Citrus-7)*

S. No	RT	Compound	Formula	Mol weight	Area %
1	3.82	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	$C_{23}H_{34}O_2$	342	0.53
2	4.35	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate	$C_{12}H_{20}O_2$	196	**31.64**
3	5.2	(5á)Pregnane-3,20á-diol, 14à,18à-[4-methyl-3-oxo-(1-oxa-4-azabutane-1,4-diyl)]-, diacetate	$C_{28}H_{43}NO_6$	489	0.21
4	5.67	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	$C_{30}H_{52}O_3Si$	488	**1.38**
5	6.5	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444	0.43
6	7.77	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.33
7	11.23	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy	$C_{30}H_{52}O_2$	444	0.14
8	11.74	á-Guaiene	$C_{15}H_{24}$	204	0.59
9	12.25	9-Methyl-S-octahydroanthracene	$C_{15}H_{20}$	200	**3.26**
10	13	9-Hexadecenoic acid, hexadecyl ester, (Z)-	$C_{32}H_{62}O_2$	478	0.28
11	13.69	á-Himachalenoxide	$C_{15}H_{24}O$	220	**2.45**
12	15.85	Cyclohexane, 1,1'-dodecylidenebis[4-methyl	$C_{26}H_{50}$	362	0.31
13	17.18	Glycine, N-[(3à,5á,12à)-3,12-dihydroxy-24-oxocholan-24-yl]-	$C_{26}H_{43}NO_5$	449	0.86
14	19.17	Lamotrigine	$C_9H_7Cl_2N_5$	255	**1**
15	20.27	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	**7.14**
16	20.62	9-Octadecenoic acid (Z)-, tetradecyl ester	$C_{32}H_{62}O_2$	478	**5.88**
17	22.36	Pregn-4-ene-3,20-dione, 11-hydroxy-, (11à)-	$C_{21}H_{30}O_3$	330	0.78
18	22.99	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444	**8**
19	23.32	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	**5.15**
20	23.77	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	$C_{36}H_{72}O_3$	552	0.48
21	24.58	Rhodopin	$C_{40}H_{58}O$	554	**1.13**
22	25.8	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4S_{i2}$	496	**8.19**
23	26.5	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	**5.53**
		Total			**85.69**

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Identification of phytochemicals in genotype 8 (Citrus-8)

The detailed result of the GC-MS analysis of genotype 8 (Citrus-8) are tabulated in *Table 8* with different analyzed parameters, the GC-MS chromatogram of the phytochemicals are given in *Figure 6*. The result showed that 26 phytochemical compounds were identified with a Percent area of 99.31% of the total compounds.

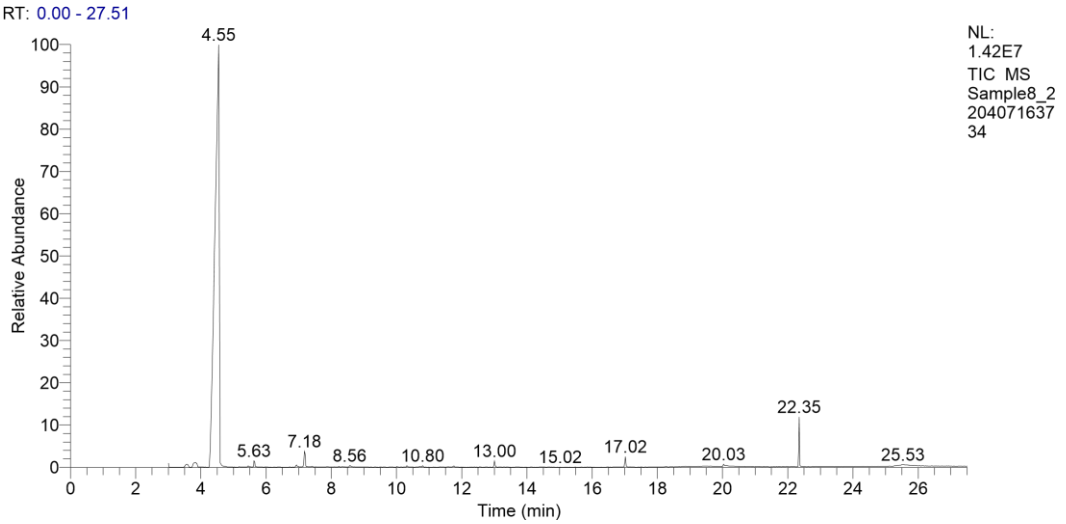

Figure 6. *GC/MS chromatogram of phytochemical compounds in genotype 8 (Citrus-8)*

Table 8. *Composition of C. aurantium fruit peel essential oil in genotype 8 (Citrus-8)*

S. No	RT	Compound	Formula	Mol weight	Area %
1	3.82	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	$C_{10}H_{16}$	136	**1.28**
2	4.49	D-Limonene	$C_{10}H_{16}$	136	**90.85**
3	5.63	1,6-Octadien-3-ol, 3,7-dimethyl	$C_{10}H_{18}O$	154	0.54
4	7.18	3-Cyclohexene-1-methanol, à,à4-trimethyl	$C_{10}H_{18}O$	154	**1.31**
5	7.89	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (E)-(ñ)-	$C_{15}H_{28}O$	224	0.02
6	8.58	1-Decanol	$C_{10}H_{22}O$	158	0.2
7	10.03	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	$C_{12}H_{20}O_2$	196	0.04
8	10.33	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{12}H_{20}O_2$	196	0.12
9	10.78	Caryophyllene	$C_{15}H_{24}$	204	0.13
10	11.76	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	$C_{15}H_{24}$	204	0.16
11	12.55	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.01
12	12.74	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	400	0.01
13	13	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	222	0.3
14	13.39	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C2_0H_{38}O_2$	310	0.01
15	14.45	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	$C_{15}H_{24}$	204	0.04
18	18.26	Olean-12-ene-3,28-diol, (3á)-	$C_{30}H_{50}O_2$	442	0.02
19	18.87	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.22
20	19.5	Stigmastan-7-one	$C_{29}H_{50}O$	414	0.47
21	20.07	Osthole	$C_{15}H_{16}O_3$	244	0.6
22	22.35	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	**2**
23	22.84	9-Octadecenoic acid (Z)-, tetradecyl ester	$C_{32}H_{62}O_2$	478	0.02
24	23.11	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z, Z, Z)-	$C_{27}H_{52}O_4Si_2$	496	0.03
25	25.55	à-Pyridone, 5-methyl-1,3,4,6-tetraphenyl	$C_{30}H_{23}NO$	413	0.91
26	26.51	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	0.02
		Total			**99.31**

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DPPH assay

During the current of 18 *C. aurantium* L. genotypes, selected genotypes Citrus-5, Citrus-6, Citrus-7, and Citrus-8, and the chemical investigation of the essential oils for genetic polymorphism were investigated through the DPPH radical-scavenging technique. *C. aurantium* L. fruit peels essential oils showed weak DPPH radicals scavenging effects. The highest DPPH scavenging capacity of essential oils was noted for Citrus-8 (43.63%) with IC_{50} value of 72 µL/mL at a concentration of 100 µL/mL, other concentrations of the same sample (Citrus-8) showed relatively less scavenging effects, at 50 µL/mL concentration (40.31%), at 25 µL/mL (36.33%), at 12.5 µL/mL (31.03%) and 6.25 µL/mL concentration the scavenging effects was (24.4%) while the IC_{50} was 72 µL/mL, followed by Citrus-7 (36%) of scavenging effects with IC_{50} value of 70 µL/mL at a concentration of 100 µL/mL, other concentrations of the same sample (Citrus-7) showed relatively less scavenging effects, at 50 µL/mL concentration (32.36%), at 25 µL/mL (29.04%), at 12.5 µL/mL (25.06%) and 6.25 µL/mL concentration the scavenging effects was (19.09%) while the IC_{50} was 70 µL/mL. However essential oils of Cit-5 showed 25.99% scavenging capability at 100 µL/mL, 22.41% at 50 µL/mL, 18.43% at 25 µL/mL, 15.78% at 12.5 µL/mL, and 10.47% at 6.25 µL/mL concentration, and the IC_{50} value was 72 µL/mL at a concentration of 100 µL/mL. The % inhibition of the Citrus-6 essential oils was recorded as 21.48% at 100 µL/mL, 19.09% at 50 µL/mL, 16.44% at 25 µL/mL, 13.12% at 12.5 µL/mL, and 9.15 at 6.25 µL/mL concentration, while the IC_{50} value was 75 µL/mL at a concentration of 100 µL/mL. The % inhibition for the standard ascorbic acid was 94.96% at 100 µL/mL, 87.40% at 50 µL/mL, 76.12 at 25 µL/mL, 65.51 at 12.5 µL/mL, and 50.92 at 6.25 µL/mL concentration and the IC_{50} value was 13% at the greatest concentration range of 100 µL/mL. The IC50 values of the tested essential oils from four genotypes of *C. aurantium* L. were much higher than the control ascorbic acid (*Table 9*).

Discussion

Different agronomic traits have been used as a prime objective to evolve new breeding materials (Ihsan et al., 2022). The initial stage in evaluating and categorizing diverse crops' germplasm is to conduct an agro morphological study (Ihsan et al., 2024; Sunil et al., 2013). For all plant breeders, the evaluation and characterization of distinct crop germplasm using morphological and phenotypic parameters are very important (Huang et al., 2022; Martins et al., 2008). That is why it is critical to develop effective techniques for collecting and evaluating germplasm resources that are locally used in various parts of the world to prevent them from becoming extinct (Balkaya et al., 2008). Looking into the genomic diversity of crop germplasm, researching every aspect of it, and storing it for future breeding programs is the order of the day (Latini et al., 2007). It is vital to improving the maintenance of the current diversity of various beneficial crops through characterization and evaluation. Morphological characters may be inexpensive to evaluate, but they are also highly flexible, varying according to growing conditions (Richards et al., 2002).

Citrus aurantium L. is one of the most important crops economically, the essential oil is used as a food flavoring agent, in skin care products and medicines. Scientists are always on the lookout for the best genotypes to develop cultivars for farmer fields. In the present study, a significant variation was found in the length of leaf lamina, the coefficient of variation for the trait was 20.49%. The leaf lamina coefficient of variation was 16.45%. Fruit diameter is a very important character, and is used by humans for

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various purposes. This character was observed from 63 mm to 78 mm, with the coefficient of variation for the trait being 6.59%. A considerable variation was found for the trait of fruit length, ranging from 64 mm to 80 mm, with a coefficient of variation of 6.83%. Fruit volume was observed from 130.53 cm^3 to 248 cm^3, with a mean value of 179.55 cm^3. The number of segments in various studied *Citrus* genotypes ranged from 7 to 11 segments per genotype, coefficient of variation for the trait was 14.08%. Our study is comparable with that of Jaskani et al. (2006), where the authors observed different morphological traits and found significant variation for most of the traits. Similarly, the studies of Gupta et al. (2020) on sweet oranges (*C. sinesis)* showed little fluctuation in our studied parameters. Further support to our results was given by the results of Ait-Mimoune et al. (2022), during their study of mandarin *(C. reticulata)*.

Table 9. *Percent DPPH free radical scavenging activity of methanolic extract of Citrus aurantium L selected genotypes*

Sample code/name	Concentration (µL/mL)	Scavenging DPPH %	IC_{50} (µL/mL)
Genotype 5 (Citrus-5)	100	25.99	72
	50	22.41	
	25	18.43	
	12.5	15.78	
	6.25	10.47	
Genotype 6 (Citrus-6)	100	21.48	75
	50	19.09	
	25	16.44	
	12.5	13.12	
	6.25	9.15	
Genotype 7 (Citrus-7)	100	36.00	70
	50	32.36	
	25	29.04	
	12.5	25.06	
	6.25	19.09	
Genotype 8 (Citrus-8)	100	43.63	72
	50	40.31	
	25	36.33	
	12.5	31.03	
	6.25	24.4	
Ascorbic acid (Standard) (Vit-C)	100	94.96	13
	50	87.40	
	25	76.12	
	12.5	65.51	
	6.25	50.92	

Among the studied parameters, a diverse correlation was found for leaf lamina width with leaf lamina length, fruit length with fruit diameter, fruit weight with fruit diameter, and fruit length, similarly fruit volume was found to correlate with fruit diameter, fruit length, and fruit weight. Koehler-Santos et al. (2003) found similar results in mandarins

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where they observed a significant correlation between the traits of leaf lamina and fruit diameter. Principal Component Analysis aids in the evaluation of diversity on multivariate scales, the current study suggested a total 98.469% cumulative variance among the 18 *Citrus aurantium* L. genotypes. The first principal component was found to have a 56.455% variation, in PC2 the total variation was 80.193%. The contribution of PC3 with a total variation of 92.512% while in PC4 the total variation was 98.469%. Tapia Campos et al. (2005) recorded 56.1% of the total variability for the five Principal Component Analyses. Further support to our studies was given by the studies of Derso et al. (2007) where they recorded similar results in the Mexican *Citrus aurantifolia*.

During the present investigation, a cluster dendrogram was constructed for 7 quantitative traits. All the genotypes were divided into two basic groups at a distance of 25% and further subdivided into four groups at 62.5%. Each group shows less differences from one another but high variation to other groups. The first group contains 5 genotypes namely Citrus-1, Citrus-5, Citrus-17, Citrus-12, and Citrus-18. The second group comprised 5 genotypes namely Citrus-2, Citrus-6, Citrus-13, Citrus-4, and Citrus-8. The third group has 3 genotypes namely Citrus-3, Citrus-7, and Citrus-11. Similarly, group 4 is also composed of 5 genotypes namely Citrus-9, Citrus-15, Citrus10, Citrus-16, and Citrus-14. Based on the cluster dendrogram, Citrus-1 and Citrus-14 were the most diverse based on morphological characterization and are placed at the extreme periphery of the dendrogram. Budiarto et al. (2021) found similar results in the morphological evaluation of different *Citrus* species on Java Island, Indonesia. Lombardo et al. (2012) also recorded similar results in the genetic analysis of *C. aurantium.*

During the present study, the GC-MS technique was used to investigate the bioactive compounds/phytochemical compounds in the essential oils of *C. aurantium* L. A total of 28 phytochemical compounds were identified in genotype 5 (Citrus-5) with a percent area of 95.68% of the total compounds. In genotype 6 (Citrus-6) a total of 28 phytochemical compounds were detected with a Percent area of 99.19% of the total compounds. In genotype 7 (Citrus-7) 23 phytochemical compounds were identified with a Percent area of 85.69% of the total compounds in which Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate have the highest area (31.64%). A total of 26 phytochemical compounds were identified in the essential oils of genotype 8 (Citrus-8) with a Percent area of 99.31% of the total compounds in which D-Limonene has the highest area (90.85%). In the study of Chaieb et al. (2018), 29 phytochemical compounds were reported with a percent area of 98.76% of the total compounds in Tunisian *C. aurantium* fruit peel's essential oils. Their studies indicated that Limonene (67.1%) is the major compound followed by Linalool (8.37%), β-pinene (4.02%), Myrcene (3.17%), β-Ocimene (2.36%), and α-pinene (1.18%). Odeh et al. (2021) identified that Limonene (96-97.7%), followed by α-pinene (0.35-1%) and β-myrcene (0.9-1.4%) in the essential oils of Syrian *C. aurantium* peels. In the studied genotypes, the composition of essentials oils showed significant variation, especially in sample 5 (Citrus-5) the major phytochemical compounds were Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)- with 64.26% area, and in genotype 7 (Citrus-7) the major compound was Cyclohexanol, 1-methyl-4-(1-methyl phenyl)-, acetate with an area of 31.64%. The result of genotype 8 (Citrus-8) is similar to that of Bendaha et al. (2016) where studies reported (88.07-92.62%) D-Limonene in the essential oils of *C. aurantium* fruit peels grown in Morocco. According to the studies of Hassiotis et al. (2010) and Sangwan et al. (2001), the environmental conditions and time of collection can affect the composition and yield of essential oils. Therefore, the differences in composition among the samples

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may be due to the differences in genetic makeup, variation in environmental cultivation conditions, or timing of sample collection.

Antioxidants play an important role in health by combating the reactive oxygen species, which play a major role in many disease processes, including atherosclerosis, coronary heart diseases, insulin resistance, and cardiovascular diseases. Butylated hydroxyl toluene and butylated hydroxyl anisole are strong synthetic antioxidants, but they are carcinogenic and toxic in animals. Therefore, plant-based phenolic compounds can be used as antioxidants to scavenge or stabilize self-ruling free radicals involved in oxidative stress. Therefore, the antioxidants in plants have become a hotspot for research (Nazir et al., 2018). During the present study, the *C. aurantium* L. fruit peels essential oils showed weak DPPH radical scavenging effects. The highest DPPH scavenging capacity of essential oils was noted for Citrus-8 (43.63%) with IC_{50} value of 72 μL/mL at a concentration of 100 μL/mL, other concentrations of the same sample (Citrus-8) showed relatively less scavenging effects. Citrus-7 has 36% of scavenging effects with IC_{50} value of 70 μL/mL at a concentration of 100 μL/mL. The % inhibition of the Citrus-6 essential oils was recorded as 21.48% at 100 μL/mL, while the IC_{50} value was 75 μL/mL at a concentration of 100 μL/mL. Marzouk et al. (2013) reported that *C. aurantium* L. has less effective antioxidant capacity than those standard antioxidants. Our report regarding % inhibition was similar to the results of Bendaha et al. (2016) their research showed that the scavenging capability of 5 genotypes of *C. aurantium* essential oils ranged from 7-15%. Furthermore, our results were also supported by the findings of (Choi et al., 2017), where 34 kinds of *citrus* oils from Japan, Korea, and Italy possess weak DPPH scavenging capability ranging from 12-17.7%.

Conclusion

In the present study, genotypes of 18 *C. aurantium* were evaluated morphologically and biochemically. Valuable diversity was found among the genotypes for various parameters, including leaf lamina width, leaf lamina length, fruit diameter, fruit weight, fruit length, and fruit volume. A considerable correlation was also observed for the majority of genotypes. The chemical composition of selected four contrasting genotypes (Citrus-5, Citrus-6, Citrus-7, and Citrus-8) of *C. aurantium* fruits peel's essential oils were also analyzed. All the essential oils showed different chemical compositions due to differences in genetic makeup, however 9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester, (Z, Z, Z)- was observed in all the four essential oils in varying amount. Furthermore, all the essential oils were observed, to have scavenging capability, and it was confirmed by DPPH assay, when the essential oils were examined for antioxidant activity, it showed little DPPH radical scavenging effects. The highest DPPH-scavenging capability of essential oils was noted for Cit-8 (43.63%), and the lowest % inhibition was observed in Cit-6 of (21.48%) scavenging effects. The findings presented in this study may prove useful in the future for the detailed characterization of *C. aurantium* genotypes that would be important for future breeding and *C. aurantium* improvement programs.

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Conflicts of interests. The authors declare no conflict of interests.

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APPENDIX

Table A1. *Passport information of studied genotypes Citrus aurantium L used in present study*

S. No	Genotype#	Name	Scientific name	Origin
1	Genotype-1	Citrus-01	*Citrus aurantium* L	KPK Pakistan
2	Genotype-2	Citrus-02	*Citrus aurantium* L	KPK Pakistan
3	Genotype-3	Citrus-03	*Citrus aurantium* L	KPK Pakistan
4	Genotype-4	Citrus-04	*Citrus aurantium* L	KPK Pakistan
5	Genotype-5	Citrus-05	*Citrus aurantium* L	KPK Pakistan
6	Genotype-6	Citrus-06	*Citrus aurantium* L	KPK Pakistan
7	Genotype-7	Citrus-07	*Citrus aurantium* L	KPK Pakistan
8	Genotype-8	Citrus-08	*Citrus aurantium* L	KPK Pakistan
9	Genotype-9	Citrus-09	*Citrus aurantium* L	KPK Pakistan
10	Genotype-10	Citrus-10	*Citrus aurantium* L	KPK Pakistan
11	Genotype-11	Citrus-11	*Citrus aurantium* L	KPK Pakistan
12	Genotype-12	Citrus-12	*Citrus aurantium* L	KPK Pakistan

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13	Genotype-13	Citrus-13	*Citrus aurantium* L	KPK Pakistan
14	Genotype-14	Citrus-14	*Citrus aurantium* L	KPK Pakistan
15	Genotype-15	Citrus-15	*Citrus aurantium* L	KPK Pakistan
16	Genotype-16	Citrus-16	*Citrus aurantium* L	KPK Pakistan
17	Genotype-17	Citrus-17	*Citrus aurantium* L	KPK Pakistan
18	Genotype-18	Citrus-18	*Citrus aurantium* L	KPK Pakistan

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