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TOPOGRAPHICALLOCATION OF ORCHARDS MODULATES THE BIOCHEMICAL COMPOSITION OF ESSENTIAL OILS IN CITRUS FRUIT PEEL

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Citrus peel is an important source of essential oil that is used in various fields such as cosmetics, perfumes, backers and medicine. The citrus peel essential oils have diversification in chemical constituents. The aim of this experiment was to observe the effect of topographical locality of orchards on composition of essential oil in citrus peel. Fruit of three citrus cultivars named as *Citrus paradisi* (Shamber grapefruit), *Citrus reticulata* (Kinnow mandarin) and *Citrus sinensis* (Musambi sweet orange) were collected from five different localities i.e. Rahim Yar Khan Layyah, Faisalabad, Sargodha and Abbottabad (Pakistan). The citrus peel essential oil extracted from steam distillation methods at Rosa Oil Extraction Plant and physio-chemical characteristics were calculated through the course of study. A noteworthy variation in bioactive compounds availability in essential oils of citrus peel were found recorded geographical locations as well as among the cultivars. Maximum oil percentage (0.321%) was measured in Shamber from Rahim Yar Khan and minimum (0.271%) was seen in Musambi fruit collected from Abbottabad. Maximum oil density (0.834 mg cm^{-3}) was noted in Shamber and Musambi peel essential oils that showed the similar values and minimum oil density of 0.822 mg cm^{-3} noted in Shamber from Abbottabad location. Limonene was the major part of citrus peel essential oils that was found in higher concentrations in all three citrus cultivars of five different localities. Layyah region observed to produce maximum peel essential oils in all citrus cultivars. All the chemical compounds except limonene showed positive correlation with the elevation while limonene showed negative correlation with elevation. Four compounds have statistically negatively correlation with maximum temperature for three citrus cultivars. In conclusion, the citrus peel essential oil has a higher amount of bioactive compounds. This composition is closely associated with the varietal and seasonal characteristics. These findings disclose that the production of essential oils of Citrus can be high value in future for satisfying local needs. As the consumption of aromatic oils is on an increasing line day by day.
Keywords: Citrus, bioactive composition, climatic conditions, essential oils, GC-MS

INTRODUCTION

Essential oil is one of the citrus by-products enticing keen interest of people (Njoroge, 2005). Fruit peel, flowers and leaves of citrus are used for the medicinal purpose (Moraes *et al.,* 2009). Citrus processing industries left a large quantity of peels, seeds and pulps of citrus after juice extraction that can be used as a likely source of valuable by-products (Silalahi, 2002; Saidani *et al*., 2004). In recent years, the citrus essential oil has been identified in different parts of fruits (preferably in fruit flavedo) as well as in leaves, showing that dl-limonene, β-myrcene, α-pinene, sabinene, Δ-3-carene, α-terpinolene and other elements are the major aromatic compounds of many citrus species (Stashenko *et al.,* 1996; Caccioni, 1998; Lota *et al*., 2001, Minh *et al.,* 2002). These aromatic compounds are relatively inexpensive and abundant raw materials with applications in flavor and food industries (Reische *et al*., 1998). They can also serve as an excellent starting material in the synthesis of fine chemicals and of new fragrances for the cosmetic industry (Lis and Hart, 1999).

The peel and leaf of citrus fruits is a potential source of essential oils (Braddock, 1999) and yield essential oil in the range of 0.50-3.0 kg ton^{-1} (Sattar and Mahmud, 1986). Essential oils sometimes called volatile oil are concentrated aromatic compounds (Arce *et al.*, 2007; Lucchesi *et al.*, 2004) that represent a small fraction of plant's composition which can be found mostly in leaves and flowers (Roldan-Gutierrez *et al*., 2008; Bousbia *et al*., 2009). These oils have natural antioxidant and antimicrobial properties (Tepe *et al*., 2005; Jayaprakasha *et al*., 2008; Viuda-Martos *et al*., 2008). Citrus peels which are considered as agro industrial waste are potential source of plant secondary metabolites in the form of essential oils (Andrea *et al*., 2003). These essential oils have a wide range of potential activities in food, perfumery, sanitary, cosmetics and pharmaceutics (Mondello *et al.*, 2005). The important application of citrus peel essential oils is due to some bioactive compounds in them which serve as alternatives to the synthetic antioxidants (Tepe *et al*., 2006; Viuda- Martos *et al*., 2008; Choi *et al.,* 2000).

Valuable phenolic compounds are available in citrus peel essential oil that are able to change the food spoilage extents of citrus peel essential oil may be used as antioxidants (Anagnostopoulou *et al.,* 2006). Due to high source of flavonoids, terpenes, coumarinsand carotenes that are antimicrobial, citrus peel essential oils have much medicinal importance (Tepe *et al.,* 2005). These oils are widely used in medicines asl, anti-diabetic, insect repellent, antioxidant, carminative, antimicrobial, antimutagenic and antihepatotoxic agent. In pharmaceutical industries they are employed as flavoring agents to mask unpleasant tastes of drugs (Kanaze *et al*., 2008). Essential oils are being consumed by retail users beside the artificial produced items and they do not produce bacterial resistance on the body skin as these oils have large range spectra (Maggi *et al*., 2009).

The chemical compositions of the essential oils is affected by several factors such as humidity, soil conditions, temperature and seasonality, causing them to be taken into consideration to choose the best time to obtain oil with the amount of the substance of interest (Evergetis *et al*., 2016; Kiazolu *et al*., 2016; Almeida *et al*., 2016; Sarrazin *et al*., 2015). Patel *et al*. (2016) revealed the difference between the essential oil contents and oil yield percentage of the essential oil extracted from five Ocimum species from two different locations of India. Environment factors diversified the oil components. The significant quantitative changes were observed in essential oils compounds nature from *H. italicum* species i.e. monoterpenes, sesquiterpenes, alcohols, esters in the plants collected from various climatic regions. However, no such type of study was reported in the past on these three citrus cultivars (Shamber, Kinnow and Musambi) regarding their physical and biochemical composition of citrus peel essential oils in relation to various geographical locations in Pakistan. Therefore, in the current study the physical and bioactive profiling of three citrus cultivars grown in Rahim Yar Khan Layyah, Faisalabad, Sargodha and Abbotabad regions of Pakistan have been investigated and metrological data of these districts have been given in Table 1.

Table 1. Metrological data about five different citrus growing regions of Pakistan.

Region	Altitude (m)	Temperature (°C)*	Rainfall (mm)*
Rahim Yar Khan	82.93	26.20	101
Layyah	143.00	25.20	195
Faisalabad	185.60	23.20	346
Sargodha	187.00	22.80	410
Abbotabad	1308.00	16.70	1145

Source: Climate Data Processing Centre, Pakistan Metrological Department, Islamabad, Pakistan (2005-15). *Means annual average.

MATERIALS AND METHODS

***Plant material*: The fresh fruit of three citrus cultivars viz. *Citrus paradisi* (Shamber grapefruit), *Citrus reticulata* (Kinnow mandarin) and *Citrus sinensis* (Musambi sweet orange) were collected from five different locations having different climatic conditions i.e. Rahim Yar Khan Layyah, Faisalabad, Sargodha and Abbottabad regions of Pakistan (Table 1). The harvested fruit immediately brought at Rosa Oil Extraction Laboratory, IHS University of Agriculture, Faisalabad, Pakistan for peel essential oil extraction. The obtained citrus peel oil samples were weighed and preserved in glass vessels at 4°C until analysis.

***Peel oil extraction method*:** All the collected fruits were peeled off for oil extraction. The peel was cleaned (free from any defects, debris or any other inert matter). The collected fruit peel and weighed carefully. Steam distillation process was adopted for extraction of peel essential oil. A sample of 12.5 kg peel was used for each run in steam distillation unit. The peel sample was placed in the steam chamber and was tightly closed. In this method the mixture of two immiscible liquids was heated while stirring to expose the surface of each liquid to the gas phase. Each component independently exerts its own vapor pressure as a function of temperature, as if the other components did not exist. As a result, the vapor pressure of the entire system increases. When the sum of the vapor pressures of the two immiscible liquids just exceeds the atmospheric pressure (sea level, about 101 kPa), boiling begins. In this way, many water-insoluble organic compounds are purified much below the point where the decomposition takes place. The steam chamber temperature was set at 105°C.

***Essential oil percentage*:** The citrus peel oil percentage was measured by weighing the extracted oil just after the oil recovery. It was calculated by analytical weighing balance and percentage was resulted by using the equation

$$\text{Peel essential oil \%} = \text{Oil weight} / \text{Peel weight} \times 100$$

***Density of essential oils*:** The density tube is a container with an exact known volume. Although it is used to determine the density or specific gravity, it measures the volume. The specific gravity tube or the density tube was filled one by one, leaving no bubbles in the vessel and then weighed. The oil density was calculated as using following formula.

$$\text{Density } (\rho) = m/V$$

***Measurement of bioactive composition*:** The extracted essential oils were subjected to the chemical analysis by GC-MS. Gas Chromatography separated the chemical compounds of the extracted essential oils while the Mass Spectrometer identified these compounds by their structures.

***Gas Chromatography Mass Spectrometry* (GC-MS):** The qualitative exploration of citrus peel essential oils was performed by the GC-MS using Agilent technologies 6890N-5975B system. Capillary column was at 30m×0.25µm. Temperature was programmed at 50°C to 280°C. Helium was used as the carrier. It was set a constant scan mode wit electron energy of 70eV. Mass range was 35-400 Da while the quadruple temperature was 150°C and source temperature was 230°C. Acquired data were analyzed by Agilent Chem

Station along with a paired NIST MS search software and AMIDIS (Automated Mass Spectral Deconvolution and Identification System). The finally the identification was made possible by the comparison of their relative retention indices and retention times with recorded values (Adams, 1995; Vagionas *et al*., 2007; Grbovic *et al*., 2010)

***Statistical analysis*:** Principal component analysis (PCA) and cluster analysis techniques were used for statistical analysis of the resulting data. PCA is one of the most important results of applying linear algebra, and perhaps the most common use is to try the first step in analyzing large data sets. After the PCA, the data were clustered. Clustering analysis has developed tools and methods for data matrices containing multivariate measurements of a large number of individuals (or objects), with the aim of building some natural subgroups or individual clusters. This is done by grouping "similar" individuals by some appropriate criteria. Once clustered, it is often useful to use some descriptive tools to describe each group (Hardle and Leopold, 2007).

RESULTS AND DISCUSSION

***Citrus essential oil percentage*:** The percentage of oil showed significant differences in three cultivars i.e., Shamber, Kinnow and Musambi in all selected locations. Maximum oil percentage was recorded in Shamber in Layyah region which was significantly different from Kinnow and Musambi. The Shamber oil percentage gradually decreased in Rahim Yar Khan, Faisalabad, Sargodha and Abbotabad, respectively. The oil percentage of Kinnow and Musambi was found maximum in Rahim Yar Khan followed by Layyah, Faisalabad, Sargodha and Abbotabad. Musambi oil percentage in Abbotabad was significantly less than other four districts.

***Essential oil density* (*mg cm^{-3}*):** The oil of all the essential oils in all three cultivars was different at all regions. Maximum oil density (0.834 mg cm^{-3}) was recorded in Shamber peel essential oil in Rahim Yar Khan and minimum (0.829 mg cm^{-3}) was recorded in the Shamber peel oil from Abbotabad. In Kinnow peel essential oil, maximum oil density of 0.834 mg cm^{-3} was recorded in Rahim Yar Khan and minimum was recorded in the Shamber peel oil from Abbotabad (0.827%). Maximum oil density in Musambi peel essential oil was recorded in Rahim Yar Khan (0.833%) and minimum was recorded in the Shamber peel oil from Abbotabad (0.822%).

***Identification and composition of essential oil compounds*:** Essential oil composition of chemical compounds identified in three citrus cultivars from five localities showed significant results (Table 2). The results showed that 26 compounds were identified in Shamber from Abbottabad region followed by Kinnow and Musambi where the numbers of identified compounds were 17 and 18, respectively. Limonene was the major chemical constituent found in all three citrus cultivars collected from five different localities (Table 2). In Faisalabad region, the number of identified compounds were 18, 12 and 19 from Shamber, Kinnow and Musambi, respectively. In Layyah, the numbers of identified chemical compounds were13, 14 and 13 in Shamber, Kinnow and Musambi, respectively. The numbers of isolated compounds in Rahim Yar Khan were 15, 14 and 15 in Shamber, Kinnow and Musambi, respectively. Similarly, in Sargodha, 15, 15 and 9 chemical compounds were isolated from Shamber, Kinnow and Musambi in respective manner (Table 2).

***Correlation of environmental factors with compounds in citrus peel essential oils*:** The cultivars from the climates with high rain fall and low average temperature resulted in more number of chemical components in their essential oils. Limonene, the major part of the citrus peel essential oils was negatively correlated with elevation. Many of the compounds were negatively correlated with average temperature e.g. ß-Citronellol, Elemol, α-Selinene, 4-terpinenol, 4, 11-Selinadiene, Carvyol acetate (z), Citronellol, Decanal, E-Nerolidol, Farnesol, Farnesyl acetate, Globulol, Linalool, Menthol and Perillaldehyde. Similarly, compounds were directly related to the wind speed and average rainfall.

***Principal component analysis*:** In the case of percentage of different compounds in essential oils extracted from three citrus cultivars collected from different localities, four PCs showed eigen values greater than one (significant) (Table 3). The other PCs exhibited non-significant variation and were not worth interpreting. The first PC showed cumulative variability of 42.67%, second PC showed 9.61 variability, third PC showed 8.10% variability and fourth PC showed 7.59% variability in different compounds percentages found in essentials oils extracted from three citrus cultivars collected from different localities (Fig.1). The method established by Johnson and Wichern (1988) was used to estimate the importance of a trait coefficient for each significant principal component. The first PC was negatively related to three citrus cultivars collected from three localities except Musambi collected from districts Abottabad and Rahim Yar Khan and Kinnow collected from district Rahim Yar Khan which are not related to first PC (Table 4). The second PC is positively related to Musambi collected from districts Abbotabad and Rahim Yar Khanand the fourth PC is positively related to Kinnow collected from district Rahim Yar Khan (Table 4)

A principal component scatter plot of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities by using steam distillation method depicted that percentages of different compounds are close together are being similar when related with other percentages of compounds. The projection of percentages of different compounds on PC1 and PC2 showed the diverse nature. To identify the better transgressive pattern of percentages of compounds in dissimilar groups of pattern, the projection of percentages of compounds on first two principal components was useful.

Table 2. List of identified chemical compounds from peel essential oils of three citrus cultivars from different locations through.

Compounds (%)	Abbotabad			Faisalabad			Layyah			R.Y. Khan			Sargodha		
	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3	C1	C2	C3
(-) -α-Panasinsene	0	0	0.23	0	0	0	0	0	0	0	0	0.13	0	0	0
(-)α-Neoclovene	0.75	0	0	0.36	0	0	0	0	0	0	0	0	0	0	0
(z) – Carveol	0	0	0	0	0	0	0	0	0	0	0.65	0	0	0	0
α-caryophyllene	0.55	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-Citronellol	0	0	1.99	0	0	0	0	0	0	0	0	0	0	0	0
Elemene	0	0	0.26	0.28	0	0	0	0	0	0	0	0	0	0	0
Elemol	0.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-farnesol	0	0	0	0.63	0	0	0	0	0	0	0	0	0	0	0
α-gurjenene	0	0	0	0	0	0	0.12	0	0	0	0	0	0	0	0
ϒ-Muurolene	0	0	0	0	0	0	0	0	0.15	0	0	0	0	0	0
β-myrcene	0	0.71	0	0	0.67	0.87	0.9	0.64	0	0	0	0	0.59	0.65	0.71
α -phellenderene	0	0	0	0	0.08	0	0.13	0	0	0	0	0	0	0	0
α -Selinene	0	0	1.07	0	0	0	0	0	0	0	0	0	0	0	0
1-Decanol	0	0	0	0	0	0	0	0	0	0	0.87	0	0	0	0
2,6-Octadiene	0	0	0	0	0	0	0	0	0	0	0	0	0.18	0	0
4-terpinenol	0.36	0.24	0	0	0	0.14	0	0	0	0	0	0.23	0	0	0
4,11-Selinadiene	0	0	0.22	0	0	0	0	0	0	0	0	0	0	0	0
α-citral	0	0.17	0	0	0	0.16	0	0	0	0	0	0	0	0	0
α-pinene	0.32	0.22	0.34	0.43	0.31	0.39	1.46	0.43	0.58	0.52	0	0	0	0.39	0.15
Anethol	0	0.44	0	0	0	0	0	0	0	0	0.53	0	0	0	0
Aromadendrene	0	0	0	0	0	0.44	0	0	0	0	0	0.51	0	0	0
Carveol	0	0	0	0	0	0	0	0	0	0	0.24	0	0	0	0
Carvyol acetate (z)	0.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllene	0.66	0.14	0.16	1.7	1.23	0	2.68	0.51	0.47	5.73	0	0	1.5	0.34	0
Caryophyllene oxide	1.33	0	0	0	0	0	0	0	0	0	0.54	0	0	0	0
Cis-Carveol	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0
Cis, Trans-Farnesol	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0.13	0
Citronellal	0.88	0.24	0	0.56	0	0.34	0	0.14	0	0	0	0.23	0.54	0.54	0.15
Citronellol	0.37	0.33	0.62	0.19	0	0.33	0	0	0.21	0.12	0	0.39	0	0	0
Copaene	0.31	0	0	0	0	0	0.65	0.24	0.27	1.82	0.47	0	0.41	0.16	0
d-cadinene	0.39	0.39	0	1.41	1.02	0.24	2.33	0.41	0.33	1.14	0	0	1.49	0.33	0.1
D-Germacrene	0.18	0	0	0	0	0	0.14	0	0	0	0	0	0	0	0
Decanal	0.79	1.71	0	0.9	1	0.3	0.17	0.15	0.22	0.17	0	0	0.73	0.85	0.12
E-Nerolidol	0	0	0.78	0	0	0	0	0	0	0	0	0	0	0	0
Estragole	0	0	0	0	0.31	0	0	0	0	0	0	0	0	0.18	0
Farnesol	0	0	0.61	0	0	0	0	0	0	0	0	0	0	0	0
Farnesyl acetate	0.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Globulol	0.28	0.21	0	0	0.12	0	0	0	0	0	0	0	0	0	0
Heneicosane	1.51	0	0	0.17	0	0	0	0.44	0	0	0	0	0.37	0	0
Heptacosane	0.65	0	0	0.48	0	0	0	0	0	0	0	0	0.26	0.12	0
Hexahydrothymol	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0
Hexyl Hexanoate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iso-Caryophyllene	0	0	0	0	0	0.12	0	0	0	0	0	0	0	0	0
Juniper Camphor	0	0	0.27	0	0	0.14	0	0	0	0	0	0.63	0	0	0
Levoverbenone	0.41	0	0	0	0	0.16	0	0	0	2.13	0.41	0.22	0.39	0	0.4
Limonene	83.17	90.05	84.73	89.21	96.91	91.66	87.34	94.04	94.83	84.45	94.61	88.04	92.01	94.34	97.36
Limonene oxide	0	0.19	0	0	0	0.19	0	0	0	0	0	0	0	0	0
Limoneneglycol	0	0	0	0	0	0	0	0	0	0	0.24	0	0	0	0
Linalool	1.02	0.67	0.37	0.44	0.23	0.77	0	0.34	0.65	0.34	0.19	0.55	0.22	0.23	0.38
Menthadien-1-ol	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0
Menthol	0	0	0.19	0	0	0	0	0	0	0	0	0	0	0	0
Nerolidol	0.7	0	0	0.48	0	0	0	0	0	0	0	0	0	0	0
Nootkatone	3	0	0.44	2.59	0	0.14	1.53	0.39	0.37	0.45	0	0.33	0.5	0.39	0
Octacosane	0	0	0	0.18	0	0	0	0.66	0	0.97	0	0	0	0.11	0
Octanal	0	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0
Perillaldehyde	0	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0
Perillyl acetate	0	0	0	0.22	0	0	0	0	0	0	0	0	0	0	0
Sabinene	0	0	0.12	0	0	0.13	0	0	0	0	0	0	0	0	0
ß-Citral	0	0	0	0	0	0.11	0	0	0	0	0	0	0	0	0
ß-cubebene	0.18	0	0	0	0	0	0.21	0	0.15	0.32	0	0	0	0	0
ß-pinene	1.21	0	0.49	0	0	0	0	0	0.54	0	0.49	0.39	0	0	0
Terpinene-4-ol	0	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0
Trans-A-Terpineol	0	0	0	0	0	0	0	0	0	0	0	0.26	0	0	0
Trans-Anethol	0	0	0	0	0	0	0	0.24	0	0	0	0	0	0	0
Trans-carane	0	0	0	0	0	0	0	0	0	0.58	0	0	0	0	0
Trans-Carveol	0	0	0	0	0	0	0	0	0	0	0.31	0	0	0	0
Trans-nerolidol	0	0	0	0	0	0	0	0	0	0	0	0.27	0	0	0
Valencene	0.35	4.12	7.31	0.58	0.2	3.7	1.54	1.26	1.22	0.83	0	6.87	0.59	1.31	0.63

Note: C1=Grapefruit, C2=Kinnow, C3=Musambi; The values presented are in percentage (%).

A principal component scatter plot of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities depicted that citrus cultivars collected are diverse to each other when compared to each other (Fig.2). The projection of three citrus cultivars from different localities on PC1 and PC2 showed the diverse nature (Fig.2). The projection of compounds on PC1 and PC2 shows population structure on the base of percentage of compounds (Fig.3). From the different compounds in essential oils extracted from three citrus cultivars collected from different localities also showed the diverse nature because they were scattered in different ordinations on the factor-plane (Fig.2).

Table 3. Eigenvalues of correlation matrix, and related statistics on the basis of percentage of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities.

	Eigenvalue	% Total – variance	Cumulative - Eigenvalue	Cumulative - %
1	6.4009	42.67	6.40	42.67
2	1.4408	9.61	7.84	52.28
3	1.2148	8.10	9.06	60.38
4	1.1386	7.59	10.20	67.97
5	0.9578	6.39	11.15	74.35
6	0.7396	4.93	11.89	79.28
7	0.6315	4.21	12.52	83.49
8	0.4832	3.22	13.01	86.72
9	0.4254	2.84	13.43	89.55
10	0.4122	2.75	13.84	92.30
11	0.3087	2.06	14.15	94.36
12	0.2803	1.87	14.43	96.23
13	0.2193	1.46	14.65	97.69
14	0.2101	1.40	14.86	99.09
15	0.1368	0.91	15.00	100.00

Table 4. Principal components of different compounds on the basis of percentage found in essentials oils extracted from three citrus cultivars collected from different localities.

	Factor 1	Factor 2	Factor 3	Factor 4
GF_Abt	-0.60998	0.00559	0.39710	0.02130
Kin_Abt	-0.65060	0.13247	-0.37904	0.05452
Mus_Abt	-0.29472	0.55633	0.21311	-0.40643
GF_Fsd	-0.65982	-0.02475	0.17626	-0.31960
Kin_Fsd	-0.61754	-0.25271	-0.42392	0.06307
Mus_Fsd	-0.61089	0.43363	-0.45692	0.04353
GF_Lya	-0.69330	-0.34403	0.08841	-0.08901
Kin_Lya	-0.80016	-0.29914	-0.00083	-0.05023
Mus_Lya	-0.77685	0.09716	0.36933	-0.03233
GF_RYK	-0.79584	0.02854	0.30307	0.00549
Kin_RYK	-0.01550	0.09951	0.33192	0.86894
Mus_RYK	-0.35014	0.75262	-0.00478	0.10676
GF_Sgd	-0.73685	-0.12630	0.05402	0.15432
Kin_Sgd	-0.79496	-0.22073	-0.00933	-0.03135
Mus_Sgd	-0.82047	0.04519	-0.31495	0.24335

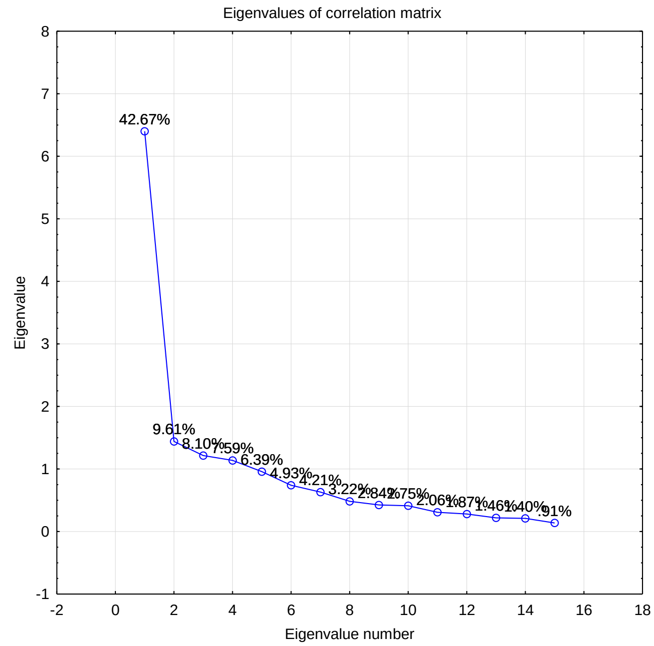

Figure 1. Scree plot between eigen values and number of principal components of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities.

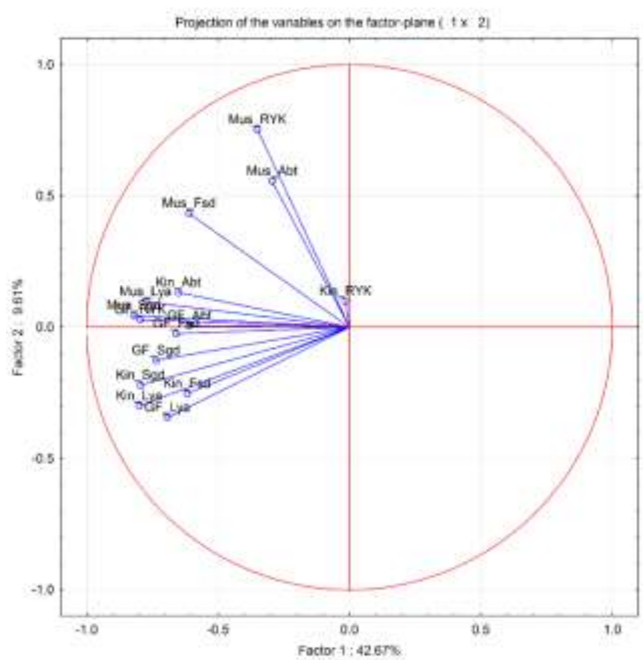

Figure 2. Two dimensional ordination of three citrus cultivars collected from different localities on PC1 and PC2.

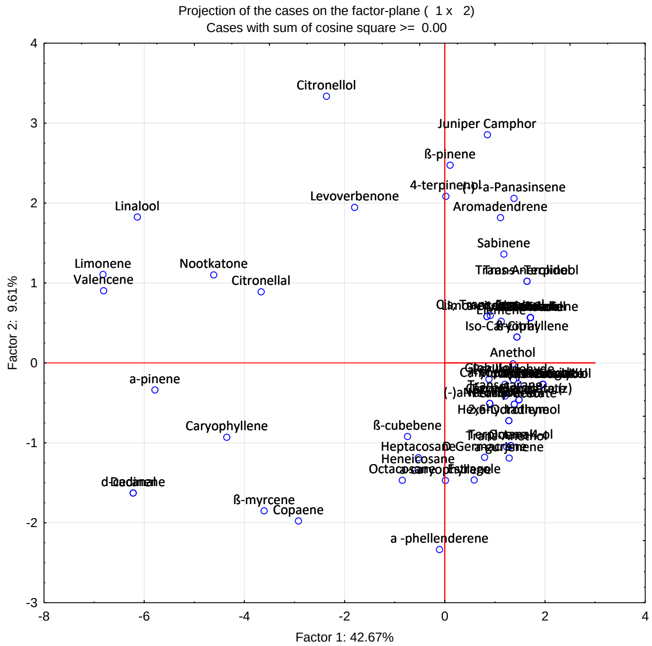

Figure 3. Two dimensional ordination of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities on the basis of percentage PC1 and PC2.

***Cluster analysis*:** Hierarchical clustering by using Ward's hierarchical method (Ward, 1963) grouped the compounds in three main clusters is represented on the hierarchical tree using Euclidean distance and showed a clear picture (Fig. 4). Cluster I included these compounds namely Limonene glycol, Menthadiene-1-ol, Trans-carveol, β-myrecene, Citronellal, α-pinene, d-cadinene, Decanal, Limonene, Linalool, Valencene, Caryophyllene, Nootkatone, Copaene, Citronellol, β-pinene and Levoverbenone. Cluster I is subdivided into two sub-clusters. Sub-cluster I of cluster I included these compounds viz., Caryophyllene, Nootkatone, Copaene, Citronellol, β-pinene and Levoverbenone. Sub-cluster II of cluster I included the compounds β-myrecene, Citronellal, α-pinene, d-cadinene, Decanal, Limonene, Linalool and Valencene.
Cluster II contained Farnesol, Menthol, (z)-Carveol, 1-Decanol, Carveol and Cis-carveol. Cluster III included these compounds (-)-α-Panasinsene, Juniper Camphor, Aromadendrene, Cis, Trans-Farnesol, Trans-α-Terpineol, Trans-nerolidol,4-terpineol, α-citral, Limonene oxide, Anethol, Perillaldehyde, Globulol, Iso-Caryophyllene, β-Citral, Sabinene, (-)α-Neoclovene, Nerolidol, Heneicosane, Heptacosane, Elemene, α-farnesol, Perillal acetate, α-caryophyllene, Trans-Anethol, Octacosane, α-gurjenene, D-Germcrene, α-phellenderene, ϒ-Muurolene, Trans-carane, β-cubebene, 2,6-Octadiene, Hexahydrothymol, Estragole, Octanal, Terpinene-4-ol, Elemol, Carvoyl acetate (z), Fernesyl acetate, Caryophyllene oxide, β-Citronellol, α-Selinene, 4,11-Selinadiene and E-Nerolidol (Fig. 4).
In each cluster, the number of compounds are different and decide the number of sub-clusters and groups on hierarchical tree. Cluster III is subdivided into two sub-clusters. Sub-cluster I of cluster III was further divided into two groups and sub-cluster II of cluster III also has two groups. Each group included different compounds. Group I of sub-cluster I of cluster III included following compounds namely α-caryophyllene, Trans-Anethol, Octacosane, α-gurjenene, D-Germcrene, α-phellenderene, ϒ-Muurolene, Trans-carane and β-cubebene. Group II of sub-cluster I of cluster III included following compounds namely Nerolidol, Heneicosane, Heptacosane, Elemene, α-farnesol (Fig. 4). Similarly group I of sub-cluster II of cluster III has following compounds included namely 4-terpineol, α-citral, Limonene oxide, Anethol, Perillaldehyde, Globulol, Iso-Caryophyllene, β-Citral and Sabinene. Group II of sub-cluster II of cluster III compounds viz., (-)-α-Panasinsene, Juniper Camphor, Aromadendrene, Cis, Trans-Farnesol and Trans-α-Terpineol (Fig. 4).

DISCUSSION

Climatic and environmental factors affect the chemical composition of the essential oils. Essential oil yield and their chemical components are highly affected by climatic and genetic factors (Rahimmalek *et al*., 2009). As the altitude increases the other factors such as rainfall and average temperature also changes. Limonene has a negative correlation with the elevation. Compounds for example ß-Citronellol, Elemol, α –Selinene, 4-terpinenol, 4,11-Selinadiene, Carvyol acetate (z), Citronellol, Decanal, E-Nerolidol, Farnesol, Farnesyl acetate, Globulol, Menthol and Perillaldehyde have positive correlation with elevation. This result is in accordance with Melito *et al*. (2016). With a place at high altitude from sea level have less temperature and more average precipitation the oil components increase in their number and percent composition. Similar results were observed by Melito *et al*. (2016). They found that altitude level and climatic condition affected the chemical composition of this species, determining the differentiation of two different chemotypes which correspond to specific environmental conditions. Fanciullino *et al*. (2006) also found the similar trend of compounds. Two major chemotypes, limonene and limonene/γ-terpinene were distinguished for peel oils. Dhouioui *et al*. (2016) observed the variation in the chemical components of the essential oils due to the climate and seasonal change; they found the different number and percentages of the chemical compounds. Some compounds (ß-Citronellol, Elemol, α-Selinene, 4-terpinenol, 4,11-Selinadiene, Carvyol acetate (z), Citronellol, Decanal, E-Nerolidol, Farnesol, Farnesyl acetate, Globulol, Linalool, Menthol and Perillaldehyde) have significant negative

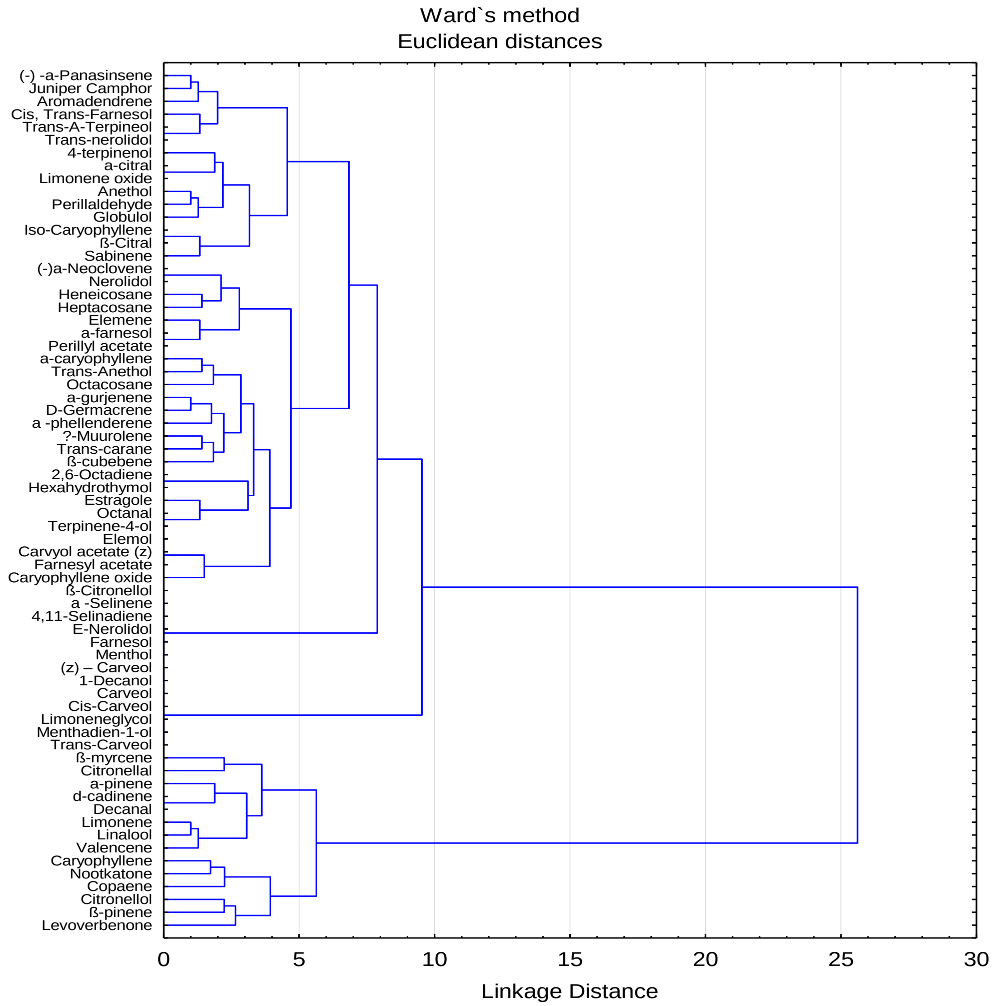

Figure 4. Diversity of different compounds found in essentials oils extracted from three citrus cultivars collected from different localities.

correlation with average temperature The change in the chemical compounds of the essential oils is also in accordance with the results of Matias *et al*. (2010). They found qualitative and quantitative change significantly in the essential oils. Some compounds (Citronellol, Decanal and Globulol) have statistically significant positive correlation with rainfall in citrus peel essential oils. The variation in the chemical constituents of the essential oils was also in accordance with the results of Aprotosoaie *et al*. (2010). They revealed that climate temperature and rainfall directly affect the composition of the compounds in the essential oils. Salamon *et al*. (2010) also observed biodiversity in essential oils in regard to influence of eco-physiological conditions i.e., biotic and abiotic factors. Kamal *et al*. (2011) resulted in the same pattern for the citrus essential oil components of different citrus cultivars. The major components were Limonene, decanal, α-pinene, caryophyllene, nootkatone and valencene. Limonene was the most prevailing constituent of the citrus peel oils. The results are according to Droby *et al*. (2008) findings of composition of peel essential oil of *Citrus sinensis*, that limonene was the predominant compound with percentage varying from 72.41 to 94.77%. Ou *et al*. (2015) also reported the same ratio of limonene and other major constituents of the citrus essential oils. These results are also in accordance with Bourgou *et al*. (2012). The availability of the major compounds in the peel essential oils of Shamber is also in accordance with the results of Kirbaslar *et al*. (2006) those were as limonene (92.5%), myrcene (2.6%) (0.8%) were □-caryophyllene (0.4%) and d-cadinene (0.2%) and nootkatone (0.2%), octanal (0.2%), decanal (0.2%) geranial (0.1%) and neral (0.1%), linalool (0.2%), □-terpineol (0.1%), neryl acetate (0.1%) and geranyl acetate (0.1%).

***Conclusion*:** Citrus peel oil is much important for Pakistan as citrus is our major fruit crop. Every year a huge amount of fruit is being exported and consumed. Pakistan is in the top ten citrus producing countries in the world. The juice industries are also on the increasing demand so the peel is being wasted in thousands of tons. In this study, three different citrus cultivars were used for essential extraction from five different geographical locations. The isolated compounds were significantly correlated with the environmental factors. Shamber was found the best citrus cultivars for essential oil.

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