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THE NOMENCLATURE OF THREE *CITRUS* VARIETIES COLLECTED IN PAKISTAN AND CHEMICALS IN ESSENTIAL OILS FROM THEIR PEELS

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Abstract

Some *Citrus* varieties are indigenous in Pakistan. In order to make use of these cherished genetic resources, a continuous study has been carried out on the essential oils from the peels. Three varieties were collected from Khyber Pakhtunkhwa, Pakistan, Their nomenclature were ascertained by the examination of the morphological characters of fruits, branches and leaves. They are identified as *Citrus karna* Raf. (Kharna khatta), *C. pseudolimon* Tanaka (Galgal), and *C. paradisi* var. *Foster*, respectively. The essential oils (EOs) extracted from the fresh peels by hydro-distillation were analyzed. In total, 40 components are identified and quantified, accounting for 98.4-99.7%. Limonene (92.1-97.7%) is the most abundant; nootkatone (tr-0.5%) is a chemical marker (CM) for Galgal and Foster; *β*-bisabolene (0-1.5%) and *α*-bergamotene (0-1.2%) are two CMs for Galgal; decanal (0.1-0.5%), valencene (0-0.3%) and selin-6-en-4*α*-ol (0-0.2%) are two CMs of Foster. The other important CMs in peels EOs of *Citrus* such as *γ*-terpinene were undetected. *α*-Bergamotene and *β*-bisabolene are two important CMs for discriminating lemons, limes and citrons from the other *Citrus* species. Nootkatone most probably only originates from *Citrus grandis*. The results of CMs in these EOs also prove the results of nomenclature.

Key words: *Citrus* L., Pakistan, Nomenclature, Peels, Essential oils, Chemical markers.

Introduction

The genus *Citrus* L. is very diverse in nature and the classification of its innumerable cultivated forms has ever been a difficult problem (Huang, 1997; Wu *et al*., 2018; Zhang & Mabberley, 2008; Hajra *et al*., 1997; Singh, 1981).Three ancestral *Citrus* species associated with commercially important types-citrons (*C. medica*), mandarins (*C. reticulata*) and pummelos (*Citrus maxima*)-are separated from lemons (*C. limon*), limes (*C. aurantifolia*), sweet oranges (*C. sinensis*), sour oranges (*C. aurantium*) and grapefruits (*C. paradisi*) as hybrids involving these three species (Wu *et al.*, 2018; Zhang & Mabberley, 2008; González-Mas *et al.*, 2019). Indian subcontinent enjoys a remarkable position in the '*Citrus* belt of the world' due to its rich wealth of *Citrus* genetic resources, both wild and cultivated. Some varieties are indigenous such as *C. karna* Raf., *C. limetta* Risso (sweet lime), *C. pseudolimon* Tanaka (Galgal), whereas, these cherished genetic resources are not esteemed and become less and less in Pakistan (Hajra *et al*., 1997; Singh, 1981; Khan & Shaukat, 2006; Yousafzai *et al*., 2021).

The essential oils (EOs) are abundant in *Citrus* and have important usage. As for sour orange, the fresh flowers yield two high-quality fragrant oils commercially known as 'oil of Neroli Bigarade' and 'orange flower water'. 'Oil of petitgrain' is another product obtained by steam-distillation of leaves and young shoots. Lemon oil is an effective remedy for scurvy, rheumatism, dysentery and diarrhoea (Hajra *et al*., 1997). The representative chemicals in these EOs include limonene, *γ*-terpinene, linalool, terpinen-4-ol, *α*-terpineol, *E*, *E*-*α*-farnesene, *E*-nerolidol, *etc.* (González-Mas *et al.*, 2019). The peels EOs from *Citrus* varieties demonstrate some kinds of chemotype due to their different chemical markers (CMs) (Lota *et al.*, 2000; Fanciullino *et al.*, 2006; Wang, 2014). The differences in the chemotype of these EOs are also related to the different genetic types of corresponding plants (González-Mas *et al.*, 2019; Wu *et al*., 2018; Wang, 2014; Haq & Wang, 2023). For example, *α*-bergamotene and *β*-bisabolene are two important CMs to discriminate citron, lemon and lime from the other *Citrus* species (Haq & Wang, 2023; González-Mas *et al.*, 2019).

Previously, five *Citrus* varieties collected in Khyber Pakhtunkhwa (KP), Pakistan were studied for the EOs extracted from peels and morphological identification (Haq & Wang, 2023). In order to utilize such resources reasonably, to explore the chemicals in peels EOs, to make clear their nomenclature, three *Citrus* varieties were collected further in KP. Their nomenclature were ascertained by the morphological characters of their fruits, branches and leaves. The EOs extracted from the fresh peels by hydro-distillation were analyzed by Gas chromatography-Mass spectrometer (GC-MS), and then the CMs were identified, which also verified the nomenclature (Haq & Wang, 2023).

Material and Methods

Plant materials: Three different *Citrus* varieties with No. 1, 2 and 3 were collected on February 4 and 11, 2022. Among them, No. 1 was collected from the University of Peshawar, Peshawar, KP; No. 2 and 3 were collected from the village of P/O Dherizardad, tehsil, district Charsadda, KP.

Chemicals: Freshly distilled water and HPLC grade *n*-Hexane (Merck) were used.

The varieties identification: The nomenclature of varieties was deduced on the morphological characteristics of fruits, branches and leaves.

Extraction of the EOs: The fresh peels from No. 1, 2 and 3, were subjected to hydro-distillation for 2-3.5 h, twice, using a Clevenger apparatus, respectively. The remaining water in these EOs was removed by anhydrous magnesium sulfate, and then the EOs were stored at -4°C until analyzed. In the end, 3 kinds of EOs were collected corresponding to No. 1, 2 and 3.

The samples preparation: The EOs 10 µL corresponding to No. 1, 2 and 3 were diluted by 100 µL of n-hexane (HPLC), respectively.

GC-MS detection: A Thermo Scientific (DSQII) GC-MS (USA) with software version 2.0.71, matched with a NIST 17 MS database, equipped with a column TR-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness), was used for detection. The oven temperature was programmed from 50°C held for 2 min to 150°C at 8°C·min^{-1}, and was further increased to 300°C at 15°C·min^{-1}, and then held for 5 min. The solvent delay was 3 min. The injector was operated in a split ratio of 20: 1 with 250°C temperature. Electron impact mass spectra were recorded with a scan from m/z 50-650 amu. The injection volume was 1 µL. The carrier gas was Helium with a flow rate of 1 mL/min.

The identification and quantitation of compounds: The peaks in total ion chromatograms (TICs) obtained by GC-MS were identified by probability-based matching (PBM). The linear retention indices (LRIs) of the semi-apolar column were gotten from the NIST (National Institute of Standards and Technology) 17 database. The peak area normalization was used to calculate the relative area percentage of each compound.

PCA (principal components analyses) and CA (clustering analyses): The chemicals detected in EOs were to be done PCA by SPSS-26 (Statistical Product and Service Solutions). The 3 samples were to be done CA by SPSS-26.

Results and Discussion

The nomenclature of varieties: The detailed morphological information of the fruits, branches and leaves are listed in (Tables 1 and 2). The detailed information of deduced varieties from literature and websites are presented in (Table 3).

The variety 1 is deduced as *Citrus aurantium* L. *'Karna'*, also named *C. karna* Raf (Kharna khatta) (Singh, 1981; Hajra *et al*., 1997; Haq & Wang, 2023), variety 2 is deduced as *C. pseudolimon* (Galgal), a variety of lemon (Hajra *et al*., 1997; Attri *et al*., 1996) and variety 3 is deduced as a variety of *C. paradisi*, maybe *C. paradisi* var. *Foster* (Foster) (Khan & Shaukat, 2006; Rattanpal *et al*., 2017; Huang, 1997).

Yields and density of EOs: For varieties 1, 2 and 3, the extraction rate (Volume of EO mL/Mass of fresh peel g, %) by hydrodistillation is 0.81, 0.34 and 0.21, respectively, the corresponding density (ρ) is 0.87, 0.83 and 0.96, respectively. In previous research, the extraction rate of *C. karna* Raf., *C. jambhiri* Lush. (Rough lemon) and Kinnow is 3.06% (or 0.94%), 0.86% and 1.76%, respectively, the corresponding density (ρ) is 0.81 (or 0.86), 0.92 and 0.79, respectively (Haq & Wang, 2023).

Chemicals in the EOs: The identified and quantified results can be seen in (Table 4).

Limonene (92.1-97.7%) as the most abundant compound in peels EOs from *Citrus* is relatively high compared with other related reports, especially for galgal since limonene usually ranges between 48-70% in lemon (González-Mas *et al*., 2019).

The *cis*-linalool oxide is detected in Foster (0.4%) and Kharna khatta (0.1%), which can reach 0.4% in *C. aurantium* and is seldom detected in grapefruits and lemons (González-Mas *et al*., 2019; Lota *et al*., 2001; Haq & Wang, 2023). It should be noted that Kamal *et al*., (2013) reported Linalool oxide (>1%) as a main component in peel EO of grapefruit produced in Pakistan. *α*-Terpineol (0.1-0.4%) is relatively higher in Foster (0.4%), which is not more than 0.1% in grapefruit (González-Mas *et al*., 2019). *β*-Citronellal is only detected in Foster (0.2%). *β*-Terpineol is only detected in Kharna khatta (tr), which was undetected in the peels EOs of grapefruits and lemons, seldom detected in *C. medica* and *C. aurantium* previously (González-Mas *et al*., 2019).

Decanal (0.1-0.5%) is relatively high in Foster (0.5%), which is also reported as a main component (>1%) in grapefruits from Pakistan (Kamal *et al.*, 2013), can reach 0.4% in *C. aurantium* (González-Mas *et al*., 2019; Lota *et al*., 2001).

Two important SHs including *α*-bergamotene and *β*-bisabolene are only detected in Galgal (1.2% and 1.5%, respectively). *α*-Bergamotene has never been reported in pummelos and seldom been recorded in mandarins, grapefruits, sour oranges and sweet oranges usually not more than 0.1%, which can be chosen as a chemical marker (CM) to distinguish citrons (up to 0.5%), limes (up to 1.0%) and lemons (up to 1.7%) from other species, to the best of our knowledge (González-Mas *et al*., 2019; Haq & Wang, 2023). *β*-Bisabolene is seldom reported in pummelos, mandarins, grapefruits, sweet oranges and sour oranges usually less than 0.1%, which can also be selected as a CM to differentiate citrons (up to 0.7%), limes (up to 2.1%) and lemons (up to 2.8%) from other species, to the best of our knowledge (González-Mas *et al*., 2019; Haq & Wang, 2023). Previously, *α*-bergamotene and *β*-bisabolene were detected in *C. pennivesiculata* (Lush.) Tan. (0.3% and 0.5%), *C. jambhiri* Lush. (0.3% and 0.5%) and *C. karna* Raf. (1.0% and 1.6%), respectively (Lota *et al*., 2002). In former study, *α*-bergamotene (0-1.4%) and *β*-bisabolene (0-1.6%) were relatively high in rough lemon (1.4% and 1.6%) but undetected in *C. karna* Raf. (Haq & Wang, 2023).

Valencene is only detected in Foster (0.3%), which is seldom detected in pummelos, limes, sour oranges and citrons, can be up to 0.41% in lemons (González-Mas *et al*., 2019; Haq & Wang, 2023; Loizzo *et al.*, 2016). It should be noted that valencene was reported as a major component (>1%) in grapefruits and Musammi (a variety of *C. sinensis*) produced in Pakistan (Kamal *et al.*, 2013). Cadina-3,9-diene is detected in Foster (0.1%), which is rarely detected in the peels EOs of *Citrus* (González-Mas *et al*., 2019). (E)-*β*-Caryophyllene (0.1-0.4%) is relatively high in Galgal (0.4%), which was detected in peels EOs of *C. pennivesiculata* (Lush.) Tan. (0.1%), *C. karna* Raf. (0.6%) and rough lemon (0.2%) previously (Lota *et al*., 2002).

Table 1. The morphological characteristics of fruits.

Three kinds of *Citrus* varieties	No.		
	1	**2**	**3**
Number of fruits	15	8	10
Pictures			
Description	Fruit is of medium size, globose, basal with radial furrows, some apex with circular ring. The external colour is red. The rind often feels tough, is of medium thickness with a smooth surface.	Fruits **ovate-oblong**, lemon-yellow, large in size, 10-12 cm in diameter, **apex slightly nippled**, basal with slight radial furrows, rind 0.9-2.4 cm thick, strongly adhering. surface smooth.	Fruit is of medium size, globose. The external colour is yellow. The rind often feels soft, is of medium thickness, and the surface is smooth and even. The pulp is blush.
The average mass of fresh fruits (g)	176.5	849.4	295.3
The average mass of fresh peels (g)	60.0	306.5	69.8
The mean length (cm)	/	13.6	7.8
The mean width (cm)	/	11	8.4
The mean length/The mean width	/	1.2	0.9
The ratio of fresh peels compared with fresh fruits (%)	34.0	36.1	23.6
The taste of pulps	Slight sweet, strong acid	Some sweet and some sour	Sweet and little acid
The width of fresh flavedo (mm)	2.3	3.5	1.8
The width of fresh mesocarp (mm)	3.5	8.7	3.4
The width of fresh peels (mm)	5.8	12.3	5.1

"/" denotes there is no related information.

Table 2. The morphological characteristics of trees, branches and leaves.

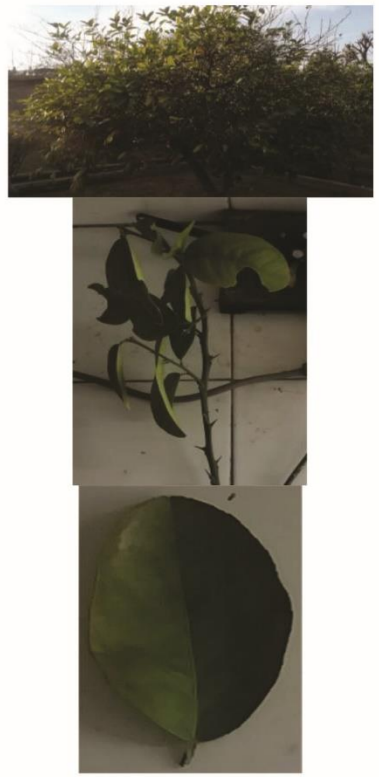
Three kinds of *Citrus* varieties		No.		
		1	2	3
Pictures				
The Trees	Description	About 5.4 m height, vigorous.	About 2.5 m height, with an irregular and loose crown	About 2.5 m tall, with an irregular and loose crown
Leaves	Number of Leaves	16	10	10
	Mean Length (cm)	9.0	6.4	7.1
	Mean Width (cm)	4.5	4.1	3.0
	Mean length/Mean width	2.0	1.6	2.4
	Color of leaves	Dark green	Green	Dark green
	Leaflets (Yes/No, its mean length cm, mean width cm, and mean length/mean width, if yes)	Yes (1.6, 0.8, 2)	No or very small	Yes (1.4, 0.5, 2.8)
	Sawteeth (Yes/No)	Yes (small)	Yes	No
	Leaf apex	Blunt	Blunt	Blunt
	Texture and shape	Leather, flat or little involved	Flat, oval-shape, some involved	Flat
Branches	Thorns (Yes/No, its length cm if yes)	Yes (0.2-0.6)	Yes (0.3-2)	Yes (very small)
	Young/old (Shape of stem)	Flat/round	Flat/round	Flat/round

Table 3. The information of deduced *Citrus* cultivars planted in Pakistan.

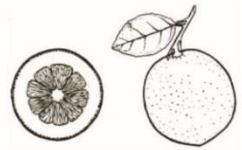
No.	Common Name	Species	Varieties	Production area	Characterization	Local usage	Pictures
1	Khatta (Singh, 1981; https://citrusvariety.ucr.edu/citrus/kharna.html); Kharna khatta, Indian lemon (https://www.plantnames.unimelb.edu.au/Sorting/Citrus_1.html; Hajra *et al*., 1997)	*C. aurantium* (Singh, 1981; https://www.plantnames.unimelb.edu.au/Sorting/Citrus_1.html; https://citrusvariety.ucr.edu/citrus/kharna.html; Hajra *et al*., 1997)	*C. aurantium* L. *'Karna'*; *C. aurantium* var. *Khatta*; *C. karna* Raf. (https://www.plantnames.unimelb.edu.au/Sorting/Citrus_1.html; https://citrusvariety.ucr.edu/citrus/kharna.html; Singh, 1981; Hajra *et al*., 1997)	KP	Fruits are ovoid-oblong or subglobose up to 12 x ca 10 cm, the surface golden-yellowish or orange-coloured, glandular, bumpy with a nipple at apex, depressed at base, the rind thick, moderately adherent, the mesocarp greenish-white, the segments 10-13, adherent, central axis solid, pulp-vesicles orange-coloured and juice abundant, sour and orange flavoured. (Hajra *et al*., 1997) May be a hybrid of sour orange and lemon or citron. The leaves and flowers similar to rough lemon but larger in size. The fruits are typical sour oranges but the flowers are red-tinted like those of the lemon. Fruits usually with broad and prominent nipple, sometimes depressed or lacking. Rind surface smooth, warty or ribbed. Flesh only moderately juicy. Tree vigorous, medium to large in size, upright-spreading, spiny; foliage lemon-like but darker green. New growth purple-tinted. Flowers medium-large and strongly purple-tinged. One bloom and crop per year. (https://citrusvariety.ucr.edu/citrus/kharna.html)	Plants are used as rootstocks for grafting other commercial *Citrus* fruits. (Hajra *et al*., 1997; https://citrusvariety.ucr.edu/citrus/kharna.html)	 (Singh, 1981)  (https://citrusvariety.ucr.edu/citrus/kharna.html)
2	**Galgal; Khtta; Hill lemon** (http://himalayanwildfoodplants.com/2020/12/citrus-pseudolimon-tanaka-galgal-%E0%A4%97%E0%A4%B2%E0%A4%97%E0%A4%B2-khtta/) Gulgul (https://citrusvariety.ucr.edu/crc4235)	*C. limon* (Hajra *et al*., 1997; http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=242313269; Attri *et al*., 1996; https://citrusvariety.ucr.edu/crc4235)	*C. pseudolimon; C. penivesiculata* (http://himalayanwildfoodplants.com/2020/12/citrus-pseudolimon-tanaka-galgal-%E0%A4%97%E0%A4%B2%E0%A4%97%E0%A4%B2-khtta/; Attri *et al*., 1996; https://citrusvariety.ucr.edu/crc4235)		A medium sized tree, 5-6.5 m tall, with an irregular and loose crown. Trunk 28 cm in diameter. Bark smooth, green, thorns numerous, stout, up to 2.3 cm log; **petioles 1-2 cm long and 0.3-0.4 cm wide**, marginally winged, distinctly articulated; leaflets broadly elliptic-ovate to oblong, **9.5-15.5 cm**, **crenate**, base cunceate-rounded, obtuse-acute to blunt or pointed; mildly fragrant. Fruits **ovate-oblong**, yellow, 10-11.5 cm in diameter, **weight 400-500 g**,	Galgal fruits are eaten raw or preserved in the form of pickle or concentrated fruit juice called "chukh" in local dialect for off season. Raw fruits are made into "chacha" a widely admired traditional preparation of	 (http://himalayanwildfoodplants.com/2020/12/citrus-pseudolimon-tanaka-galgal-%E0%A4%97%E0%A4%B2%E0%A4%97%E0%A4%B2-khtta/)

Table 3. (Cont'd.).

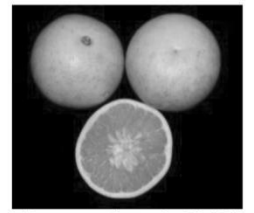
					apex slightly nippled, rind 0.6-0.8 cm thick, strongly adhering. (http://himalayanwildfoodplants.com/2020/12/citrus-pseudolimon-tanaka-galgal-%E0%A4%97%E0%A4%B2%E0%A4%97%E0%A4%B2-khtta/; http://www.fruitipedia.com/2018/11/galgal_citrus-pseudolimon/)	Western Himalayas for winter season. (http://himalayanwildfoodplants.com/2020/12/citrus-pseudolimon-tanaka-galgal-%E0%A4%97%E0%A4%B2%E0%A4%97%E0%A4%B2-khtta/)	 (https://citrusvariety.ucr.edu/crc4235)
3	Foster (Rattanpal *et al*., 2017)	Grapefruit *C. paradisi* (Khan & Shaukat, 2006; Rattanpal *et al*., 2017; http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=250071511; Huang, 1997)	*C. paradisi* var. *Foster* (Rattanpal *et al*., 2017)	Punjab (Rattanpal *et al*., 2017)	Its fruits are medium to large, oblate to spherical, having a large number of seeds with 40-50 seeds per fruit. Primary colour is pale to light yellow, but sometimes rind blushed with pink, extending into the albedo. Rind medium-thick and surface smooth. Primary flesh colour is light with grey tinge and pink colouration, flesh texture tender and juicy with good flavour. Fruit ripens in November-December. Tree vigorous, large, and productive. (Rattanpal *et al*., 2017; https://citrusvariety.ucr.edu/citrus/fosterpink.html; Huang, 1997)	The grapefruit is used to develop resistance against colds and influenza (Holdsworth, 1987). Grapefruit has highly revitalizing and elevating effect on the emotion. It is also very detoxifying and has a sharp, zesty fragrance. Grapefruit is processed to prepare marmalade in Australia. (Morton, 1987). Aromatherapy oil is prepared from grapefruit in Europe. The fruit juice is said to be very good in heart problems. (Khan & Shaukat, 2006)	 (Rattanpal *et al*., 2017)  (https://citrusvariety.ucr.edu/citrus/fosterpink.html)

Table 4. The identified and quantified results.

Chemicals	CAS	LRIs	Retention time (RT) (min)	Kharna khatta	Galgal	Foster
				%		
Sabinene	3387-41-5	974	3.57	/	/	tr
β-Myrcene	123-35-3	991	4.02	1.1	0.9	0.6
Limonene	138-86-3	1030	4.49	97.7	92.1	94.7
cis-Linalool oxide	5989-33-3	1074	5.26	0.1	/	0.4
Linalool	78-70-6	1099	5.83	0.3	0.4	0.4
trans-p-Mentha-2,8-dien-1-ol	7212-40-0	1123	6.04	/	/	tr
β-Terpineol	138-87-4	1153	6.36	tr	/	/
β-Citronellal	106-23-0	1153	6.57	/	/	0.2
α-Terpineol	98-55-5	1189	7.28	0.1	0.1	0.4
Decanal	112-31-2	1206	7.52	0.1	0.2	0.5
β-Citronellol	106-22-9	1228	7.83	tr	/	/
Neral	106-26-3	1240	8.03	/	0.1	0.1
trans-Geraniol	106-24-1	1255	8.26	/	0.1	/
Citral	5392-40-5	1276	8.54	0.1	0.2	0.3
Undecanal	112-44-7	1307	9.07	/	tr	/
δ-EIemene	20307-84-0	1338	9.64	tr	0.1	/
Nerol acetate	141-12-8	1364	10.19	tr	tr	/
Geranyl acetate	105-87-3	1382	10.46	0.1	0.1	0.2
(E)-*β*-Caryophyllene	87-44-5	1419	10.92	0.1	0.4	0.2
α-Bergamotene	17699-05-7	1435	11.19	/	1.2	/
α-Humulene	6753-98-6	1454	11.46	tr	/	tr
Germacrene D	23986-74-5	1481	11.9	tr	/	/
Valencene	4630-07-3	1492	12.02	/	/	0.3
β-Bisabolene	495-61-4	1509	12.33	/	1.5	/
Cadina-3,9-diene	523-47-7	1518	12.49	/	/	0.1
Elemol	639-99-6	1549	12.76	/	/	tr
E-Nerolidol	40716-66-3	1564	13.19	tr	0.2	/
Octanoic acid, hexyl ester	1117-55-1	1581	13.23	/	tr	tr
Globulol	51371-47-2	1583	13.67	/	tr	/
γ-Eudesmol	1209-71-8	1631	14.22	/	/	tr
Selin-6-en-4α-ol	118173-08-3	1636	14.41	/	/	0.2
α-Bisabolol	515-69-5	1684	15.06	/	0.1	/
E, E-Farnesol	106-28-5	1722	15.73	/	0.1	0.1
Nootkatone	4674-50-4	1808	17.1	tr	0.5	0.4
Heneicosane	629-94-7	2100	19.6	/	/	tr
Docosane	629-97-0	2200	20.28	/	/	tr
Tricosane	638-67-5	2300	20.99	/	tr	tr
Tetracosane	646-31-1	2400	21.46	/	/	tr
Pentacosane	629-99-2	2500	22.11	/	/	tr
Bis(2-ethylhexyl) phthalate	117-81-7	2529	22.37	tr	tr	tr
MHs (3)				98.9	93.0	95.3
MAlcs (7)				0.5	0.6	1.2
MAlds (3)				0.1	0.3	0.6
MEsters (3)				0.1	0.1	0.3
SHs (8)				0.1	3.2	0.6
SAlcs (7)				tr	0.5	0.3
SKets (1)				tr	0.5	0.4
Aliphatic esters (1)				/	tr	tr
Aromatic Esters (1)				tr	tr	tr
Alkanes (5)				/	tr	tr
Aliphatic alds (2)				0.1	0.2	0.5
In total (40)				99.7	98.4	99.1

Note: tr means less than 0.05%. "/" denotes there is no related information. MHs, MAlcs, MAlds, MEsters, SHs, SAlcs, SKets and alds, refer to Monoterpene hydrocarbons, Monoterpene alcohols, Monoterpene aldehydes, Monoterpene esters, Sesquiterpene hydrocarbons, Sesquiterpene alcohols, Sesquiterpene ketones and aldehydes, respectively.

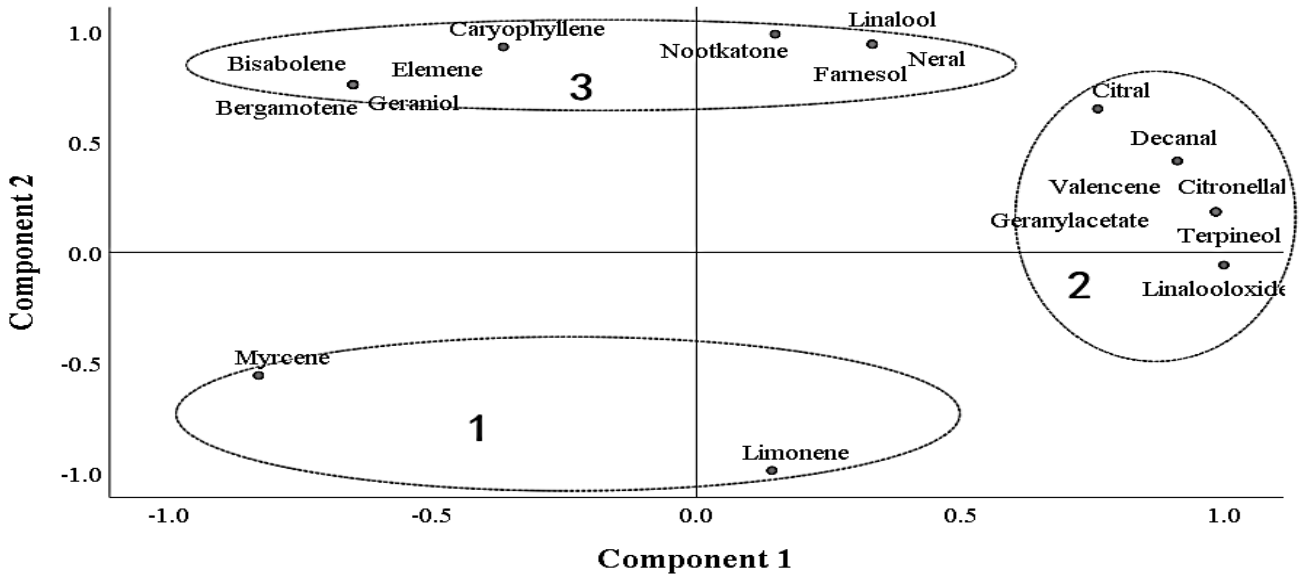


Fig. 1. PCA result of components. Cadina-3,9-diene and selin-6-en-4*α*-ol belonging to class 2, *E*-nerolidol and *α*-bisabolol belonging to class 3 are not displayed.

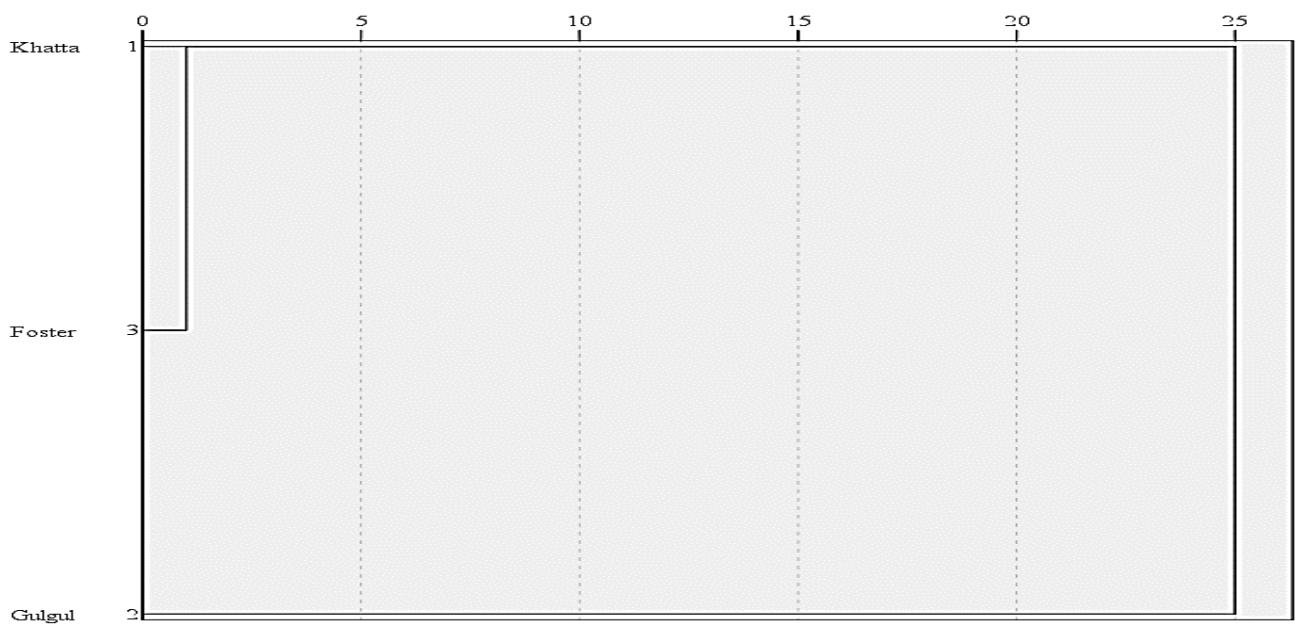


Fig. 2. Clustering analysis result of three samples.

E-Nerolidol (0-0.2%), selin-6-en-4*α*-ol (0-0.2%), *α*-bisabolol (0-0.1%) and *E*, *E*-farnesol (0-0.1%) belonging to SAlcs are prominent. E-Nerolidol is relatively high in Galgal (0.2%), which was undetected in *C. pennivesiculata* (Lush.) Tan, was detected in Kharna khatta (tr-0.1%) produced in KP, Pakistan, previously (Haq & Wang, 2023). Selin-6-en-4*α*-ol is only detected in Foster (0.2%), which was only reported in the peels EOs of mandarin (0-0.5%) previously, to the best of our knowledge (González-Mas *et al*., 2019; Wang *et al*., 2013; Han *et al*., 2020). *α*-Bisabolol is only detected in Galgal (0.1%), which is undetected in pummelos, mandarins and grapefruits, usually not more than 0.2% in other species, is a characteristic component of citron, lemon and lime (Loizzo *et al*., 2016; González-Mas *et al*., 2019). *E, E*-farnesol is relatively high in Foster and Galgal (both 0.1%), which is undetected in mandarins, limes and citrons, is rarely detected in other species usually with tr content (González-Mas *et al*., 2019). It should be also noted that farnesol was reported as a major component in the peels EOs of *C. reticulata* var. *kinnow* and grapefruit produced in Pakistan (Kamal *et al*., 2013).

Nootkatone (tr-0.5%) is relatively high in Galgal (0.5%) and Foster (0.4%), which was seldom detected in mandarins, sour oranges and citrons usually with tr content, was undetected in lime, to the best of our knowledge (González-Mas *et al*., 2019), was reported as a major component (>1%) in grapefruit produced in Pakistan (Kamal *et al*., 2013). Since limes are the hybrid of pure mandarins and citrons, lemons are a sour oranges-citrons hybrid, grapefruits are a sweet oranges-pummelos hybrid, sour oranges and sweet oranges are the hybrid of mandarins and pummelos (Wu *et al*., 2018), nootkatone most probably only originates from pummelos.

Heneicosane, docosane, tetracosane and pentacosan are detected in Foster with tr content. Tricosane is detected in Galgal and Foster with tr content. Docosane, tricosane, tetracosane and pentacosane were recorded in mandarin with 0-0.5%, 0-1.0%, 0-0.4% and 0-0.6%, respectively; heneicosane was detected in Kinnow (2.4%) and *C. paradisi* var. *Shamber* (4.5%) (Khan *et al*., 2012; Wang *et al*., 2013; Han *et al*., 2020; González-Mas *et al*., 2019; Wang, 2023).

Octanoic acid, hexyl ester is detected in Galgal (tr) and Foster (tr), which was also reported in peels EOs of *C.grandis* and Lisbon lemon in tr (González-Mas *et al*., 2019; Zhang *et al*., 2017; Cannon *et al*., 2015).

Bis(2-ethylhexyl) phthalate (tr) is detected in each sample, which is a commonly used plasticizer in synthetic polymers (Gross, 2015).

In total, 40 chemicals are quantified, of which 22 are to be done PCA after the other 18 with 0 or tr are excluded. The 22 components can be classified into 3 classes (Fig. 1). The three samples can be divided into 2 clusters (Fig. 2). The sample from Galgal is quite different from the other two samples, which is the same as the previous study (González-Mas *et al*., 2019).

The chemicals in variety 1 are close to those reported in *C. karna* Raf. collected in KP previously, which further ascertains that No. 1 should be Kharna khatta (Haq & Wang, 2023). Linalyl acetate as a characteristic component in sour orange, which can reach 5% (González-Mas *et al*., 2019), was undetected in variety 1, but was reported (0.2%) previously (Haq & Wang, 2023).

Conclusion

Varieties 1, 2 and 3 are identified as *C. karna* Raf. (Kharna khatta), *C. peseudolimon* (Galgal) and *C. paradisi* var. *Foster*. In total, 40 components are identified and quantified. Limonene is a CM of them; nootkatone is a CM of Galgal and Foster; β-bisabolene and α-bergamotene are CMs of Galgal, which can differentiate citrons, limes and lemons from other species; decanal, valencene and selin-6-en-4α-ol are CMs of Foster. Nootkatone most probably only originates from pummelos. To the best of our knowledge, there is no report on the detection of sabinene, β-terpineol, neral, undecanal, cadina-3,9-diene, elemol, octanoic acid, hexyl ester, selin-6-en-4α-ol, docosane, tetracosane and pentacosane from peels EOs of *Citrus* produced in Pakistan previously.

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References

Attri, B.L. and S.B. Maini. 1996. Pectin from Galgal (*Citrus pseudolimon* Tan.) peel. *Biores. Technol*., 55: 89-91.

Cannon, R., A. Kazimierski, N.L. Curto, J. Li, L. Trinnaman, A.J. Janczuk, D. Agyemang, N.C. Da Costa and M.Z. Chen. 2015. Identification, synthesis, and characterization of novel sulfur-containing volatile compounds from the in-depth analysis of Lisbon lemon peels (*Citrus limon* L. Burm. f. cv. *Lisbon*). *J. Agric. Food Chem*., 63: 1915-1931.

Fanciullino, A.L., F. Tomi, F. Luro, J.M. Desjobert and J. Casanova. 2006. Chemical variability of peel and leaf oils of mandarins. *Flavour Frag. J*., 21: 359-367.

González-Mas, M.C., J.L. Rambla, M.P. López-Gresa, M.A. Blázquez and A. Granell. 2019. Volatile compounds in Citrus essential oils: A comprehensive review. *Front. Plant Sci*., 10: 12

Gross, J.H. 2015. Mass spectrometry. Science Press, Beijing, China, in page 299.

Hajra P.K., V.J. Nair and P. Daniel. 1997. Rutaceae. In: Botanical Survey of India (Ed.), *Flora of India*, Vol 4: *Malpighiaceae-Dichapetalaceae*. Calcutta, India, pp. 276-293.

Han, T.L., I. Ullah and J. Wang. 2020. Chemical variation of essential oils from peels of *Citrus kinokuni* Tanaka, *C. reticulata* 'Dahongpao', and *C. reticulata* 'Zhang Shuensis'. *Acta Sci. Agric*., 4: 4-26.

Haq, A.U. and J. Wang. 2023. The varieties identification and biomarkers analyses on essential oils from peels of *Citrus* L. collected In Pakistan. *Pak. J. Bot*., 55: 1407-1418.

Holdsworth, D. 1987. Traditional medicinal plants of the central province of Papua New Guinea. *Int. J. Crude Drug. Res*., 25: 103-112.

Huang, C.C. 1997. Rutaceae. In: *Flora Reipublicae Popularis Sinicae*. Science Press, Beijing, China, 43.2: 169-209.

Kamal, G.M., M.Y. Ashraf, A.I. Hussain, A. Shahzadi and M.I. Chughtai. 2013. Antioxidant potential of peel essential oils of three Pakistani *Citrus* species: *Citrus reticulata*, *Citrus sinensis* and *Citrus paradisi*. *Pak. J. Bot*., 45: 1449-1454.

Khan, D. and S.S. Shaukat. 2006. The fruits of Pakistan: Diversity, distribution, trends of production and use. *Int. J. Biol. Biotech*., 3: 463-499.

Khan, M.M., M. Iqbal, M.A. Hanif, M.S. Mahmood, S.A. Naqvi, M. Shahid and M.J. Jaskani. 2012. Antioxidant and antipathogenic activities of *Citrus* peel oils. *J. Essent. Oil Bear. Plants*, 15: 972-979.

Loizzo, M.R., R. Tundis, M. Bonesi, G.D. Sanzo, A. Verardi, C.G. Lopresto, A. Pugliese, F. Menichini, R. Balducchi and V. Calabro. 2016. Chemical profile and antioxidant properties of extracts and essential oils from *Citrus x limon* (L.) Burm. cv. Femminello comune. *Chem. Biodiv*., 13: 571-581.

Lota, M.L., D.R. Serra, F. Tomi and J. Casanova. 2000. Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco. *Biochem. Syst. Ecol*., 28: 61-78.

Lota, M.L., D.R. Serra, C. Jacquemond, F. Tomi and J. Casanova. 2001. Chemical variability of peel and leaf essential oils of sour orange. *Flavour Fragr. J*., 16: 89-96.

Lota, M.L., D.R. Serra, F. Tomi, C. Jacquemond and J. Casanova. 2002. Volatile components of peel and leaf oils of lemon and lime species. *J. Agric. Food Chem*., 50: 796-805.

Morton, J. 1987. Grapefruit. In: Fruits of warm Climates (Julia F. Morton). *Miami. Fl*., 152-158.

Rattanpal, H.S., G. Singh, S. Singh and A. Arora. 2017. *Citrus* cultivation in Punjab. Additional director of communication for Punjab agricultural university, Ludhiana.

Singh, B. 1981. Establishment of first gene sanctuary in India for *Citrus* in Garo hills. Concept Publishing Company, New Delhi, pp. 29-41, 53-60, 62.

Wang, J., Y.P. Liu, H.P. Chen, L. Chen, Z. Wei, R. Liu, F. Long and D.G. Wan. 2013. Chemical changeability of essential

oils in Chenpi and Qingpi from the same origin by Gas Chromatography-Mass Spectrometry compiled with Automated Mass spectral Deconvolution and Identification System. *Asian J. Chem*., 25: 6434-6442.

Wang, J. 2014. The essential oil chemotype of *Citrus reticulata* Blanco peel. *J. Chem. Pharm. Res*., 6: 16-18.

Wang, J. 2023. Alkanes and chemical markers identified in the essential oil from pericarp of Nanfengmiju (*Citrus kinokuni* Hort. ex Tanaka). *J. Mex. Chem. Soc.*, 67: 82-93.

Wu, G.A., J. Terol, V. Ibanez, A. López-García, E. Pérez-Román and C. Borredá. 2018. Genomics of the origin and evolution of *Citrus*. *Nature*, 554: 311-316.

Yousafzai, A.U.H., U. Anwar, Y.M. Su, H.X. Yang and J. Wang. 2021. How to make use of the *Citrus* peels in Pakistan. *Acta Sci. Agric*., 5: 06-08.

Zhang, H., Y. Xie, C. Liu, S. Chen, S. Hu, Z. Xie, X. Deng and J. Xu. 2017. Comprehensive comparative analysis of volatile compounds in *Citrus* fruits of different species. *Food Chem*., 230: 316-326.

Zhang, D. and D.J. Mabberley. 2008. CITRUS Linnaeus. In: *Flora of China, Rutaceae,* Vol.11. (Eds.): D. Zhang, T.G. Hartley and D.J. Mabberley. Science press, Beijing, China, pp. 90-96.

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