JJBS

Volume 17, Number 1,March 2024
ISSN 1995-6673
Pages 9 – 19
https://doi.org/10.54319/jjbs/170102

Jordan Journal of Biological Sciences

Comparative Profiling of Volatile Compositions of Fresh and Dehydrated Rinds and Leaves of Different Indian *Citrus* Species

Susmita Das[1,*], Rinku Saha[2], Moumita Biswas[1], Sritopa Mondal[2], Gargi Mahapatra[2]

[1]*Phytochemistry and Pharmacognosy Research Laboratory, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Kolkata - 700 019, West Bengal, India;* [2]*Lady Brabourne College, Beniapukur, Kolkata, West Bengal, 700017*

Received: February 4, 2023; Revised: May 20, 2023; Accepted: June 16, 2023

Abstract

Citrus is an essentially important fruit that grows in diverse parts of the world. India is one of the chief producers of *Citrus* species. The most important varieties cultivated in West Bengal, India are: Paati, Gondhoraaj, Kaghchi, Batapi, Rangpur, Kamala and Musambi. This research aimed at profiling the volatile organic compositions of the essential oils (EOs) of a few popularly cultivated *Citrus* sps, isolated, in their fresh and dehydrated conditions, both from leaves as well as from fruit rinds. GC-MS (Gas Chromatography - Mass Spectrometry) analyzed a total of 78 metabolites belonging to different classes. This study has established a comprehensive volatile profile of *Citrus* species. The essential oils (EOs) isolated using hydro-distillation method from the discarded rinds and leaves can be used as a potential source of aroma and flavour compounds for the emerging nutritional market. The PLS-DA (Partial Least Squares – Discriminant Analysis) and HCA (Hierarchical Cluster Analysis) showed distinct clusters for dehydrated and fresh rind and leaf samples of all the studied species.

Keywords: volatile organic compounds, fruit rind, GC-MS, PLS-DA, HCA

1. Introduction

Among the different horticultural fruit crops, Lemon (*Citrus* sp.) is one of the chief fruit crops cultivated around the globe, with universal agricultural produce exceeding 80 million tons / year (Marin et al., 2007), and is the largest genus belonging to the family Rutaceae with approximately 70 species (Mahato et al., 2020). The *Citrus* sps and varieties are a prospective source of essential oils worldwide, utilised in flavour industries of alcoholic and non-alcoholic beverages, confectionaries, cookies, desserts and also in perfumery, cosmeceutical and nutraceutical industries. In the pharmaceutical industry, the volatile essential components play a major role in masking the disagreeable bitter tastes of medicines (Steuer et al., 2001; Nguyen et al. 2009). EOs may improve the olfactory properties viz., flavour, odour and colour when added to food substances (Maroid, 2016). The rinds (flavedo) of the fruits contain oil glands that contain essential oil fractions composed of several important volatile and semi-volatile compounds (Dugo and Mondello, 2011; Tranchida et al., 2012; Sarrou et al., 2013). The *Citrus* essential oils are mostly obtained from the flavedo or fruit rinds, but flowers and foliages are also exploited. The most studied *Citrus* EO compositions from rinds, leaves and flowers of *Citrus* sp. comprise *C.* x *sinensis* (L.) Osb. (Sweet orange), *C. reticulata* Bl. (Mandarin), *C. paradise* Macfad. (Grapefruit), *C. grandis* (L.) Osb. (*C. maxima* Burm. pummelo), *C. limon* (L.) Burm.f. (Lemon), *C. medica* L. (Citron), *C.* x *aurantifolia* (Christm.) Swingle (Lime), *C. aurantium* (Bitter orange), *C. bergamia* Rissoet Poit. (Bergamot orange) and *C. junos* Sieb. ex. Tanaka (yuzu) (Gonzalez-Mas et al., 2019).

The different species of *Citrus* fruits are cheaply available throughout India and they are very popular fruits because of the economical source of nutritive juices, rich in vitamins and minerals. After consumption of the edible parts of *Citrus* fruits, the fruit rinds are discarded as waste. The discarded rinds could be a source of essential oils needed for various industrial purposes. Massive amounts of *Citrus* waste, especially rinds, are generated internationally, and these are an ecological menace in several areas of the globe. Moreover, essential oils present in the rinds of oranges are fatal to yeasts (Murdock and Allen, 1960) and deter the progress of yeasts, molds and bacterial growth (Subba et al., 1967). Various studies have shown that incorporating *Citrus* rinds as powder or as EOs into food products may enhance the food's quality without negatively affecting the sensory attributes when added at the right amount (Ademosun, 2022). So, it is noteworthy to use *Citrus* wastes scientifically in food-nutrients industries and other areas (Tripodo et al., 2004; De Gregorio et al., 2002; Lo Curto et al., 1992).

The EOs present in oil glands are found at diverse layers in the rinds and cuticles of *Citrus* fruits and leaves. The EOs are released when oil glands are squashed, smashed or broken. These essential oils are used for flavouring ingredients in drinks, ice creams and other food products, also used in the preparation of toilet soaps, perfumes, cosmetics and other home and health care products (Raeissi et al., 2008).

Some plants' essential oils (EOs) are ranked among the most bioactive EOs in the world (Gherairia et al., 2022) and *Citrus* EOs are very common around the world. The

* Corresponding author. e-mail: susouravipar@gmail.com.

isolation of EOs from *Citrus* vegetal materials (rinds and leaves) is based on hydro-distillation principally in Clevenger-type hydro-distillation (Asikin et al., 2015; Fancello et al., 2016, Ben Hsouna et al., 2017). So for this purpose, fresh lemon rinds are used to obtain essential oils which possess the characteristic aroma and flavour (Fierascu et al., 2019). Many researchers have deciphered the volatile profile of essential oil from the lemon rinds, but information on the comparison of fresh and dehydrated rinds as well as their leaves of the studied *Citrus* samples is not yet reported.

Recently, the idea of valorization of agro-industrial bio-wastes following the recently developed extraction processes has been increasingly applied as an emerging tool to manage and recover value-added products (Zema, Calabro et al., 2018) including *Citrus* rinds and pulps (Zema, Folino et al., 2018; Forney and Song, 2017; Zhang et al., 2019). *Citrus* rinds and leaves are under-exploited owing to the availability of scant information for its recycling and valorization. These products deserve to be reconnoitred as a promising and valuable source of aroma and flavour compounds significant for the flavour and fragrance, nutraceutical, cosmeceutical and functional food industries. So, the focus of this study was to detect and quantify the volatile profiles of a few popularly cultivated species of *Citrus* from the essential oils (EOs) of their rinds and leaves using the hydro-distillation method under fresh and dehydrated conditions in order to compare the yield and their essential oil compositions. From this study, enough information could be assimilated about the chemical variability of the rinds and leaves of these *Citrus* species of West Bengal, India.

2. 2. Materials and Methods

2.1. Collection of samples and preparation

Healthy fully mature ripened fruits of 2.5 to 3 kilograms each of the seven different species of *Citrus* (Fig.1) were used in the current study: *Citrus* x *aurantifolia* (Christm.) Swingle (Paati), *Citrus medica* L. (Bir-jara / Kaghchi), *Citrus* x *sinensis* (L.) Osbeck cv. *Mosambi* [Godhadi type (thick skinned)], *Citrus reticulata* Blanco (Darjeeling Mandarin), *Citrus limon* (L.) Osbeck (Gondhoraj), *Citrus* x *limonia* Osbeck (Rangpur/Gora), *Citrus maxima* Merr. (Batapi pomelo). *Citrus reticulata* Blanco and *Citrus* x *sinensis* (L.) Osbeck were collected from the local market in East Kolkata, West Bengal, India. The collected samples of *Citrus reticulata* (Darjeeling Mandarin) and *Citrus* x *sinensis* cv. *mosambi* were harvested from fruit farms in Darjeeling and West Medinipur district, West Bengal respectively, as confirmed by the vendor. All the other species viz., *Citrus* x *aurantifolia* (Paati), *Citrus medica* (Kaghchi), *Citrus limon* (Gondhoraaj), *Citrus maxima* (Batapi pomelo) were collected from the garden of Lady Brabourne College, South Kolkata, and *Citrus* x *limonia* (Gora) was collected from a local garden in Salt Lake, East Kolkata, West Bengal. The collection of the fruits was done under the same climatic conditions. The climatic condition at the time of collection was mild winter, with temperatures ranging between 20 and 25° C. in the months of February and March. The *Citrus* fruit species were identified by Prof. Pinaki Acharya, Professor in the Department of Agriculture, University of Calcutta. After collection, the *Citrus* fruit species were washed thoroughly under tap water to remove the suspended particulate matters (SPMs) and dirt. The fruits were then peeled off manually, but very carefully. 100g of the lemon rinds were kept for drying at room temperature for 2 - 3 days and 50g of the rinds were kept fresh. Both dried and freshly scraped out rinds were recycled for the isolation of EOs. The fresh rinds of *C.* x *aurantifolia* could not produce isolatable essential oil (but the oil droplets were found suspended in the hydro-distilled water) and the dried rinds of *C. medica*, *C.* x *sinensis*, *C. limon* and *C.* x *limonia* could not be preserved for EO isolation. The fresh leaves of *Citrus* x *limonia* and *Citrus limon* were also collected, cleaned and torn into pieces before the extraction of essential oils. Some of the fresh leaves were left at room temperature for 2-3 days to dehydrate. The leaves of other species could not be collected in appreciable amounts for EO isolation.

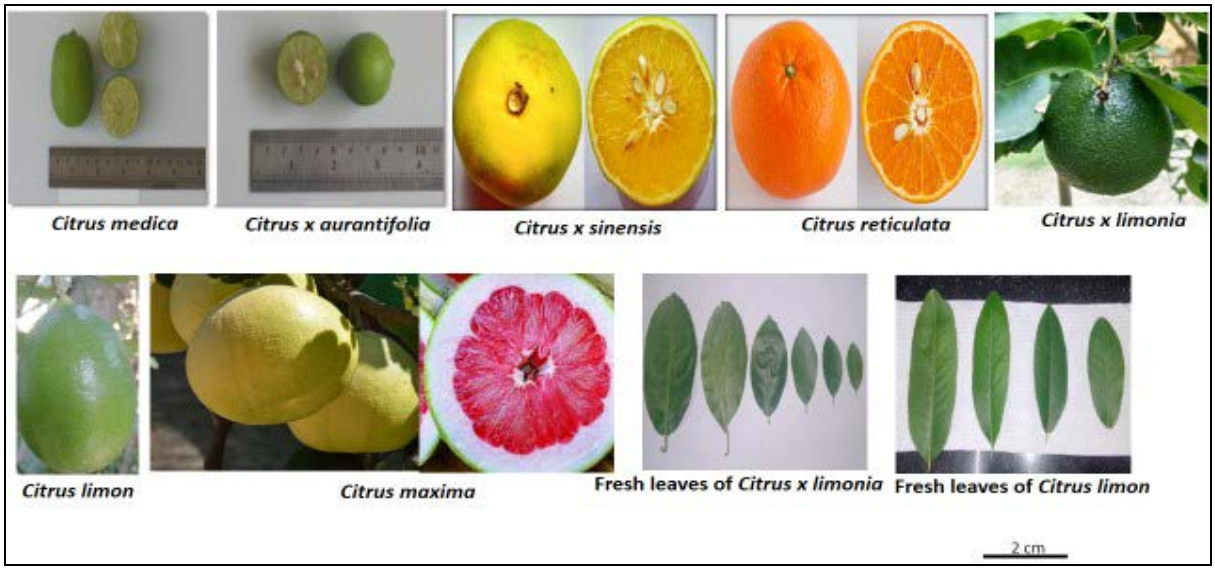

Figure 1. Fruits and leaves of the experimental samples

2.2. Isolation of essential oils

For the isolation of essential oils, 50g of fresh rinds and 25g of dry rinds of each *Citrus* fruit were taken separately in a 500 mL flask with 100 mL and 60 mL of double - distilled water respectively. Similarly, 60g of fresh leaves and 40g of dry leaves were taken separately with 200 mL and 150 mL of distilled water, respectively. The optimization of isolation as well as yield of volatile oils was done by several factors, including EO extraction time, temperature, water to plant material (rind / leaf) ratio and also the sample size. As per literature (Bardakei et al., 2019), the essential oils were isolated by the hydro-distillation method using a Clevenger-type apparatus for 3 hrs at 100° C. The time of EO isolation was determined by counting from the moment when the plant materials in the flask started to boil and the first drop was distilled. The hydro-distillation system was heated by a heating mantle, placed under the flask containing the plant material and distilled water. The condenser of the Clevenger was attached to a running tap water (Bardakei et al., 2019). Once the mixture started boiling, the steam-volatile components of the samples were condensed, and the insoluble, lighter than water, volatile oil was separated and collected on the surface of the water. The essential oils isolated were collected in 2 ml Eppendorff (EP) tubes, dehydrated over anhydrous sodium sulphate (Na_2SO_4). Thereafter, the essential oils were taken in fresh EP tubes and preserved in a -20° C. freezer (Bardakei et al., 2019) with proper sealing until GC/MS analyses were conducted. The EO extraction yields [average yields in mL/g, absolute yields in g and % yield (v/w)] were recorded. The percentage yield of EOs was computed using the below-mentioned equation:

$$y = x/z * 100 \quad \text{(i)}$$

Where y is the EO yield (mL/g), x is volume of the isolated EO (mL) and z is the mass of plant sample (g).

2.3. Identification of volatile compounds

For the detection of volatile components, the EOs were analyzed and identified using GC/MS. From each isolated anhydrous EO of fresh and dried lemon rinds and leaves, only 5 µL were diluted with 500 µL of *n*-hexane of HPLC grade. To it 1µL of 0.66% methyl myristate (methyl tetradecanoate) mixed in *n*-hexane was used as an internal standard and injected into GC via split-less mode. The separation of EO components was done using a DB-5-MS capillary column (Agilent J & W; GC columns, USA) of 30 m length, 0.25 mm diameter and 0.25 mm narrow-bore film of an Agilent 7890A GC equipped with a 5795C inert MSD with Triple Axis Detector. The analysis was done under the temperature programme: the oven temperature ramp was set at 60° C (5-minute hold time) to 220° C at the rate of 4° C / minute and held for 10 minutes, pressure 8.232 psi, purge flow 24 mL /minute, 55 minutes of run time. The injection temperature was set at 230° C, the MS transfer line at 280° C and the ion source at 250° C. Helium was used as the carrier gas at a constant flow rate of 1 mL /minute (carrier linear velocity of 36.623 cm/sec). Samples (1 µL) were injected with a standard septum purge flow mode, maintaining 3 minutes of solvent delay to prevent sample overload. MS detector was operated on the Electron Ionization (EI) method at 70eV (Karak et al., 2016).

The constituents of EOs were detected by aligning the fragmentation configuration of the mass spectral data of samples with those of the G1033A NIST 2011 (National Institute of Standards and Technology, USA: Agilent PMB Search format) mass spectral library entries. Only the compound hit that showed the highest matching factor (MF) and reverse MF (RMF) (≥ 650) was considered (Wahyuni et al., 2013). Confirmation of identification was also done based on the minimum deviation from the Retention Index (RI) value entries in the NIST database. Once the retention times of the alkane standards were properly determined, the RI of each compound was calculated.

Metabolites were further confirmed by computing the Arithmetic Index (AI) value by comparing the AI relative to alkane standards (C_{11} to C_{28}) with reported literature (Adams, 2009). When temperature programming is done, an Arithmetic Index (AI) would be more appropriate than a logarithm-based index. The Arithmetic Index (AI) was computed exploiting the formula:

$$AI\ (unknown) = 100\ P_Z + 100\ [RT_{(unknown)} - RT\ (P_z)] / (RT\ (P_{z+1}) - RT\ (P_z)] \quad \text{(ii)}$$

Where, P_z = the no. of carbon atoms in the smaller alkane, $RT_{unknown}$ = the retention time of the unknown compound, RT (P_z) = the retention time of the smaller alkane, RT (P_{z+1}) = the retention time of the larger alkane. An RI deviation of < 50 units and AI deviation of < 20 units were considered as reliable for the identification of components. The quantitation of individual identified volatile components was determined as a percentage of peak area relative to the total peak area from the GC/MS study of the samples.

2.4. Hierarchical Cluster Analysis

In this study, the results reported are the average values of three biological replicates. The chemical compositions of the *Citrus* EOs obtained from fresh and dry rinds as well as leaves of different species were subjected to cluster analysis. The EO compositions could be used as operational taxonomic units (OTUs), and the relative responses of the components detected were used to define the chemical fingerprints between the volatile organic compounds (VOCs) of the EOs of different *Citrus* species using Hierarchical Cluster Analysis (HCA) using Metaboanalyst 5.0 version. Dissimilarities were measured using Euclidean distance and cluster analysis was done using Ward's method. PCA and PLS-DA were accomplished using the same software.

Multivariate analysis (MVA) approaches for example PCA and PLS are used to decipher the importance in metabolomics raw datasets, where spectral characters participating mostly for distinction or discrimination are acknowledged for additional analysis (Worley & Powers, 2015).

3. Results and Discussion

3.1. Variability in the EOs' yield

In this study, different species of *Citrus*, popularly cultivated and available in plentiful amounts in West Bengal, India were picked for evaluating the variability of yield and characterisation in their essential oils. The total volume (mL), absolute yield (g) and percentage yield (w/v) of 50g of fresh fruit rinds of *C. reticulata*, *C.* x

sinensis, *C. limon*, *C.* x *limonia*, *C. medica*, and *C. maxima*, 25g of dehydrated rinds of *C.* x *aurantifoila*, *C. reticulata* and *C. maxima*, 60g of fresh leaves of *C. limon* and *C.* x *limonia* and 40g of dry leaves of *C. limon* were compared correspondingly (Table 1).

Table 1. Variability of EOs' yield in various *Citrus* species

Plant materials	(fresh / dry)	Weight of sample (g)	Total volume (mL)	Absolute yield (g)	Percentage yield (v/w) (%)
Fresh rinds	*C. reticulata*	50	5.48	4.658	9.316
	*C.*x *sinensis*	50	3.174	3.332	6.664
	C. limon	50	1.03	0.999	1.998
	*C.*x *limonia*	50	1.66	1.709	3.418
	C. medica	50	0.625	0.613	1.225
	C. maxima	50	0.3	0.261	0.522
Dehydrated rinds	*C.* x *aurantifoila*	25	0.945	0.926	3.704
	C. reticulata	25	3.67	3.927	15.708
	C. maxima	25	0.1	0.085	0.34
Fresh leaves	*Citrus limon*	60	0.63	0.611	1.108
	Citrus x *limonia*	60	0.49	0.505	0.841
Dry leaves	*C. limon*	40	0.879	0.835	2.087

3.2. Variability in VOCs

In this study, the variability of VOCs from the EOs obtained from the studied *Citrus* species was investigated by GC/MS study. A total number of 78 VOCs were recognised from the EOs of different *Citrus* species studied. The normalized data of the relative responses of individual compounds was deposited in Metaboanalyst 5.0. PCA (Fig. 2) and PLS-DA (Fig. 3) was performed and the metabolites responsible for their differentiation were determined based on VIP scores. Normalised values were also subjected to ANOVA and Tukey's HSD Post-hoc test, designated 74 significant metabolites ($p < 0.05$) out of the 78 identified compounds. A Dendrogram was produced based on generalized logarithm transformed dataset.

The volatile metabolites identified were 11 monoterpenes, 12 sesquiterpenes, 10 aldehydes, 25 alcohols, 8 ethers, 4 esters, 3 ketones, 2 hydrocarbons and 3 unknown compounds presented in Table 2.

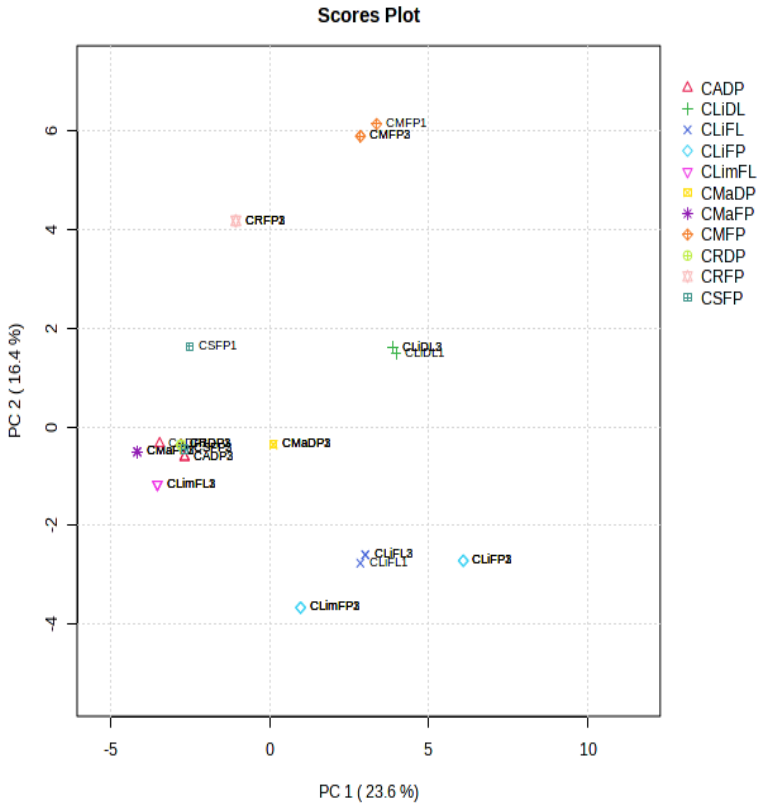

Figure 2. Principal Component Analysis (PCA) of the *Citrus* species studied; *C.* x *aurantifolia* dry peel (CADP); *C. limon* dry leaf (CLiDL); *C. limon* fresh leaf (CLiFL); *C.* x *limonia* fresh peel (CLiFP); *C.* x *limonia* fresh leaf (CLimFL); *C. maxima* dry peel (CMaDP); *C. maxima* fresh peel (CMaFP); *C. medica* fresh peel (CMFP); *C. reticulata* dry peel (CRDP); *C. reticulata* fresh peel (CRFP); *C.* x *sinensis* fresh peel (CSFP)

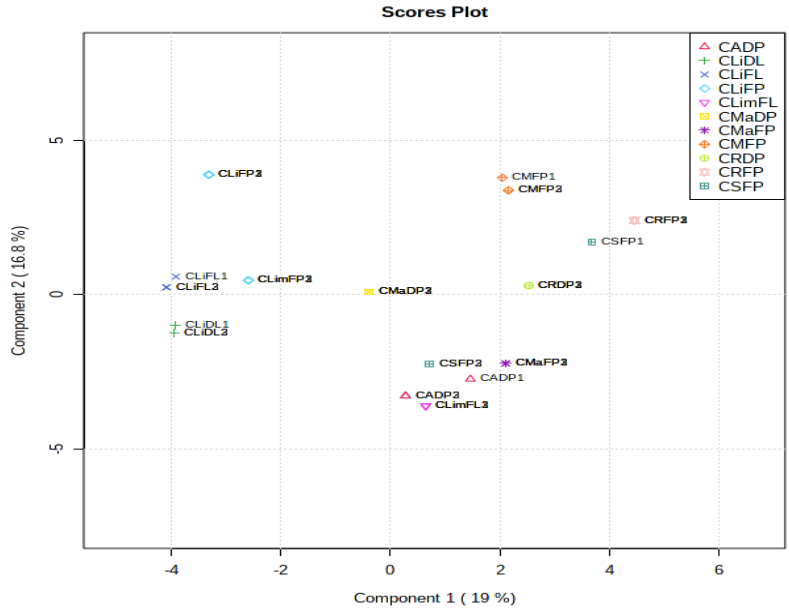

Figure 3. PLS-DA 2-D scores plot of the *Citrus* species studied; *C.* x *aurantofolia* dry peel (CADP); *C. limon* dry leaf (CLiDL); *C. limon* fresh leaf (CLiFL); *C.* x *limonia* fresh peel (CLiFP); *C.* x *limonia* fresh leaf (CLimFL); *C. maxima* dry peel (CMaDP); *C. maxima* fresh peel (CMaFP); *C. medica* fresh peel (CMFP); *C. reticulata* dry peel (CRDP); *C. reticulata* fresh peel (CRFP); *C.* x *sinensis* fresh peel (CSFP)

Table 2. List of volatile organic compounds identified in the seven *Citrus* species studied; RT = retention time, AI calculated = experimental arithmetic index; Adam's AI = literature Adam's index, nd = not detected

					Area percentage					
					Orange *Citrus reticulata* dry peel	Orange *Citrus reticulata* fresh peel	Musambi *Citrus* x *sinensis* fresh peel	Kaghchi *Citrus* x *aurantifolia* dry peel	Paati *Citrus medica* fresh peel	Batapi pomelo *Citrus maxima* dry peel
Chemical classes	Metabolites	RT	AI calculated	Adam's AI	CRDP	CRFP	CSFP	CADP	CMFP	CMaDP
Monoterpene	α-Pinene	10.919	933	932	nd	0.31 ± 0.01	0.54 ± 0.01	0.21 ± 0.002	0.002 ± 0.01	0.23 ± 0.01
Bicyclic monoterpene	Sabinene	12.55	974	969	nd	nd	nd	0.17 ± 0.001	nd	nd
Monoterpene	β-Pinene	13.102	988	974	1.196 ±0.01	0.53 ± 0.02	1.09 ± 0.01	0.39 ± 0.00	0.09 ± 0.01	1.14 ± 0.001
Bicyclic monoterpene	delta-3-Carene	13.865	1007	1008	nd	nd	nd	0.40 ± 0.01	nd	nd
Cyclic monoterpene	D-Limonene	15.041	1036	1024	84.48 ± 0.001	88.40 ± 0.002	92.18 ± 0.001	97.30 ± 0.01	90.25 ± 0.00	89.89 ± 0.003
Monoterpene	trans-β-Ocimene	15.468	1047	1044	nd	nd	nd	nd	nd	nd
Monoterpene	α-Ocimene	16.292	1067		nd	nd	nd	nd	nd	nd
Aldehyde	Bergamal	16.379	1069	1051	nd	nd	nd	nd	nd	nd
Ether	α-Pinene oxide	16.824	1080	1099	nd	nd	nd	nd	nd	nd
Monoterpene	γ-Terpinene	17.093	1086	1054	nd	7.18 ± 0.003	3.09 ± 0.01	nd	nd	0.20 ± 0.02
Hydrocarbon	p-Mentha-1,4(8)-diene	17.286	1091	1080	8.05 ± 0.02	nd	nd	nd	nd	0.05 ± 0.002
Ketone	Chrysanthenone	17.433	1096	1124	nd	nd	nd	nd	nd	nd
Hydrocarbon	α-Terpinolene	17.618	1099	1086	nd	0.18 ± 0.001	0.12 ± 0.00	0.04 ± 0.01	nd	nd
Aldehyde	n-Nonanal	18.007	1109	1100	nd	nd	nd	nd	nd	nd
Ether	Rose oxide	18.027	1110	1106	nd	nd	nd	nd	nd	nd
Alcohol	β-Linalool	17.831	1105	1095	3.00 ± 0.01	1.13 ± 0.01	1.23 ± 0.02	0.74 ± 0.01	3.68 ± 0.01	0.89 ± 0.01
Monoterpene	p-Mentha-1,3,8-triene	18.541	1123	1108	nd	nd	0.02 ± 0.001	nd	nd	nd
Monoterpene	Allo-Ocimene	18.818	1130	1128	nd	nd	nd	nd	nd	nd
Monoterpene	Neo-allo-ocimene	18.844	1130	1140	nd	nd	nd	nd	nd	0.03 ± 0.001
Ethers	cis-Limonene oxide	19.088	1137	1132	nd	nd	nd	nd	nd	nd

Ethers	trans- Limonene oxide	19.339	1144	1137	nd	0.02 ± 0.001	nd	nd	nd	nd
Alcohol	trans-3(10)-Caren-2-ol	19.434	1146		nd	nd	nd	nd	nd	0.07 ± 0.01
Aldehydes	β-Citronellal / (R)-(+)-Citronellal	19.757	1155	1148	nd	0.052 ± 0.001	0.11 ± 0.01	0.05 ± 0.01	0.13 ±0.001	0.23 ± 0.001
Cyclic nonaromatic alcohol	L-isopulegol	19.882	1158	1145	0.16 ± 0.001	nd	nd	nd	nd	nd
Alcohol	cis-Verbenol	20.14	1165	1137	nd	nd	nd	nd	nd	0.17 ± 0.01
Ether	Limonene oxide, cis-	20.916	1185	1132	nd	nd	nd	nd	nd	0.52 ± 0.002
Alcohol	L-terpinen-4-ol	21.002	1187	1174	0.72 ± 0.001	0.13 ± 0.001	0.39 ± 0.01	0.26 ± 0.02	1.08 ± 0.001	nd
Aldehyde	1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde	21.127	1190		nd	nd	nd	nd	nd	nd
Alcohol	cis-p-mentha-1(7),8-dien-2-ol	21.272	1194	1227	nd	nd	nd	nd	nd	nd
Alcohol	L-α-Terpineol	21.509	1200	1186	0.68 ± 0.002	0.16 ± 0.01	0.34 ± 0.001	0.11 ± 0.001	2.42 ± 0.001	0.12 ± 0.02
Aldehyde	n-Decanal	21.79	1208	1201	0.34 ± 0.001	0.16 ± 0.01	0.17 ± 0.01	0.10 ± 0.02	nd	0.16 ± 0.03
Ketone	D-Verbenone	22.075	1215	1204	nd	nd	nd	0.08 ± 0.002	nd	nd
Alcohol	cis-Carveol	22.447	1226	1226	0.41 ± 0.001	nd	0.23 ± 0.01	nd	nd	nd
Alcohol	Citronellol	22.522	1228	1223	nd	0.09 ± 0.001	nd	nd	nd	nd
Aldehyde	cis-Neral/ cis-Citral	22.946	1240	1235	0.10 ± 0.01	0.02 ± 0.001	0.07 ± 0.001	0.06 ± 0.001	0.95 ± 0.002	2.20 ± 0.002
Ketone	(-)-Carvone	23.229	1248	1239	nd	0.02 ± 0.002	nd	nd	0.33 ± 0.002	nd

					Area percentage					
					Batapi pomelo	Gora lebu	Gora lebu	Gondhoraj	Gondhoraj	Gondhoraj
					Citrus maxima	*Citrus* x *limonia*	*Citrus* x *limonia*	*Citrus limon*	*Citrus limon*	*Citrus limon*
					fresh peel	fresh peel	leaf fresh	dry leaf	fresh leaf	peel fresh
Chemical classes	Metabolites	RT	AI calculated	Adam's AI	CMaFP	CLiFP	CLimLF	CLiDL	CLiFL	CLiFP
Monoterpene	α-Pinene	10.919	933	932	nd	nd	nd	0.27 ± 0.002	nd	nd
Bicyclic monoterpene	Sabinene	12.55	974	969	nd	nd	6.77 ± 0.04	nd	nd	nd
Monoterpene	β-Pinene	13.102	988	974	0.35 ± 0.01	0.16 ± 0.001	nd	0.64 ± 0.02	0.21 ± 0.004	0.28 ± 0.002
Bicyclic monoterpene	delta-3-Carene	13.865	1007	1008	nd	nd	nd	nd	nd	nd
Cyclic monoterpene	D-Limonene	15.041	1036	1024	95.75 ± 0.002	9.49 ± 0.001	26.39 ±0.004	74.98 ± 0.04	17.41 ± 0.01	61.59 ± 0.003
Monoterpene	trans-β-Ocimene	15.468	1047	1044	nd	0.20 ± 0.04	0.29 ± 0.01	nd	nd	nd
Monoterpene	α-Ocimene	16.292	1067		nd	nd	nd	nd	nd	0.03 ± 0.02
Aldehyde	Bergamal	16.379	1069	1051	nd	nd	nd	nd	nd	0.01 ± 0.01
Ether	α-Pinene oxide	16.824	1080	1099	nd	nd	nd	nd	nd	0.03 ± 0.002
Monoterpene	γ-Terpinene	17.093	1086	1054	nd	nd	nd	nd	nd	nd
Hydrocarbon	p-Mentha-1,4(8)-diene	17.286	1091	1080	0.04 ± 0.02	nd	nd	nd	nd	nd
Ketone	Chrysanthenone	17.433	1096	1124	nd	nd	nd	nd	nd	0.02 ± 0.002
Hydrocarbon	α-Terpinolene	17.618	1099	1086	nd	nd	nd	nd	nd	nd
Aldehyde	n-Nonanal	18.007	1109	1100	nd	nd	nd	0.41 ± 0.02	nd	nd
Ether	Rose oxide	18.027	1110	1106	nd	0.05 ± 0.002	nd	nd	nd	nd
Alcohol	β-Linalool	17.831	1105	1095	2.97 ± 0.01	nd	6.09 ± 0.002	nd	nd	0.14 ± 0.002
Monoterpene	p-Mentha-1,3,8-triene	18.541	1123	1108	nd	nd	nd	nd	nd	nd
Monoterpene	Allo-Ocimene	18.818	1130	1128	nd	nd	nd	0.12 ± 0.02	nd	nd

Monoterpene	Neo-allo-ocimene	18.844	1130	1140	nd	nd	nd	nd	nd	0.07 ± 0.01
Ethers	cis-Limonene oxide	19.088	1137	1132	nd	nd	nd	0.09 ± 0.01	nd	0.20 ± 0.001
Ethers	trans- Limonene oxide	19.339	1144	1137	nd	nd	nd	nd	nd	nd
Alcohol	trans-3(10)-Caren-2-ol	19.434	1146		nd	nd	nd	nd	nd	nd
Aldehydes	β-Citronellal / (R)-(+)-Citronellal	19.757	1155	1148	0.08 ± 0.03	81.53 ± 0.04	55.51 ± 0.03	19.73 ± 0.001	72.76 ± 0.003	14.48 ± 0.02
Cyclic nonaromatic alcohol	L-isopulegol	19.882	1158	1145	nd	nd	nd	nd	nd	0.003 ± 0.00
Alcohol	cis-Verbenol	20.14	1165	1137	nd	nd	nd	0.05 ± 0.02	nd	nd
Ether	Limonene oxide, cis-	20.916	1185	1132	nd	nd	nd	nd	nd	nd
Alcohol	L-terpinen-4-ol	21.002	1187	1174	0.12 ± 0.03	nd	1.06 ± 0.002	0.07 ± 0.02	nd	nd
Aldehyde	1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde	21.127	1190		nd	nd	nd	nd	nd	0.42 ± 0.02
Alcohol	cis-p-mentha-1(7),8-dien-2-ol	21.272	1194	1227	nd	nd	nd	nd	nd	0.08 ± 0.002
Alcohol	L-α-Terpineol	21.509	1200	1186	0.28 ± 0.003	nd	0.12 ± 0.002	nd	nd	0.12 ± 0.002
Aldehyde	n-Decanal	21.79	1208	1201	nd	0.25 ± 0.001	nd	0.28 ± 0.001	0.41 ± 0.002	0.30 ± 0.01
Ketone	D-Verbenone	22.075	1215	1204	nd	0.08 ± 0.02	nd	nd	nd	nd
Alcohol	cis-Carveol	22.447	1226	1226	nd	nd	nd	nd	nd	nd
Alcohol	Citronellol	22.522	1228	1223	0.15 ± 0.001	1.43 ± 0.001	3.03 ± 0.002	1.30 ± 0.001	2.63 ± 0.01	13.93 ± 0.003
Aldehyde	cis-Neral/ cis-Citral	22.946	1240	1235	0.08 ± 0.001	nd	0.32 ± 0.02	0.31 ± 0.03	nd	nd
Ketone	(-)-Carvone	23.229	1248	1239	nd	nd	nd	nd	nd	nd

To better understand the relationships among the VOCs present in the different *Citrus* samples (fresh as well as dehydrated rinds and leaves), PCA (Fig. 2) and PLS-DA (Fig. 3) were applied to the experimental results. It was very easy to understand that there was substantial variability in the chemical components of VOCs in the studied samples.

The HCA was performed on the VOCs of *Citrus* species and the dendrogram showed two main clusters (Fig. 4). Cluster II was constituted of six samples including *C.* x *sinensis* fresh rind, *C.* x *aurantifolia* dry rind, *C.* x *limonia* fresh leaf, *C. maxima* fresh rind, *C. reticulata* dry rind, and *C. maxima* dry peel. Cluster I was composed of six *Citrus* samples including *C. limon* fresh leaf, *C. limon* dry leaf, *C.* x *limonia* fresh rind, *C. maxima* fresh rind, *C. reticulata* fresh rind, and *C. limon* fresh rind.

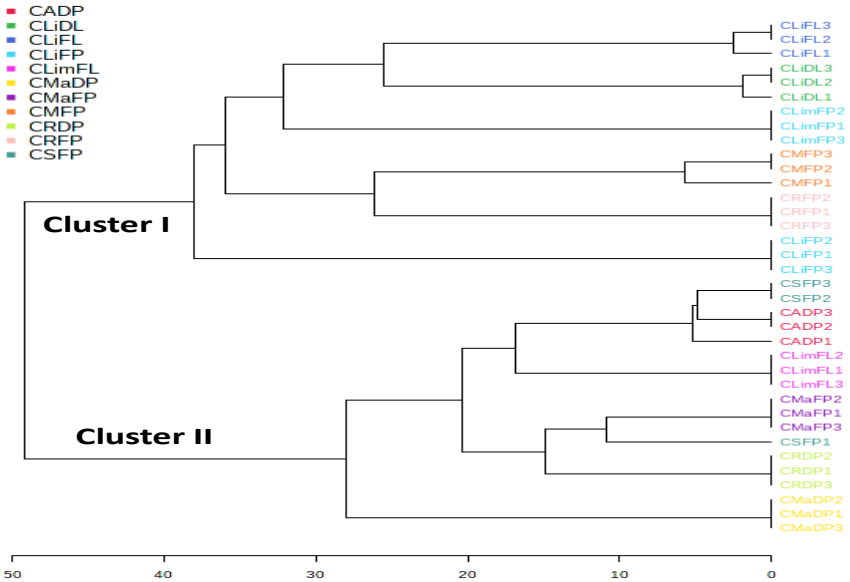

Figure 4. Dendogram obtained by hierarchical cluster analysis of VOCs of the *Citrus* species under study based on Ward's method using the Euclidean distances. *C.* x *aurantofolia* dry peel (CADP); *C. limon* dry leaf (CLiDL), *C. limon* fresh leaf (CLiFL), *C.* x *limonia* fresh peel (CLiFP), *C.* x *limonia* fresh leaf (CLimFL); *C. maxima* dry peel (CMaDP); *C. maxima* fresh peel (CMaFP); *C. medica* fresh peel (CMFP); *C. reticulata* dry peel (CRDP); *C. reticulata* fresh peel (CRFP); *C.* x *sinensis* fresh peel (CSFP); 1 - 4 depicted 4 biological replicates of same sample.

Based on Fig. 5, we could be able to identify the top 15 VOCs for which the different *Citrus* species and samples under study were different from each other. The top most 15 metabolites based on VIP (Variable importance of projection) scores of PLS-DA were: α-humulene, L-perillaldehyde, β-citronellal, α-bisabolol, caryophyllene, cedr-8-en-13-ol, isolongifolol, δ-elemene, n-dodecanol, α-terpinolene, cis-neral, α-bisabolene, (-)-carvone, cis-limonene-oxide, and trans-limonene oxide.

Based on this study, we find that α-humulene is present in the highest concentration in *C. limon* dry leaf, and then in *C. limon* fresh leaf and next in *C. limon* fresh peel. L-perillaldehyde is present in the highest relative concentration in *C. reticulata* fresh peel, followed by *C. reticulata* dry peel. β – citronellal was present in its highest concentration in *C. reticulata* fresh peel and next in *C. maxima* fresh peel. α-bisabolol, caryophyllene, cedr-8-en-13-ol, isolongifolol and α-bisabolene were present in the highest concentration in *C. limon* fresh leaf. δ-elemene was found its highest concentration in *C. limon* dry leaf. *n*-dodecanal was found in maximum amounts both in *C. medica* fresh peel and *C. reticulata* fresh peel. α-terpinolene was found to be present in the highest concentration in *C.* x *sinensis* fresh peel. Cis-neral, (-) -carvone and trans-limonene oxide were found in the highest amounts in *C. reticulata* fresh peel.

Based upon percentage peak area calculated, the cyclic monoterpene, D-limonene was estimated as the chief volatile component in the EOs extracted, ranging from 97.302 ± 0.01% in dry peel of *C.* x *aurantifolia* > 95.747 ± 0.002% in *C. maxima* fresh peel > 90.251± 0.00% in *C. medica* fresh peel > 89.89 ± 0.003% in *C. medica* dry peel > 88.392 ± 0.002% in *C. reticulata* fresh peel > 88.392 ± 0.002% in *C. reticulata* fresh peel > 84.48 ± 0.001% in *C. reticulata* dry peel > 74.981 ± 0.04% in *C. limon* dry leaf > 61.489 ± 0.003% in *C. limon* fresh peel > 26.389 ± 0.004% *C.* x *limonia* fresh leaf > 17.412 ± 0.01% in *C. limon* fresh leaf > 9.494 ± 0.001% in *C.* x *limonia* fresh peel. It was the only compound found in all the studied samples, both in fresh and dehydrated conditions. The monoterpene, β -pinene was detected in all the experimental samples except in the EO of *C.* x *limonia* fresh leaf. The alcohol, β-linalool was found in all except in *C. limon* dehydrated as well as fresh leaf samples. The highest percentage of peak area of β-linalool was calculated in *C.* x *limonia* fresh leaf (6.092 ± 0.002%). *C. medica* fresh rind and *C. maxima* fresh rind also contained β-linalool in 3.675 ± 0.01% and 2.974 ± 0.01% respectively. *C. reticulata* dry and fresh peel contained 3.001 ± 0.01% and 1.131 ± 0.01 % of β-linalool. *C.* x *sinensis* contained 1.233 ± 0.02% of β-linalool in its EO. The aldehyde β-citronellal was present in a high percentage peak area in *C.* x *limonia* fresh rind (81.533 ± 0.004%) > in *C. limon* fresh leaf (72.757 ± 0.003%) > *C.* x *limonia* fresh leaf (55.51 ± 0.03%) > *C. limon* dry leaf (19.733 ± 0.001 %) > *C. limon* fresh rind (14.479 ± 0.02%).

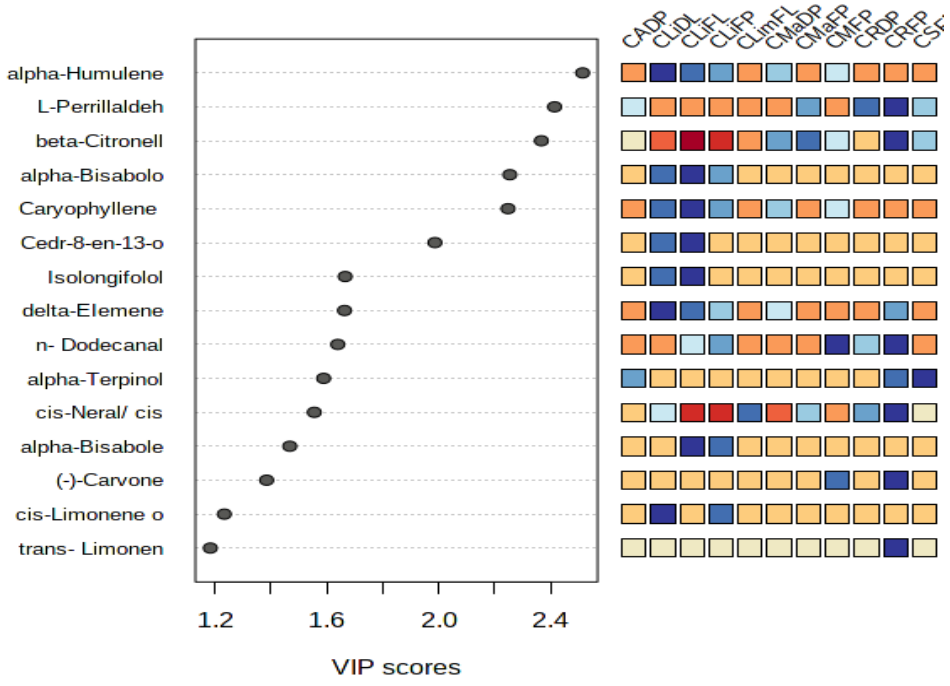

Figure 5. VIP scores of PLS-DA showing top 15 metabolites. The coloured boxes on the right indicate the relative concentrations of the corresponding metabolites in *Citrus* species under study. *C.* x *aurantifolia* dry peel (CADP), *C. limon* dry leaf (CLiDL), *C. limon* fresh leaf (CLiFL); *C.* x *limonia* fresh peel (CLiFP); *C.* x *limonia* fresh leaf (CLimFL); *C. maxima* dry peel (CMaDP); *C. maxima* fresh peel (CMaFP); *C. medica* fresh peel (CMFP); *C. reticulata* dry peel (CRDP); *C. reticulata* fresh peel (CRFP); *C.* x *sinensis* fresh peel (CSFP)

Previously EOs from the rinds of Malta (*C. limetta*, a cultivar of *C. limon*), Mousambi (*C.* x *sinensis*), Grapefruit (*C. paradisi*) and Eureka lemon (*C. limon*) were isolated employing the cold press method, and the chemical composition of the EOs of the species was investigated by GC/FID on a Carbowax 20 M packed glass column. It was reported that composition of the EOs varied significantly among the species, which may be due to their variation in genetic makeup (Ahmad et al., 2006). The volatile aroma and flavour metabolies of flower, leaf, rind and "Page" mandarin juice were scrutinised and the compounds were separated by ultrasound water bath apparatus, water distillation method, by utilizing poly dimethyl siloxane membranes (PDMS) and cold press technique, respectively, and then eluted by *n*-pentane: diethylether

(1:2), *n*-hexane, pentane: dichloromethane (2:1) and *n*-hexane respectively and were analyzed by GC-FID and GC/MS, and it was reported that the percentage of flavour molecules was significantly different from organ to organ (Darjazi, 2011). The EO components from the leaf and fruit rinds of *C. reticulata* Blanco cv. Santra (Santra mandarin) cultivated in Egypt were assessed qualitatively and quantitatively using GLC and GLC/MS and 131 components were identified and quantified. The Egyptian Santra mandarin chemotype was discriminated for the presence of limonene in rind oil and sabinene and linalool in leaf oil (Hamdan et al., 2016). The difference in yield, chemical characteristics and detections in solvent-assisted oils extracted from the dehydrated rinds and seeds of the two different *Citrus* samples from Isinbode-Ekiti, Ekiti-State, Nigeria –*C. sinensis* var. Shamuti and *C. paradisi* var. Marsh planted in a cocoa farm was investigated, and it was found that the raw rinds and seeds have a lower yield and a higher percentage of metabolites that serve as compound detection for the *Citrus* family, whereas the dehydrated rinds and seeds have alcohol components like spathulenol, linalool, nerol, α-terpeniol and farnesol, which are not present in the fresh samples (Adebisi, 2014).

In a review report by Gonzales Mas et. al., 2019, based upon quantitative insight, the greatest copious metabolites in EO of *C. reticulata* were found monoterpene hydrocarbons. Among these, the chief pertinent is limonene, usually indicating about 95% of the total EO, but sometimes down to 60% in some analysis (Fanciullino et al., 2006; Tao et al., 2014). The following compounds in large quantity are γ-terpinene, sometimes accomplished above 15% (Mondello et al., 2003; Petretto et al., 2016), β-myrcene (7.43–0.1%) (Fanciullino et al., 2006; Tao et al., 2014), α-pinene (3.93–0.1%) (Fanciullino et al., 2006; Tao et al., 2014) or β-pinene (4% - traces) (Fanciullino et al., 2006). Linalool and β-citronellal were reported to contain up to 2.9% and 0.6%, respectively (Tao et al., 2014). Amongst minor metabolites with great quantities approximately between 0.7 and 0.1%, sesquiterpene α-sinensal, the non-terpene aliphatic compounds octanal and decanal, and the aromatic compound methyl N-methylanthranilate were reported (Gonzales Mas et. al., 2019).

As per Gonzales Mas et al., 2019; the VOCs of *C.* x *sinensis* peel EO is the most analyzed *Citrus* species along with those of *C. reticulata* and *C. limon*. This species seems to be higher in the diversity of sesquiterpene hydrocarbons such as aromadendrene (Njoroge et al., 2005; Hosni et al., 2010) and sesquiphellandrene (Ruberto and Rapisarda, 2002; Sawamura et al., 2005) than other species as *C. reticulata* or *C. limon*. The major VOCs in *C. sinensis* are very identical to that of *C. reticulata*, with analogous proportions. Limonene is generally affirmed between 90 and 97% in *C.* x *sinensis*, although this percentage decreased down to 64% in some analyses (Chen et al., 2014).

The most plentifully present compound in *C. medica* is limonene, but its % can drop down to 51% (Verzera et al., 2005), while other monoterpene compounds such as γ-terpinene, β-pinene or camphene are present at greater concentration, in comparison with species of *C. reticulata* cluster where these compounds are usually described in percentages below 1%. Thus, in *C. medica* oil, γ-terpinene, β-pinene or camphene can reach upto 31%, 9.7%, and 10%, respectively (Aliberti et al., 2016; Petretto et al., 2016). Also *C. medica* represents higher amounts of some sesquiterpenes, as is the case of (E)-α-bergamotene (Aliberti et al., 2016) or germacrene D (Petretto et al., 2016), although their copiousness is usually lower than 0.5%.

Many mono- and sesquiterpene hydrocarbons and oxygenated monoterpenes have been reported in *C. aurantifolia* rind. The % of limonene may decrease to 39.9% in the oil of *C. aurantifolia* (Lota et al., 2002), and the prevalence of other terpene compounds is improved, such as β-pinene, neryl acetate, geranyl acetate, β-bisabolene, (E)- α-bergamotene, germacrene D and β-caryophyllene (Lota et al., 2002; Minh Tu et al., 2002a).

Quantitatively, the chief compound of *C. limon* EO is limonene, at levels usually ranging between 70 and 48%. Geranial and neral are some of the more richly present compounds reported so far (Lota et al., 2002; Loizzo et al., 2016).

Our results substantiate the earlier studies on some species of the *Citrus* EOs, and still we report several other VOCs much more elaborately and also some marker metabolites for each of the studied species for the first time with the help of multivariate analytical approaches.

4. Conclusions

In conclusion, based on this study, it can be said that an increasing amount of fruit rinds and other agricultural wastes can be useful as a source of bioactive substances. This idea of utilizing agricultural wastes may increase the financial strength of farmers and decrease the problem of agricultural waste management. Moreover, in India, the organic wastes generated from food processing industries are highly hazardous to the environment and can be a potential source for extraction of bioactive compounds.

Citrus fruits have a very high-water content, making them difficult to dry through common conventional methods or industrial drying devices. Before disposing of the peel waste in and around landfills, extraction of EOs from peels is very important, because EOs adversely influence fermentation and bacterial degradation. Expulsion of waste into waterbodies may cause pollution and devastation of aquatic life. Wastes released into urban garbage or sewage structure can contaminate aquatic resources below the surface, cause impairment to pumps and piping, choke gravel beds, and produce froth in primary settling reservoirs. Thus, direct disposal of *Citrus* waste without proper processing causes environmental hazard.

In this study, we also find a clear understanding of the VOCs of *Citrus* rind and leaf wastes (both in fresh and dehydrated conditions), many of which are bioactive VOCs and may have medicinal benefits against various diseases. In this research we have determined different classes of VOCs, namely monoterpene (Cyclic, bicyclic), aldehyde, ketone, ether, alcohols, cyclic nonaromatic alcohols, hydrocarbons and their variation in content in the studied *Citrus* species. The present study also focused on the simple hydro-distillation extraction method of various beneficial value-added metabolites obtained from their EOs from these wastes, which are extremely costly and time-consuming to produce using typical chemical approaches. The EOs and their isolated components may be exploited in therapeutic implementation and as plant-based value-additives for functional foods.

This work also represents the identification of VOCs which are species specific biomarkers based on which the species could be useful for food, flavor, aroma and therapeutic industries. This research provides significant facts and figures in the selection of *Citrus* species for volatile chemicals for pharmaceutical, food, beverages, flavor, fragrances, cosmeceuticals and nutraceticals etc.

5. Author Contributions

SD and RS conceptualized the experimental design and SD performed the data analyses and wrote the manuscript. RS, collected the samples. SM, GM and MB performed the extraction and isolation of the EOs from different samples and injected the EOs in GC/MS. All authors contributed to the article and approved the submitted version.

Acknowledgements

GC-MS based work supported by DST-FIST Programme, Govt. of India [Grant Number: SR/FST/LS1-459/2010] is acknowledged.

References

Adebisi O. 2014. Comparative study of essential oil composition of fresh and dry peel and seed of Citrus sinensis (L) Osbeck var Shamuti and Citrus paradise Macfadyen var Marsh. *IJS.***16 (2)**: 211-217.

Adams RP. 2009. Identification of Essential Oil Components by Gas chromatography/Mass Spectrometry, 4th ed.; Allured Business Media: Illinois.

Ademosun AO. 2022. Citrus peels odyssey: From the waste bin to the lab bench to the dining table. *Appl. Food Res.* **2**: 100083.

Ahmad MM, Rehman SU, Anjum FM and Sultan JI. 2006. Genetic Variability to Essential Oil Composition in Four Citrus fruit species. *Pak. J. Bot.* **38 (2)**: 319-324.

Aliberti L, Caputo L, de Feo V, de Martino L, Nazzaro F. and Souza LF. 2016. Chemical composition and in vitro antimicrobial, cytotoxic, and central nervous system activities of the essential oils of Citrus medica L. cv. 'Liscia' and C. medica cv. 'Rugosa' cultivated in Southern Italy. *Molecules* **21**: E1244.

Asikin Y, Maeda G, Tamaki H, Mizu M, Oku H and Wada K. 2015. Cultivation line and fruit ripening discriminations of Shiikuwasha (*Citrus depressa* Hayata) peel oils using aroma compositional, electronic nose and antioxidant analyses. *Food Res. Int.* **67**: 102-110.

Bardakei H, Servi H and Polatoglu K. 2019. Essential oil composition of *Salvia candidissima* Vahl. *occidentalis* Hedge, *S. tomentosa* Miller and *S. heldreichiana* Boiss. Ex Benthum from Turkey. *J. Essent. Oil - Bear. Plants.* **22 (6)**: 1467-1480.

Ben Hsouna A, Ben Halima N, Smaoui S and Hamdi N. 2017. Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. *Lipids Health Dis.***16**: 1-11.

Chen Y. Wu J, Xu Y, Fu M, and Xiao G. 2014. Effect of second cooling on the chemical components of essential oils from orange peel (Citrus sinensis). *J. Agric. Food Chem.* **62**: 8786–8790.

Darjazi BB. 2011. Comparison of volatile components of flower, leaf, peel and juice of 'Page' mandarin [(Citrus reticulate var 'Dancy' x Citrus paradise var 'Duncan') x Citrus clementina]. *Afr. J. Biotechnol.* **10 (51)**: 10437-10446.

De Gregorio A, Mandalari G, Arena N, Nucita F, Tripodo M M and Lo Curto, R B. 2002. SCP and crude pectinase production by slurry-state fermentation of lemon pulps. *Bioresour Technol.* **83**: 89–94.

Dugo P and Mondello L. 2011. Citrus Oils: Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity, 49. Boca Raton, FL: CRC Press

Fancello F, Petretto G L, Zara S, Sanna M L, Addis R, Maldini M. et al. 2016. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of Citrus limon var. pompia leaf essential oil. *LWT Food Sci. Technol.* **69**: 579-585.

Fanciullino AL, Tomi F., Luro, F., Desjobert, J. M., and Casanova, J. (2006). Chemical variability of peel and leaf oils of mandarins. Flavour Frag. J. **21:** 359–367.

Fierascu RC, Fierascu I, Avramescu SM and Sieniawska E. 2019. Recovery of natural antioxidants from agro-industrial side streams through advanced extraction techniques. *Molecules*, **24**: 1-29.

Forney C and Song J. 2017. Flavors and aromas: chemistry and biological functions. In Fruit and Vegetable Phytochemicals; Wiley: Hoboken, NJ, USA,

Gherairia N, Boukerche S, Mustapha MA and Chefrour A. 2022. Effects of biotic and abiotic factors on the yield and chemical composition of essential oils from four Thymus species wild growing in Northeastern Algeria. *JJBS*, **15(2)**: 173 – 181.

Gonzalez-Mas MC, Rambla JL, Lopez-Gresa MP, Blazquez MA and Granell A. 2019. Volatile compounds in Citrus Essential oils: A comprehensive review. *Front. Plant Sci.* **10**: 1-18.

Hamdan DI, Mohamed ME and El-Shazly AM. 2016. Citrus reticulata Blanco cv. Santra leaf and fruit peel: A common waste products, volatile oils composition and biological activities. *J. Med. Plant res.* **10 (30)**: 457-467

Hosni K, Zahed N, Chrif R, Abid I., Medfei W, Kallel M, et al. (2010). Composition of peel essential oils from four selected Tunisian Citrus species: evidence for the genotypic influence. *Food Chem.* **123**: 1098–1104.

Karak S, Bhattacharya P, Nandy A, Saha A and De B. 2016. Metabolite Profiling and Chemometric Study for Varietal Difference in Piper Betle L. Leaf. *Curr. Metabolomics* **4(2)**: 129–140.

Lo Curto RB, Tripodo MM, Leuzzi U, Giuffre D and Vaccarino C.1992. Flavonoids recovery and SCP production from orange peel. *Bioresour Technol.* **42**: 83–87.

Loizzo MR, Tundis R, Bonesi M, Di Sanzo G, Verardi A, Lopresto CG, et al. (2016). Chemical profile and antioxidant properties of extracts and essential oils from Citrus x limon (L.) BURM. cv. Femminello comune. *Chem. Biodiversity* **13**: 571–581.

Lota ML, de Rocca Serra, D., Tomi, F., Jacquemond, C., and Casanova, J. (2002). Volatile components of peel and leaf oils of lemon and lime species. *J. Agric. Food Chem*. **50**: 796–805. doi: 10.1021/jf010924l

Mahato N, Sharma K, Sinha M, Baral E R, Koteswararao R. et al. 2020. Biosorbents, industrially important chemicals and novel materials from citrus processing waste as a sustainable and renewable bioresource: A review. *J. Adv. Res.* **23**: 61-82.

Marin FR, Soler-Rivas C, Benavente-Garcia O, Castillo J and Perez-Alvarez J. 2007. By-products from different citrus processes as a source of customized functional fibres. *Food Chem.* **100 (2)**: 736-741.

Mariod AA. 2016. Effect of Essential oils on organoleptic (Smell, Taste and Texture) properties of food. Essential oils in food preservation, flavor and safety, Elsevier Inc., London UK, Academic Press, 131-137.

Minh Tu NT, Onishi Y, Choi HS, Kondo Y, Bassore SM, Ukeda H. et al. 2002. Characteristic odor components of Citrus sphaerocarpa Tanaka (Kabosu) cold-pressed peel oil. J. Agric. *Food Chem.* **50:** 2908–2913.

Mondello L, Casilli A, Tranchida PQ, Cicero L, Dugo P, and Dugo G. 2003. Comparison of fast and conventional GC analysis for Citrus essential oils. *J. Agric. Food Chem.* **51:** 5602–5606.

Murdock DI. and Allen WE. 1960. Germicidal effect of orange peel oil and d-limonene in water and orange juice: Fungicidal properties against yeast. *Food Technol.* **14**: 441–445.

Nguyen H, Campi E M, Roy Jackson W and Patti AF. 2009. Effect of oxidative deterioration on flavour and aroma components of lemon oil, *Food Chem.***112 (2)**: 388–393.

Njoroge SM, Koaze H, Karanja PN, and Sawamura M. (2005). Essential oil constituents of three varieties of Kenyan sweet oranges (Citrus sinensis). *Flavour Frag. J.* **20**: 80–85

Petretto GL, Sarais G, Maldini MT, Foddai M, Tirillini B, Rourke JP. et al. 2016. Citrus monstruosa discrimination among several Citrus species by multivariate analysis of volatiles: a metabolomic approach. *J. Food Process. Preserv.* **40**: 950–957.

Raeissi S, Diaz S, Espinosa S, Peters CJ and Brignole EA. 2008. Ethane as an alternative solvent for supercritical extraction of orange peel oils. *J. Supercrit. Fluid.* **45**: 306–313.

Ruberto G. and Rapisarda P. 2002. Essential oils of new pigmented citrus hybrids: Citrus sinensis L. Osbeck x C. clementina Hort. ex Tanaka. *J. Food Sci.* **67**: 2778–2780.

Sarrou E, Chatzopoulou P, Dimassi-Theriou K and Therios I. 2013. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of Citrus aurantium L. growingin Greece. *Molecules.* **18**:10639–10647.

Sawamura M, Onishi Y, Ikemoto J, Minh Tu NT, and Lan Phi NT. 2006. Characteristic odour components of bergamot (Citrus bergamia Risso) essential oil. *Flavour Frag. J.* **21**: 609–615.

Subba MS, Soumithri TC and Suryanarayana R. 1967. Antimicrobial action of citrus oils. *J Food Sci.* **32**: 225–227.

Steuer B, Schulz H and L¨ager E. 2001. Classification and analysis of citrus oils by NIR spectroscopy. *Food Chem.***72 (1)**: 113–117.

Tranchida PQ, Bonaccorsi I, Dugo P, Mondello L and Dugo G. 2012. Analysis of Citrus essential oils: state of the art and future perspectives. A review. *Flavour Frag. J.* **27**: 98–123.

Tao N, Jia L, and Zhou H. (2014). Anti-fungal activity of Citrus reticulata Blanco essential oil against Penicillium italicum and Penicillium digitatum. *Food Chem.* **153**: 265–271.

Tripodo MM, Lanuzza F, Micali G, Coppolino R and Nucita F. 2004. Citrus waste recovery: a new environmentally friendly procedure to obtain animal feed. *Bioresour Technol.* **91**: 111–115.

Verzera A, Trozzi A, Zappalá M, Condurso C and Cotroneo A. 2005. Essential oil composition of Citrus meyerii Y. Tan. and Citrus medica L. cv. Diamante and their lemon hybrids. *J. Agric. Food Chem.* **53**: 4890–4894.

Worley B, and Powers R. 2013. Multivariate Analysis in Metabolomics. *Curr. Metabolomics.* **1(1)**: 92–107.

Wahyuni Y, Ballester AR, Tikunov Y, de Vos RCH, Pelgrom KTB, Maharijaya A, Sudarmonowati E, Bino RJ and Bovy AG. 2013. Metabolomics and Molecular Marker Analysis to Explore Pepper (Capsicum sp.) Biodiversity. *Metabolomics* **9**: 130–144.

Zema DA, Calabrò PS, Folino A, Tamburino V, Zappia G and Zimbone SM. 2018. Valorisation of citrus processing waste: A review. *Waste Manage.* **80**: 252-273.

Zema DA, Fòlino A, Zappia G, Calabrò PS, Tamburino V and Zimbone SM. 2018. Anaerobic digestion of orange peel in a semi-continuous pilot plant: An environmentally sound way of citrus waste management in agro-ecosystems. *Sci. Total Environ.* **630**: 401-408.

Zhang B, Chen J, He Z, Chen H and Kandasamy S. 2019. Hydrothermal liquefaction of fresh lemon-peel: parameter optimization and product chemistry. *Renew. Energy.***143**: 512–519.