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Volatilomic fingerprinting from edible flowers. Unravelling some impact compounds behind its attractiveness

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ABSTRACT

In recent years edible flowers emerged in gourmet cuisine, giving any dish the beauty of attractive colours, freshness, texture, and aromatic notes. Moreover, they also constitute a potential source of phytochemical compounds associated with beneficial effects on human health. In this work, the volatilomic fingerprinting of 4 different species of edible flowers [blue mallow (*Malva sylvestris* L.), pomegranate flower (*Punica granatum* L.), hibiscus (*Hibiscus rosa-sinensis* L.), and nasturtium (*Tropaeolum majus* L.)] used in gourmet dishes, was established, and comparatively investigated. The volatile metabolites were extracted by solid-phase microextraction in headspace mode and identified by gas chromatography-mass spectrometry to understand the chemistry behind its attractiveness better. A total of 78 volatile metabolites, belonging to diverse chemical groups were identified. Blue mallow is mainly characterised by sesquiterpenoids (61.5% of the total volatile fraction), whereas in flowers from pomegranate, hibiscus, and nasturtium, terpenoids (56.6%), carbonyl compounds (88.0%) and organosulfur compounds (98.0%) are the dominant chemical groups, respectively. In blue mallow flowers, τ-muurolene and valencene are the dominant volatiles, followed by α-cubebene and δ-cadinene. Pomegranate flowers are rich in furfural and linalool, while the aldehydes 2-hexenal, hexanal and 2-octenal are dominant volatile metabolites in hibiscus. Benzyl isothiocyanate, a potent antimicrobial agent, accounts for 98% of the total volatile fraction of nasturtium flowers. In addition to flavour notes, some of the identified volatile metabolites present bioactive properties, which could be explored for application in the food, pharmaceutical and cosmetic industries. The volatile metabolites profiles combined with unsupervised principal component analysis facilitated the differentiation of the edible flowers under investigation, revealing the most related volatile metabolites of each sample, which can be used as markers for the authentication of these valuable food samples.

1. Introduction

Since ancient times, foods of plant origin have been part of the human diet. Later, in the early days of civilisation, an essentially vegetarian diet predominated. With the evolution of times, humans directed their diet to foods of animal origin that lasted centuries. Although this type of diet prevails today, consumers are increasingly interested in changing this paradigm, adopting a diet based on plant origin foods. This change is essentially due to the harmful effects associated with the excessive consumption of foods of animal origin, especially when processed. In recent years, the use of edible flowers has emerged in the highly sophisticated gourmet cuisine, basically intending to make the dish more appetising and attractive to the consumer due to the diversity of colours, flavours and shades they present, thus enhancing the sensory quality and nutritional value of the dish.

From orange-red to purple-blue ones (depending on pH, co-pigment and metal ion), these attractive colours are attributed to the presence of a well-known family of pigments such as flavylium compounds like anthocyanins and anthocyanins-derived pigments, in addition to carotenoids (e.g., β-carotene, lutein and zeaxanthin), phenolic acids and flavonoids (e.g., apigenin, catechin, epigallocatechin gallate, luteolin, quercetin) (Fig. 1) (de Araújo et al., 2021).

Apart from beauty, the pigments play roles in plant reproduction by attracting pollinators, defending against biotic and abiotic stressor agents, and absorb excess UV–Vis light, preventing free radical formation. Volatile metabolites (VOMs) have also been shown to exhibit some

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of these characteristics.

The VOMs from biological systems are a series of more than 1700 molecules, generally lipophilic substances with low molecular weight, low boiling point, and high vapour pressure, from different chemical classes, originating from primary and secondary pathways (Dudareva et al., 2013). According to their origin, function, and biosynthesis, VOMs can be organised into three major clusters: terpenoids, phenylpropanoids, and fatty acid derivatives (Ramya et al., 2020).

In humans, the pigments and VOMs are associated with several health benefits, including antioxidant, anti-inflammatory, antidiabetic, neuroprotective and hepatoprotective effects (Daniloski et al., 2022; Delfine et al., 2017; Dowek et al., 2020; Mousavi et al., 2021). Indeed, some have been proven helpful in the prevention of several non-communicable diseases such as cardiovascular disease (CVD), cancer and neurodegenerative diseases (NDD) (Fig. 2).

The increasing consolidation in the international market of edible flowers is also related to the potential health effects associated with the presence of a wide variety of phytochemicals with pharmaceutical effects (Delfine et al., 2017). Lin et al. (2015) reported the induction of apoptotic death of human hepatocellular carcinoma (HepG2) cells by an ethanolic extract from *Litchi chinensis* Sonn. flower.

Native to Western Europe, North Africa and Asia, *Malva sylvestris* L. is widely cultivated as an ornamental and medicinal plant with anti-inflammatory, antioxidant, anticancer and antimicrobial properties (Mousavi et al., 2021). This species belongs to the *Malvaceae* family and presents edible parts like leaves and flowers. Zuo et al. (2017) evaluated the cardioprotective effect of *M. sylvestris* L. on myocardial ischemic/reperfusion (MI/R) in rats, and Xiao et al. (2020) revealed a novel circular RNA involved in the protective effect of *M. sylvestris* L. on myocardial ischemic/re-perfused injury. The anti-inflammatory effects of *M. sylvestris* alcoholic extracts by measuring the pro-inflammatory mediators PGE_2 and PGD_2 in desferrioxamine-stimulated phorbol 12-myristate 13-acetate-differentiated U937 cells, were evaluated by Martins et al. (2014).

Belonging to the Lythraceae family, *Punica granatum* L. (pomegranate) is a deciduous shrub native to the Persia region. The leaves, flowers, rinds, seeds, and roots from *P. granatum* L. are all edible. Pomegranate constituents are used for various ailments (Jurenka, 2008), due to their reported anticarcinogenic, antioxidant, and anti-inflammatory properties (Vučić et al., 2019). Its effects on the treatment and prevention of cancer (Ozkan et al., 2021), diabetes, CVD (Wang et al., 2018), erectile dysfunction (Jurenka, 2008), dental conditions, antibiotic resistance, and UV radiation-induced skin damage (Pacheco-Palencia et al., 2008), have been investigated. Other studies demonstrated pomegranate's potential utility for treating Alzheimer's (Rojanathammanee et al., 2013) and Parkinson's diseases (Fathy et al., 2021), arthritis (Danesi & Ferguson, 2017), infant brain ischemia (Jurenka, 2008), and obesity (Wu & Tian, 2019).

Hibiscus rosa-sinensis L. (hibiscus) is an ancient plant native to warm-temperate, subtropical, and tropical regions worldwide and belongs to *Malvaceae* family. Research has uncovered a range of health benefits linked to drinking hibiscus tea, showing that it may lower blood pressure (Amthaghri et al., 2022), fight against bacteria (Ngan et al., 2021) and promote weight of loss (Lingesh et al., 2019). Nade et al. (2010) investigated the neuroprotective potential of *H. rosa sinensis* L. in a bilateral common carotid artery (BCCA) occlusion model of global cerebral ischaemic reperfusion. The results suggested that *H. rosa-sinensis* administration prevented the oxidative stress and biochemical changes associated with the cerebral ischaemic reperfusion injury. The observed protection may be due to the *H. rosa-sinensis*-mediated augmentation of

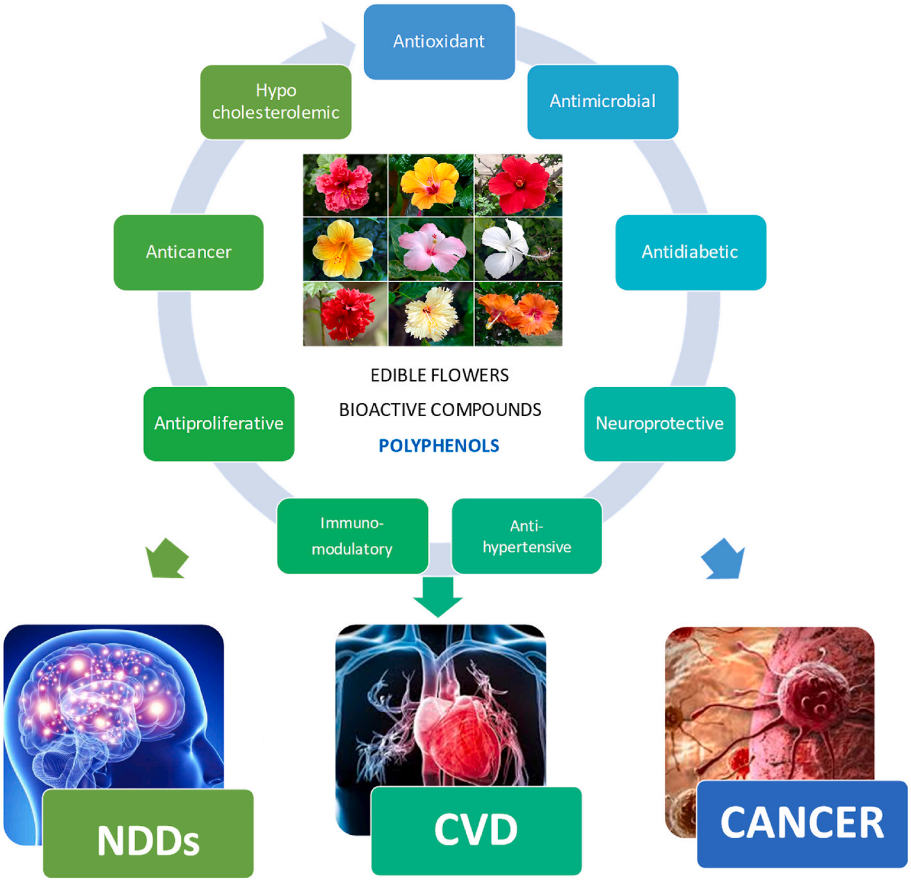

Fig. 1. Biological properties of edible flowers bioactive components and their effects on disease prevention. Legend: NDDs: neurodegenerative diseases; CVD: cardiovascular diseases.

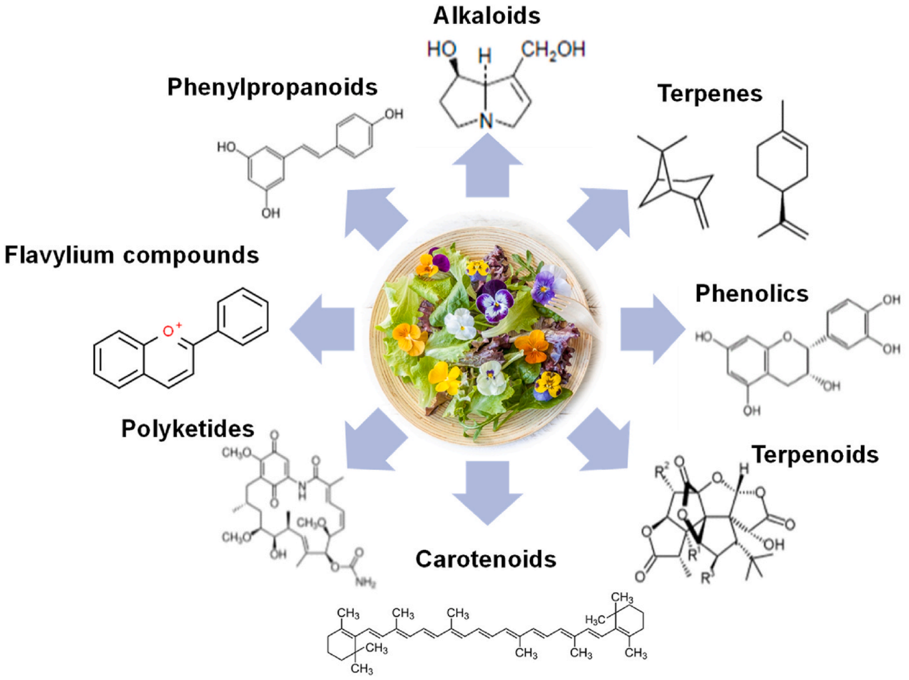

Fig. 2. Chemical groups responsible for the diversified properties of edible flowers – from attractive colours to organoleptic characteristics. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cellular antioxidant enzymes such as GSH, SOD and CAT expression, which stimulates cerebral adaptation (Nade et al., 2010). The inhibitory effect of polyphenolic compounds from flowers of *H. rosa-sinensis* on alkaline phosphatase enzyme activity was investigated in vitro by Salib et al. (2014).

Tropaeolum majus L. (nasturtium flowers) belongs to Tropaeolaceae family and is native to South America. Numerous healing properties, including antimicrobial, antifungal, hypotensive, expectorant, and anticancer, have been attributed to this plant. These properties are associated with nasturtium flowers' high concentrations of phytochemical compounds such as anthocyanins, polyphenols, and vitamin C (Jakubczyk et al., 2018). The in vitro anticancer properties of benzyl isothiocyanate, isolated from *T. majus*, against various human and murine tumour cell lines were assayed by Pintão et al. (1995).

For the extraction and pre-concentration of VOMs, headspace solid-phase microextraction (HS-SPME) has emerged as an efficient extraction procedure able to establish the volatilomic fingerprinting of edible flowers (Lo et al., 2021; Najar et al., 2019). Since this technique does not require solvent, it is environmentally and analyst friendly. It requires relatively short extraction times and minimal sample handling, combining the extraction and concentration into a single step. This extraction process comprises the absorption/adsorption of analytes onto a fused silica fibre coated with an appropriate stationary phase and their subsequent thermal desorption into the instrument injection system prior to chromatographic analysis (Najar et al., 2019).

Although, the characterisation of the bioactive compounds and bioactive activities of some edible flowers has been reported earlier in the literature (González-Barrio et al., 2018; Koike et al., 2015; Pashazadeh et al., 2021; Xiang et al., 2022), there are only a few publications concerning the volatile fingerprint of edible flowers consumed in Portugal and Europe, such as blue mallow (*M. sylvestris* L.), pomegranate (*P. granatum* L.), hibiscus (*H. rosa-sinensis* L.), and nasturtium (*T. majus* L.). Therefore, the present study aimed to establish a comprehensive volatilomic profile of some edible flowers commonly used in gourmet dishes in Iberian Peninsula (Spain and Portugal) by HS-SPME to extract the VOMs followed by GC-MS analysis for their identification. For each investigated edible flower, the dominant VOMs were identified, as well as the organoleptic and bioactive properties associated to most dominant VOMs. This approach provides insights into understanding the chemistry behind some bioactive potential and flavour properties of edible flowers, increasing their commercial value and providing consumers quality and safety guarantees.

2. Material and methods

2.1. Chemical and reagents

Sodium chloride (NaCl, 99.5%) and 3-octanol (internal standard, IS) were obtained from Sigma-Aldrich (Madrid, Spain), whereas the GC carrier gas, helium of purity 5.0, was obtained from Air Liquide, Portugal. Ultrapure water was obtained from a Milli-Q® system (Millipore, Bedford, MA, USA). The SPME fibre was coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm), an SPME holder for manual sampling and glass vials were purchased from Supelco (Bellefonte, PA, USA). The alkane series, C_8 to C_{20}, with a concentration of 40 mg/L in *n*-hexane used to determine the Kovats index (KI), was supplied from Fluka (Buchs, Switzerland).

2.2. Edible flower samples

Blue mallow and pomegranate flowers were purchased from a local market in Funchal (Madeira Island, Portugal), while the other two, hibiscus and nasturtium, were collected from different locations in the Madeira Island at Ribeira da Janela, located in the GPS coordinates: 32° 50′ 52.55″ N and 17° 9′ 9.85″ W, and at Funchal, located in the GPS coordinates: 32° 38′ 30.05″ N and 16° 55′ 29.51″ W, respectively. The blue mallow and pomegranate flowers were purchased dried from a local market, while the fresh hibiscus and nasturtium flowers were freeze-dried (LyoQuest-85 Plus, Telstar, Terrassa, Spain). Samples were milled and homogenised using a grinder (A11 Basic analytical mill, IKA, Staufen, Germany). The samples were also sieved to achieve a homogeneous particle size and stored at room temperature until analysis.

2.3. HS-SPME procedure

With slight modifications, the HS-SPME procedure was performed based on the conditions described by Figueira et al. (2014). For headspace sampling, 1 g of edible flower powder, 0.3 g of NaCl (to promote the "salting-out" effect by decreasing the solubility of volatile metabolites in the water-based phase) and 6 mL of ultra-pure Milli-Q water were placed into a 20 mL amber headspace glass vial containing a magnetic stirring microbar. Before sealing the vial with a PTFE-faced silicone septum, 5 μL of 3-octanol (102 μg/mL) was added. Then, the vial was placed in a thermostatic bath at 45 ± 1 °C under constant magnetic stirring (450 rpm). HS-SPME extractions were carried out by exposing the SPME fibre (DVB/CAR/PDMS) to the sample's headspace for 50 min. Finally, the fibre was withdrawn into the holder needle, removed from the vial and immediately introduced into the GC-MS injection system at 250 °C for 6 min, where the analytes were thermally desorbed. All analyses were carried out in triplicate ($n = 3$). The SPME fibre was thermally conditioned according to the manufacturer's instructions before use, and daily a conditioning for 10 min was carried out before the first extraction to ensure the absence of carryover.

2.4. GC-qMS analysis

Chromatographic separations of VOMs and semi-volatile organic metabolites (SVOMs) from edible flowers were performed using an Agilent Technologies 6890N (Palo Alto, CA, USA) gas chromatography system equipped with a SUPELCOWAX® 10 fused silica capillary column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) supplied by Supelco (Bellefonte, PA, USA). Helium (Helium N60, Air Liquid, Portugal) was used as the carrier gas at a flow rate of 1 mL/min (column-head pressure: 13 psi). The injector temperature was fixed at 250 °C, and a splitless injector equipped with an insert of 0.75 mm i.d. was attached. The temperature program was run as follows: initial temperature 40 °C for 1 min, 2.5 °C/min ramp until 220 °C and then held isothermally at 220 °C for 10 min. MS detection was performed in full scan in an Agilent 5975 quadrupole inert mass selective detector, the ion energy used for the electron impact (EI) was 70 eV, and a source temperature of 230 °C. The electron multiplier was set to the autotune procedure. The mass acquisition range, made in full scan mode, was 30–300 m/z. VOM tentatively identification was achieved by comparing the mass spectra results with the National Institute of Standards and Technology (NIST) MS 05 spectral database (Gaithersburg, MD, USA) and determining the Kovats Index (KI) values of each identified VOM according to the van Den Dool and Kratz (1963) equation. A C_8–C_{20} n-alkane series was used to determine the KI, and the values were compared, when available, to values reported in the literature for similar columns (Cejudo-Bastante et al., 2013; Perestrelo et al., 2019; Spínola et al., 2015) and databases available online (the Pherobase and Flavornet). Each sample was analysed in triplicate.

2.5. Multivariate statistical analysis

The multivariate data analysis (MVDA) was performed using the MetaboAnalyst 5.0 web-based tool (Chong et al., 2018; Pang et al., 2021). The raw GC-qMS data were pre-processed to remove VOMs with missing values and then normalised (data transformation by cubic root and data scaling by autoscaling). Then, the data were subjected to a one-way analysis of variance (ANOVA) followed by Fisher's test for post-hoc multiple comparisons of means from four edible flower varieties data at p-value < 0.001 to identify significant differences. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to provide insights into the separations among the edible flowers under study and to detect VOMs that may represent differences among the sample sets. Importantly, PLS-DA can identify VOM sets that best discriminate among the different edible flowers analysed by reducing the size of the data matrix by eliminating redundant variables. VOMs with variable importance in the projection (VIP) score ≥1.55 and differentially expressed in the univariate analysis were potential candidates for characterising edible flower varieties. Hierarchical cluster analysis (HCA) was carried out using the 15 most significant VOMs identified in edible flowers samples obtained by ANOVA and was generated through Ward's algorithm and Euclidean distance analysis, aiming to identify clustering patterns for the characterisation of the edible flowers analysed.

3. Results and discussion

3.1. Volatilomic fingerprinting from edible flowers

VOMs have a marked influence on the typical aroma of natural products. Furthermore, the aroma of the edible flowers analysed also depends on the synergistic effects that VOMs may have with other non-volatile compounds, on their chemical nature and their relative quantity and odour threshold, which may be related to the composition of the flower, as well as with the growing geographic region. Fig. S1 (Supplementary Material) shows the typical chromatogram of the investigated pomegranate flowers, obtained by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS. A total of 78 VOMs, including 24 terpenoids, 18 sesquiterpenoids, 16 carbonyl compounds, 4 hydrocarbons, 4 furanic compounds, 4 volatile phenols, 3 alcohols, 2 sulfur compounds, 2 nitrogenous compounds and 1 ester were identified. The detailed list of all VOMs identified in the analysed edible flowers and their respective obtained data, including retention times, KIs, molecular formula (MF), chemical families, and relative peak area, is shown in Table 1. The chemical structures of the most representative VOMs in quantitative terms and the characteristic odour associated with each VOM are presented in Fig. 3a.

3.1.1. Blue mallow

A total of 35 VOMs were tentatively identified, being mainly characterised by sesquiterpenoids (61.5%), followed by hydrocarbons (22.1%) and terpenoids (7.4%) (Fig. 3b). The major VOMs identified in blue mallow flowers include 1-decyne (25), γ-muurolene (54), valencene (58), α-cubebene (35), δ-cadinene (60), β-bisabolene (57) and calamenene (64) representing 61.4% of its total volatile fraction. Farnesene isomer (50), caryophyllene isomer (45), spathulenol (75), α-selinene (52), and eugenol (76), were also identified, although in lower amounts (3.29%, 3.25%, 2.96%, 2.36% and 2.23%, respectively).

3.1.2. Pomegranate flowers

38 VOMs were tentatively identified in pomegranate flowers (*P. granatum* L.). Terpenoids (56.6%), furanic compounds (25.5%), and carbonyl compounds (10.7%) are the most abundant chemical groups determined in pomegranate flowers, representing over 92.0% of the total volatile fraction (Fig. 3b). Such abundance is mainly provided by furfural (36), followed by linalool (40), carvone (59), anethole (63), hexanal (10), terpinen-4-ol (44) and *p*-cymene (28), which are the most abundant VOMs, in terms of the relative peak area, identified in pomegranate flowers. Other VOMs were also identified, although with lower contribution, such as limonene (21), 5-methyl-2-furfural (43), α-terpineol (53), anisaldehyde (71), methyl cinnamate (73) and thymol (77) (3.29%, 3.29%, 3.02%, 1.50%, 1.47% and 1.46%, respectively). Terpene and terpenoids are the main VOMs found in forest trees and their therapeutic potential for inflammatory diseases has been studied (Kim et al., 2020).

3.1.3. Hibiscus

A total of 20 VOMs were identified in hibiscus flowers. The total volatile profile of these flowers is highly influenced by carbonyl compounds, representing more than 88.0% of the total volatile fractions (Fig. 3b). Hydrocarbons and terpenoids were also identified but contribute to a lesser extent to the volatile profile of hibiscus flowers (5.5% and 3.9%, respectively). The most abundant VOMs in the

Table 1

Volatile metabolites (VOMs) identified in studied edible flowers by HS-SPME$_{DVB/CAR/PDMS}$/GC-qMS.

Peak nº	RT[a] (min)	KI_{cal}[b]	KI_{lit}[c]	Volatile metabolites	MF[d]	Chemical family	Relative area (% RSD)			
							Blue mallow	Pomegranate	Hibiscus	Nasturtium
1	8.02	770	777	Dimethyl sulfide	C_2H_6S	SC	–	–	0.07 (14)	–
2	9.16	818	839	Butanal	C_4H_8O	CC	–	–	0.05 (13)	–
3	10.8	883	885	2,4-Dimethyl-1-heptene	C_9H_{18}	HC	–	–	0.06 (13)	–
4	12.1	923	931	2-Methyl-butanal	$C_5H_{10}O$	CC	–	0.10 (16)	0.06 (22)	–
5	12.2	927	936	3-Methyl-butanal	$C_5H_{10}O$	CC	–	0.15 (13)	0.03 (23)	–
6	13.4	958	957	2,2,4,6,6-Pentamethyl-heptane	$C_{12}H_{26}$	HC	–	0.19 (5)	–	–
7	13.6	961	–	2-Ethyl-furan	C_6H_8O	FC	–	–	0.02 (23)	–
8	16.7	1029	1028	α-Pinene	$C_{10}H_{16}$	T	–	0.03 (12)	–	–
9	19.2	1075	1069	2-Methyl-1-penten-3-one	$C_6H_{10}O$	CC	0.05 (22)	–	–	–
10	20.0	1089	1082	Hexanal	$C_6H_{12}O$	CC	0.10 (17)	0.91 (17)	1.52 (19)	2.17 (8)
11	21.4	1120	1118	β-Pinene	$C_{10}H_{16}$	T	0.04 (17)	–	0.04 (14)	–
12	22.1	1131	1142	4-Methyl-2-hexanone	$C_7H_{14}O$	CC	0.12 (21)	–	–	–
13	22.2	1133	1132	Sabinene	$C_{10}H_{16}$	T	–	–	0.07 (7)	–
14	23.8	1161	1140	3-Carene	$C_{10}H_{16}$	T	–	0.13 (9)	–	–
15	24.5	1173	1174	β-Myrcene	$C_{10}H_{16}$	T	–	0.05 (12)	–	–
16	24.8	1177	1176	α-Phellandrene	$C_{10}H_{16}$	T	0.04 (11)	0.17 (5)	–	–
17	25.5	1188	–	1,4-Cineole	$C_{10}H_{18}O$	T	–	0.05 (21)	–	–
18	25.7	1191	1190	α-Terpinene	$C_{10}H_{16}$	T	–	0.04 (11)	–	–
19	25.9	1192	1195	2-Heptanone	$C_7H_{14}O$	CC	–	–	0.03 (7)	–
20	26.0	1195	1196	Heptanal	$C_7H_{14}O$	CC	–	–	0.13 (5)	–
21	26.8	1210	1210	Limonene	$C_{10}H_{16}$	T	0.08 (7)	0.51 (8)	0.10 (9)	–
22	27.4	1220	1223	Eucalyptol	$C_{10}H_{18}O$	T	–	0.13 (23)	–	–
23	27.5	1222	1223	β-Phellandrene	$C_{10}H_{16}$	T	0.08 (6)	0.15 (9)	–	–
24	28.2	1234	1224	2-Hexenal	$C_6H_{10}O$	CC	–	–	3.44 (12)	7.19 (10)
25	28.3	1236	1234	1-Decyne	$C_{10}H_{18}$	HC	1.84 (10)	–	–	–
26	28.7	1243	1241	2-Pentyl-furan	$C_9H_{14}O$	FC	–	0.16 (10)	–	–
27	31.0	1282	–	4-Methyl-2-hexanol	$C_7H_{16}O$	A	0.05 (19)	–	–	–
28	31.3	1286	1284	*p*-Cymene	$C_{10}H_{14}$	T	0.06 (11)	0.65 (6)	0.05 (12)	–
29	32.3	1301	1298	Octanal	$C_8H_{16}O$	CC	–	–	0.07 (19)	–
30	33.6	1326	1325	2-Penten-1-ol isomer	$C_5H_{10}O$	A	–	–	0.05 (17)	–
31	35.1	1352	1341	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	CC	–	0.09 (1)	–	–
32	40.2	1439	1421	3-Ethyl-2-methyl-1,3-hexadiene	C_9H_{16}	HC	–	–	0.31 (6)	–
33	40.7	1448	1427	2-Octenal isomer	$C_8H_{14}O$	CC	–	–	0.38 (14)	–
34	41.3	1459	1463	β-Ionone	$C_{13}H_{20}O$	T	0.08 (6)	–	–	–
35	42.2	1474	1481	α-Cubebene	$C_{15}H_{24}$	ST	0.61 (22)	–	–	–
36	42.6	1483	1486	Furfural	$C_5H_4O_2$	FC	–	3.24 (9)	–	–
37	42.9	1476	1477	Copaene	$C_{15}H_{24}$	ST	0.14 (8)	–	–	–
38	46.2	1547	1540	Camphor	$C_{10}H_{16}O$	T	–	0.09 (18)	–	–
39	46.4	1552	1558	Benzaldehyde	C_7H_6O	CC	–	–	0.06 (21)	49.10 (24)
40	46.5	1553	1552	Linalool	$C_{10}H_{18}O$	T	–	1.82 (12)	–	–
41	46.8	1559	1558	β-Cubebene	$C_{15}H_{24}$	ST	0.09 (20)	–	–	–
42	47.3	1567	1563	β-Terpineol	$C_{10}H_{18}O$	T	–	0.20 (12)	–	–
43	48.9	1597	1591	5-Methyl-2-furfural	$C_{10}H_{18}O$	FC	–	0.51 (15)	–	–
44	50.1	1611	1611	Terpinen-4-ol	$C_{10}H_{18}O$	T	–	0.75 (8)	–	–
45	50.4	1614	1612	Caryophyllene isomer	$C_{15}H_{24}$	ST	0.27 (5)	0.10 (12)	–	–
46	50.9	1619	1639	Alloaromadendrene	$C_{15}H_{24}$	ST	0.08 (5)	–	–	–
47	51.8	1628	1631	Menthol	$C_{10}H_{20}O$	T	0.06 (12)	0.16 (17)	–	–
48	52.1	1632	1641	γ-Elemene	$C_{15}H_{24}$	ST	0.09 (4)	–	–	–
49	53.2	1643	1645	Acetophenone	C_8H_8O	CC	–	–	0.12 (15)	–
50	53.4	1645	1646	Farnesene isomer	$C_{15}H_{24}$	ST	0.27 (7)	–	–	–
51	53.9	1650	1650	Estragole	$C_{10}H_{12}O$	T	–	0.04 (14)	–	–
52	54.2	1653	1664	α-Selinene	$C_{15}H_{24}$	ST	0.20 (19)	–	–	–
53	54.8	1711	1710	α-Terpineol	$C_{10}H_{18}O$	T	–	0.47 (11)	–	–
54	54.8	1712	1725	γ-Muurolene	$C_{15}H_{24}$	ST	0.66 (6)	–	–	–
55	55.2	1720	1698	Borneol	$C_{10}H_{18}O$	T	0.12 (11)	0.09 (12)	–	–
56	56.1	1739	1731	β-Selinene	$C_{15}H_{24}$	ST	0.08 (12)	–	–	–
57	56.5	1747	1741	β-Bisabolene	$C_{15}H_{24}$	ST	0.39 (4)	–	–	–
58	56.7	1751	1726	Valencene	$C_{15}H_{24}$	ST	0.64 (5)	–	–	–
59	57.4	1764	1755	Carvone	$C_{10}H_{14}O$	T	–	1.64 (14)	–	–
60	58.2	1779	1767	δ-Cadinene	$C_{15}H_{24}$	ST	0.60 (23)	0.12 (14)	–	–
61	58.8	1791	1777	α-Curcumene	$C_{15}H_{22}$	ST	0.14 (20)	0.11 (23)	–	–
62	59.5	1807	1811	Cadina-1,4-diene	$C_{15}H_{24}$	ST	0.14 (16)	–	–	–
63	61.7	1855	1845	Anethole	$C_{10}H_{12}O$	T	0.07 (3)	1.34 (23)	–	–
64	62.0	1862	1859	Calamenene	$C_{15}H_{22}$	ST	0.37 (12)	0.17 (10)	–	–
65	63.6	1897	1898	Benzyl alcohol	C_7H_8O	A	–	–	–	2.38 (6)
66	63.9	1906	1903	Safrole	$C_{10}H_{10}O_2$	T	–	0.17 (20)	–	–
67	65.9	1953	1926	α-Calacorene	$C_{15}H_{20}$	ST	0.10 (11)	–	–	–
68	66.4	1963	–	Benzyl nitrile	C_8H_7N	NC	–	–	–	38.28 (16)
69	67.9	1997	2003	2-Acetylpyrrole	C_6H_7NO	NC	0.10 (13)	–	–	–
70	69.4	2010	2012	Methyl eugenol	$C_{11}H_{14}O_2$	VP	0.15 (19)	–	–	–
71	70.9	2021	2045	Anisaldehyde	$C_8H_8O_2$	CC	–	0.23 (19)	–	–
72	71.5	2025	2017	Cinnamaldehyde	C_9H_8O	CC	–	0.16 (15)	–	–
73	72.8	2034	2024	Methyl cinnamate	$C_{10}H_{10}O_2$	E	–	0.23 (20)	–	–

(*continued on next page*)

Table 1 (*continued*)

Peak nº	RT[a] (min)	KI$_{cal}$[b]	KI$_{lit}$[c]	Volatile metabolites	MF[d]	Chemical family	Relative area (% RSD)			
							Blue mallow	Pomegranate	Hibiscus	Nasturtium
74	74.1	2143	2131	Benzyl isothiocyanate	C_8H_7NS	SC	–	–	–	5280.73 (23)
75	74.5	2145	2166	Spathulenol	$C_{15}H_{24}O$	ST	0.25 (5)	–	–	–
76	76.3	2158	2150	Eugenol	$C_{10}H_{12}O_2$	VP	0.19 (12)	–	–	–
77	76.4	2158	2157	Thymol	$C_{10}H_{14}O$	VP	–	0.23 (8)	–	–
78	77.9	2168	2183	Carvacrol	$C_{10}H_{14}O$	VP	–	0.18 (9)	–	–
Sum of the total relative area						SC	–	0.00	0.07	5280.73
						CC	0.27	1.65	5.89	58.46
						HC	1.84	0.19	0.36	–
						FC	–	3.91	0.02	–
						T	0.62	8.67	0.26	–
						A	0.05	–	0.05	2.38
						ST	5.12	0.51	–	–
						NC	0.10	–	–	38.28
						VP	0.33	0.40	–	–
						E	–	0.23	–	–
						Total	8.33	15.56	6.65	5379.85

SC: Sulfur compound; CC: Carbonyl compound; HC: Hydrocarbon; FC: Furanic compound; T: Terpenoid; A: Alcohol; ST: Sesquiterpenoid; NC: Nitrogen compound; VP: Volatile phenol; E: Ester; -: Not detected.

[a] RT: Retention time.

[b] Kovat index relative *n*-alkanes (C_8 to C_{20}) on a SUPELCOWAX® 10 capillary column.

[c] Kovat index relative reported in literature for equivalent capillary column (Cejudo-Bastante et al., 2013; Perestrelo et al., 2019; Spínola et al., 2015).

[d] MF: Molecular formula.

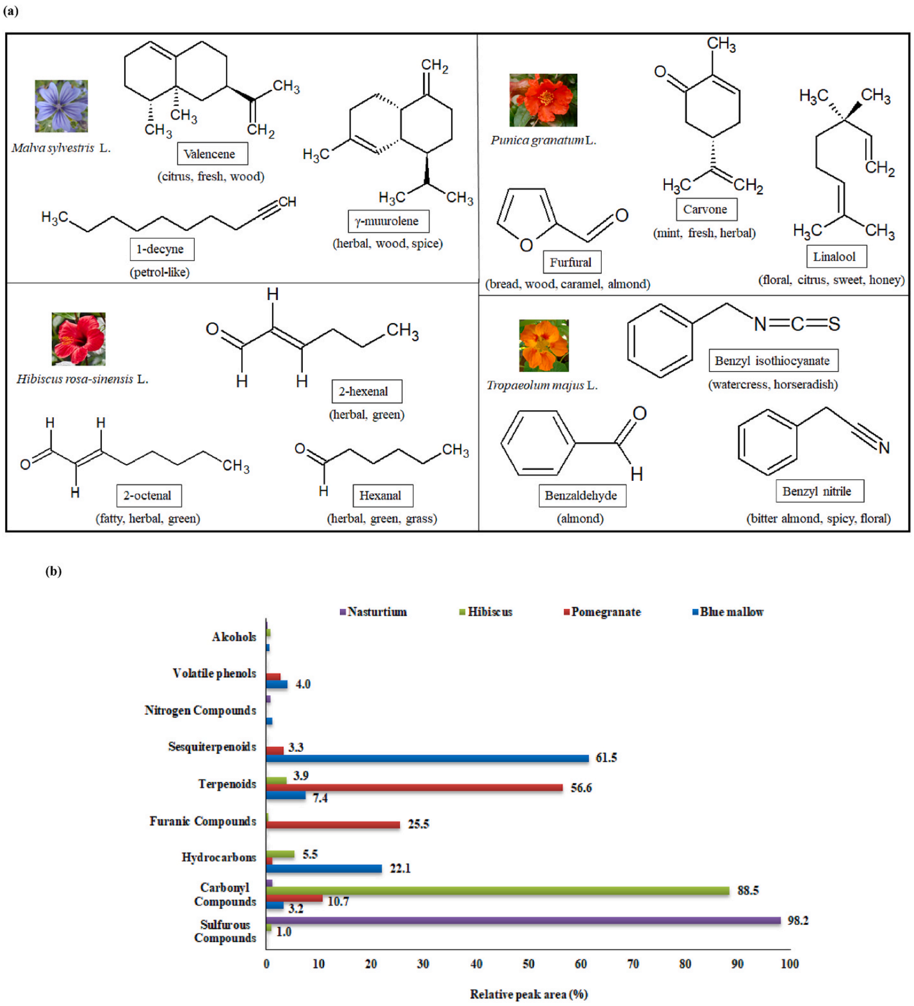

Fig. 3. (**a**) Chemical structures and characteristic odour associated with the major volatile organic metabolites (VOMs) identified in the investigated edible flowers; and (**b**) Distribution of chemical families identified in blue mallow, pomegranate, hibiscus, and nasturtium edible flowers. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

investigated hibiscus flowers are the aldehydes 2-hexenal (24), hexanal (10), and 2-octenal isomer (33), which account for 51.7%, 22.9%, 5.8% of the total volatile fraction, respectively; and the hydrocarbon 3-ethyl-2-methyl-1,3-hexadiene (32) which accounts 4.6% (Table 1). In smaller quantities, heptanal (20), acetophenone (49), limonene (21), octanal (29), dimethyl sulphide (1) and sabinene (13) were also identified, with relative peak areas of 1.94%, 1.75%, 1.54%, 1.06%, 1.04% and 1.03%, respectively (Table 1).

3.1.4. Nasturtium

This edible flower is slightly aromatic, agreeing with its simple volatile profile containing only 6 VOMs. The benzyl isothiocyanate (74) is the dominant VOM representing over 98% of the volatile fraction in nasturtium flowers (Fig. 3b; Table 1). In general, organosulfur compounds are related to the aroma, flavour, and bioactive properties of some vegetables and fruits (Aguiar et al., 2021; Cecchi et al., 2020; Neves et al., 2021). Many of these VOMs have been shown to have beneficial effects in protecting against several diseases, including cancer (Aguiar et al., 2021; Nicastro et al., 2015). Several studies have demonstrated the anticancer effects of benzyl isothiocyanate (74) by inhibiting initiation, growth, and metastasis of human cancers through different mechanisms of action (Batra et al., 2010; Boreddy et al., 2011; Xie et al., 2017). In addition, Sofrata et al. (2011) demonstrated the powerful antibacterial effect of benzyl isothiocyanate (74), which exhibited a strong bactericidal effect against oral pathogens as well as against other Gram-negative bacteria, while Gram-positive bacteria mainly displayed growth inhibition or remained unaffected (Sofrata et al., 2011). The carbonyl compounds [benzaldehyde (39; 0.91%), 2-hexenal (24; 0.13%), and hexanal (10; 0.04%)], a nitrogenous compound benzyl nitrile (68; 0.71%) and the benzyl alcohol (65; 0.04%) complete the volatile composition of nasturtium.

3.2. Bioactive potential of volatiles identified in the edible flowers

Many of the VOMs identified in the edible flowers analysed have been previously reported in other food samples with a significant number of biological activities such as antioxidant (Hou et al., 2022; Mahomoodally et al., 2018), anti-inflammatory (Li et al., 2017), antidiabetic (Alam et al., 2019), antimicrobial (Dănilă et al., 2018), cytotoxic (da Silva et al., 2018), antitumor (Assmann et al., 2018) and antiproliferative activities (Elkady & Ayoub, 2018). Therefore, these VOMs could help prevent and treat diseases like cancer (Nicastro et al., 2015), inflammatory diseases (Kim et al., 2020), diabetes (Nazaruk & Borzym-Kluczyk, 2015), CVDs (Yang et al., 2020), and neurological disorders. Table 2 shows the potential bioactive effects of some VOMs identified in the edible flowers under investigation.

Among these VOMs, terpenoids are one of the most dominant chemical families found in fruits and vegetables (Aguiar et al., 2021), which agrees with the results obtained in this work. Terpenoids, biosynthesised through isopentyl diphosphate, and the methylerythritol phosphate and mevalonate pathways (El Hadi et al., 2013) are the major contributors to the total volatile composition of the blue mallow and pomegranate flowers (Fig. 3b). Monoterpenes have several beneficial biological effects reported in several works (Brahmkshatriya & Brahmkshatriya, 2013; Cho et al., 2017; Kim et al., 2020). Terpenoids, such as α-pinene (8), sabinene (13), β-myrcene (15), α-phellandrene (16), α-terpinene (18), limonene (21), β-phellandrene (23), *p*-cymene (28), linalool (40), menthol (47), α-terpineol (53), borneol (55) and thymol (77) possess a high potential antioxidant, antimicrobial, antibacterial, anti-inflammatory and immunostimulant (Ayseli & Ayseli, 2016; Cho et al., 2017; Guimarães et al., 2019; Kim et al., 2020; Ku & Lin, 2013; Yang et al., 2020). Sieniawska et al. (2018) reported terpene-mediated have protective effects against tuberculosis. However, the most remarkable bioactivity of terpenoids is related to their anticancer potential, acting at different stages of tumour development and in different mechanisms of action (inhibition, regulation of intracellular signalling pathways) (Ansari & Akhtar, 2019; Cho et al., 2017). The chemopreventive and chemotherapeutic properties of limonene against human cancers were widely demonstrated by Paduch et al. (2007) and Kris-Etherton et al. (2002).

Organosulfur compounds, including dimethyl sulphide (1) and benzyl isothiocyanate (74), were identified in the hibiscus and nasturtium flowers. These compounds are highly reactive phytochemical metabolites and are usually present in the composition of cruciferous vegetables (Putnik et al., 2019). In addition, they also have interesting properties and bioactivities, mainly antibacterial, antiproliferative, cytotoxic and anticancer effects (Putnik et al., 2019).

On the other hand, carbonyl compounds are present in all analysed edible flowers, in greater or lesser amounts, but standing out above all in the hibiscus flowers, where the contribution of carbonyl compounds to the total volatile profile was higher than 88%. 2-Hexenal (24) and hexanal (10) were the most prominent carbonyl compounds identified in hibiscus flowers and even in greater quantity in nasturtium flowers, where the benzaldehyde (39) is also present in high amount (49.1 relative peak area). Both VOMs (2-hexenal and hexanal) generally confer an herbaceous odour like freshly cut green grass to the fruits, vegetables and flowers in which it is present such as strawberry, blueberry, olive oil, apple, hops, grape and tomato, among others (Ayseli & Ayseli, 2016). Furthermore, these compounds exhibited antimicrobial activity against pathogenic microorganisms (Ayseli & Ayseli, 2016). Benzaldehyde is used in aroma formulations and as a food flavouring due to its bitter almond, sweet, floral and spice-like odour. It is naturally present in many products such as bitter almonds, peach, apricot kernel, cheeses, and black tea (Dionísio et al., 2012).

3.3. Multivariate PCA and PLS of GC-MS data. Characterisation of edible flowers

To evaluate the performance of HS-SPME/GC–qMS to characterise edible flowers in terms of volatile profile, a statistical analysis of the volatilomic data matrix was performed using MetaboAnalyst 5.0 web-based tool (Pang et al., 2021). The PCA and PLS-DA were applied as multivariate analysis, as described in Section 2.5. The PCA is an unsupervised method that was performed to visualise the difference/similarity among sample profiles and detect significant variables (i.e., VOMs) that contribute to these discrepancies. Fig. 4a and b shows the PCA score plot and biplot from the analysed edible flower samples. The variances of the first and second principal components (PC1 and PC2) were 46.6% and 33.0%, respectively, representing 79.6% of the total VOCs variability of data, allowing a good differentiation of the edible flowers.

The hibiscus flowers, in PC1 the and PC2 negative quadrants, were chiefly characterised by dimethyl sulphide (1), butanal (2), 2,4-dimethyl-1-heptene (3), 2-ethyl-furan (7), sabinene (13), 2-heptanone (19), heptanal (20), octanal (29), 2-penten-1-ol isomer (30) and 3-ethyl-2-methyl-1,3-hexadiene (32), whereas nasturtium flowers also projected in PC1 and PC2 negative quadrants were characterised by benzyl alcohol (65), benzyl nitrile (68) and benzyl isothiocyanate (74). Blue mallow flowers were placed in the PC1 negative quadrant and PC2 positive quadrant and were characterised by 1-decyne (25), α-cubebene (35), β-farnesene isomer (50), α-selinene (52), γ-muurolene (54), β-bisabolene (57), valencene (58), methyl eugenol (70), spathulenol (75), and eugenol (76), among other VOMs. Finally, pomegranate flowers (PC1 and PC2 positive quadrants) were characterised by furfural (36), linalool (40), 5-methyl-2-furfural (43), terpinen-4-ol (44), α-terpineol (53), and carvone (59), among other VOMs.

PLS-DA was used as a supervised clustering method and revealed differentiation among edible flowers (Fig. 4c). Total variance of 61.4% was obtained by the first two principal components obtained from PLS-DA. Furthermore, 15 differently expressed VOMs were found with presented VIP score ≥ 1.55, being the most relevant compounds and having the greatest discriminatory power to characterise the four edible flowers

Table 2

Potential bioactive effects of some important VOMs identified in the studied edible flowers.

Peak nº	Volatile organic metabolites	Potential bioactive effects[a]								Edible flowers	References
		Antibacterial	Antidiabetic	Anti-inflammatory	Antifungal	Antioxidant	Antiproliferative	Antitumor	Cytotoxic		
8	α-Pinene		x		x	x	x	x	x	Pomegranate	(Aguiar et al., 2021; Hou et al., 2022; Li et al., 2017; Lubinska-Szczygieł et al., 2018; Mahomoodally et al., 2018)
11	β-Pinene				x	x	x	x		Blue mallow, hibiscus	
13	Sabinene	x						x	x	Hibiscus	
14	3-Carene	x				x		x		Pomegranate	
15	β-Myrcene		x	X	x	X	x	x	x	Pomegranate	
18	α-Terpinene	x			x			x	x	Pomegranate	
21	Limonene	x	x	X	x	X	x	x	x	Blue mallow, pomegranate, hibiscus	
22	Eucalyptol			X						Pomegranate	
23	β-Phellandrene	x	x			X			x	Blue mallow, pomegranate	
28	*p*-Cymene	x		X	x	X			x	Blue mallow, pomegranate, hibiscus	
34	β-Ionone						x	x		Blue mallow	
40	Linalool	x	x	X		x	x			Pomegranate	
45	Caryophyllene isomer	x	x			x	x		x	Blue mallow, pomegranate	
53	α-Terpineol	x			x	x		x	x	Pomegranate	
54	γ-Muurolene							x	x	Blue mallow	
74	Benzyl isothiocyanate						x	x	x	Nasturtium	
76	Eugenol	x				x		x		Blue mallow	
77	Thymol	x		X	x	x				Pomegranate	

[a] Potential bioactive effect indicates the type of bioactive effects reported for each of the volatile organic metabolites (VOMs) referred in the Table 1.

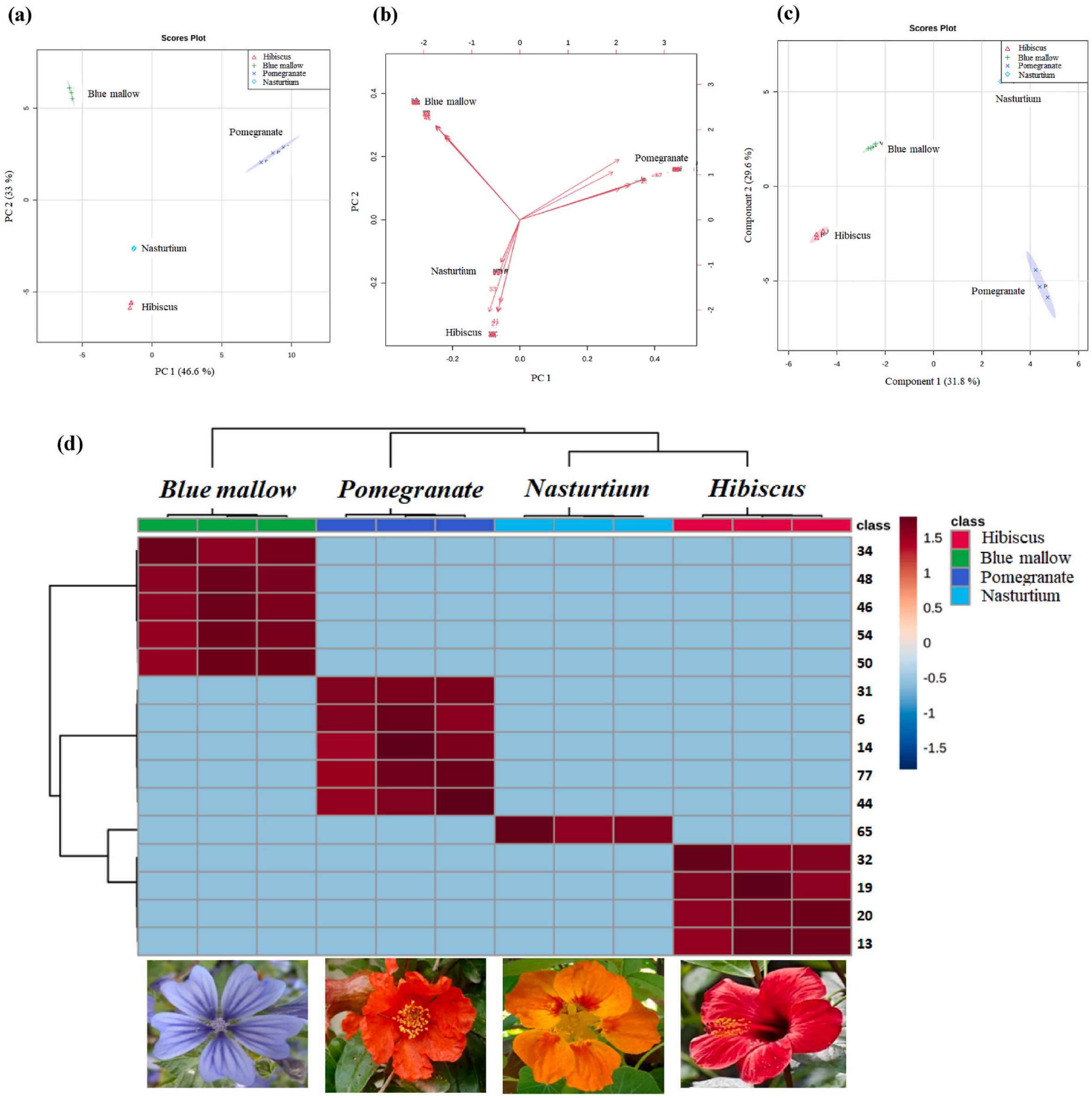

Fig. 4. Multivariate statistical analysis (MVSA) using principal component analysis (PCA) and partial least square-discrimination analysis (PLS-DA) of the volatile signature of edible flowers. **(a)** PCA score plot and **(b)** biplot. **(c)** PLS-DA score plot and **(d)** Hierarchical cluster analysis (HCA) performed using the 15 most significant volatile organic compounds (VOMs) profiles identified in four edible flower samples (blue mallow, pomegranate, hibiscus and nasturtium flowers) obtained by ANOVA. The columns in the heatmap represent samples and the rows indicate VOMs. The color gradient ranging from dark blue through white to dark red indicates low, middle, and high abundance of a VOM, respectively. The resulting dendrogram associated with heat map was generated by Ward's algorithm and Euclidean distance analysis. Numbers in the heatmap are identified in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

studied (Fig. S2). Among these 15 significant VOMs, six carbonyl compounds [heptanal (20), 2-heptanone (19), butanal (2), octanal (29), 2-octenal isomer (33) and acetophenone (49)], two hydrocarbons [3-ethyl-2-methyl-1,3-hexadiene (32) and 2,4-dimethyl-1-heptene (3)], two sulphurous compounds (dimethyl sulphide (1) and benzyl isothiocyanate (74)), two alcohols [benzyl alcohol (65) and 2-penten-1-ol isomer (30)), one terpenoid (sabinene (13)], one nitrogen compound [benzyl nitrile (68)] and one furanic compound [2-ethyl-furan (7)] were found. The p_{values} obtained by one-way ANOVA with Fisher post-hoc test ($p < 0.001$) indicated that 66 of 78 VOMs identified were significantly different among the investigated edible flowers. Moreover, HCA was also performed using the 15 most significant VOMs identified in edible flower samples obtained by ANOVA. The resulting dendrogram associated with the heat map was performed by Euclidean distance through Ward's clustering method (Fig. 4d), providing intuitive visualization of the data set, which along with the statistical analyses carried out previously, allows better identification of the inherent clustering patterns between each edible flower.

4. Conclusions

The HS-SPME/qGC-MS method has shown to be effective in extracting and identifying a total of 78 VOMs belonging to different chemical families emitted by *M. sylvestris* L., *P. granatum* L., *H. rosa-sinensis* L. and *T. majus* L. flowers. Remarkable qualitative and semi-quantitative differences were found among the analysed edible flowers. In qualitative terms, the pomegranate and blue mallow flowers showed the highest number of identified VOMs, with 38 and 35 VOMs, respectively. In contrast, 20 and 6 VOMs were observed in hibiscus and nasturtium flowers, respectively. Thus, our results present a complex volatilomic fingerprinting. Blue mallow is mainly characterised by sesquiterpenoids (61.5% of the total volatile fraction), whereas pomegranate flowers, hibiscus, and nasturtium, are dominated by terpenoids (56.6%), carbonyl compounds (88.0%) and organosulfur compounds (98%), respectively.

The application of multivariate statistical analysis enabled the visualization of clustering trends among the investigated edible flowers and identified the VOMs responsible for discriminating each group based on the genus. Moreover, 15 VOMs, including six carbonyl compounds, two hydrocarbons, two sulphurous compounds, two alcohols, one terpenoid, one nitrogen compound and one furanic compound, were responsible for this differentiation. Overall, our findings demonstrated that several VOMs identified in edible flowers have relevant biological effects on human health and sensory properties associated with volatiles at levels higher than the limits of olfactive perception. Thus, the data obtained point to the probable valuable effects of including edible flowers in the human diet.

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Credit authorship contribution statement

Sergio Izcara: Conceptualization, Formal analysis, Investigation, Writing - Original Draft, Writing. *Rosa Perestrelo*: Conceptualization, Formal analysis, Investigation and Writing - Original Draft. *Sonia Morante-Zarcero*: Writing - Review & Editing and Visualization. *Isabel Sierra*: Supervision, Conceptualization, Review & Editing. *José S. Câmara*: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration and Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2022.102188.

References

Aguiar, J., Gonçalves, J. L., Alves, V. L., & Câmara, J. S. (2021). Relationship between volatile composition and bioactive potential of vegetables and fruits of regular consumption—an integrative approach. *Molecules, 26*, 3653. https://doi.org/10.3390/molecules26123653

Alam, F., Shafique, Z., Amjad, S. T., & Bin Asad, M. H. H. (2019). Enzymes inhibitors from natural sources with antidiabetic activity: A review. *Phytotherapy Research, 33*, 41–54. https://doi.org/10.1002/ptr.6211

Amthaghri, S., Amssayef, A., Slaoui, M., & Eddouks, M. (2022). Antihypertensive and vasorelaxant effects of *Hibiscus rosa-sinensis* through angiotensin-converting enzyme-2 (ACE-2), and Ca2+ channels pathways. *Cardiovascular & Haematological Disorders - Drug Targets, 22*. https://doi.org/10.2174/1871529X22666220329190331

Ansari, I. A., & Akhtar, M. S. (2019). Current insights on the role of terpenoids as anticancer agents: A perspective on cancer prevention and treatment. In *Natural bioactive compounds: Chemistry, pharmacology and health care practices* (pp. 53–80). Singapore: Springer. https://doi.org/10.1007/978-981-13-7205-6_3, 978-981-13-7205-6.

de Araújo, F. F., Farias, D. P., Neri-Numa, I. A., & Pastore, G. M. (2021). Polyphenols and their applications: An approach in food chemistry and innovation potential. *Food Chemistry, 338*, Article 127535. https://doi.org/10.1016/j.foodchem.2020.127535

Assmann, C. E., Cadoná, F. C., Bonadiman, B.d. S. R., Dornelles, E. B., Trevisan, G., & da Cruz, I. B. M. (2018). Tea tree oil presents in vitro antitumor activity on breast cancer cells without cytotoxic effects on fibroblasts and on peripheral blood mononuclear cells. *Biomedicine & Pharmacotherapy, 103*, 1253–1261. https://doi.org/10.1016/j.biopha.2018.04.096

Ayseli, M. T., & Ayseli, Y. I. (2016). Flavors of the future: Health benefits of flavor precursors and volatile compounds in plant foods. *Trends in Food Science & Technology, 48*, 69–77. https://doi.org/10.1016/j.tifs.2015.11.005

Batra, S., Sahu, R. P., Kandala, P. K., & Srivastava, S. K. (2010). Benzyl isothiocyanate-mediated inhibition of histone deacetylase leads to NF-kappaB turnoff in human pancreatic carcinoma cells. *Molecular Cancer Therapeutics, 9*, 1596–1608. https://doi.org/10.1158/1535-7163.MCT-09-1146

Boreddy, S. R., Pramanik, K. C., & Srivastava, S. K. (2011). Pancreatic tumor suppression by benzyl isothiocyanate is associated with inhibition of PI3K/AKT/FOXO pathway. *Clinical Cancer Research, 17*, 1784–1795. https://doi.org/10.1158/1078-0432.CCR-10-1891

Brahmkshatriya, P. P., & Brahmkshatriya, P. S. (2013). Terpenes: Chemistry, biological role, and therapeutic applications. In *Natural products* (pp. 2665–2691). Berlin, Heidelberg: Springer, ISBN 978-3-642-22144-6. https://doi.org/10.1007/978-3-642-22144-6_120.

Cecchi, L., Ieri, F., Vignolini, P., Mulinacci, N., & Romani, A. (2020). Characterization of volatile and flavonoid composition of different cuts of dried onion (*Allium cepa* L.) by HS-SPME-GC-MS, HS-SPME-GC×GC-TOF and HPLC-DAD. *Molecules, 25*, 408. https://doi.org/10.3390/molecules25020408

Cejudo-Bastante, M. J., Durán, E., Castro, R., Rodríguez-Dodero, M. C., Natera, R., & García-Barroso, C. (2013). Study of the volatile composition and sensory characteristics of new Sherry vinegar-derived products by maceration with fruits. *LWT - Food Science and Technology, 50*, 469–479. https://doi.org/10.1016/j.lwt.2012.08.022

Cho, K. S., Lim, Y. R., Lee, K., Lee, J., Lee, J. H., & Lee, I. S. (2017). Terpenes from forests and human health. *Toxicological Research, 33*, 97–106. https://doi.org/10.5487/TR.2017.33.2.097

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., Wishart, D. S., & Xia, J. (2018). MetaboAnalyst 4.0: Towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research, 46*, W486–W494. https://doi.org/10.1093/nar/gky310

Danesi, F., & Ferguson, L. R. (2017). Could pomegranate juice help in the control of inflammatory diseases? *Nutrients, 9*, 958. https://doi.org/10.3390/nu9090958

Dănilă, E., Moldovan, Z., Popa, M., Chifiriuc, M. C., Kaya, A. D., & Kaya, M. A. (2018). Chemical composition, antimicrobial and antibiofilm efficacy of *C. limon* and *L. angustifolia* EOs and of their mixtures against Staphylococcus epidermidis clinical strains. *Industrial Crops and Products, 122*, 483–492. https://doi.org/10.1016/j.indcrop.2018.06.019

Daniloski, D., D'Cunha, N. M., Speer, H., McKune, A. J., Alexopoulos, N., Panagiotakos, D. B., Petkoska, A. T., & Naumovski, N. (2022). Recent developments on Opuntia spp., their bioactive composition, nutritional values, and health effects. *Food Bioscience, 47*, Article 101665. https://doi.org/10.1016/j.fbio.2022.101665

Delfine, S., Marrelli, M., Conforti, F., Formisano, C., Rigano, D., Menichini, F., & Senatore, F. (2017). Variation of *Malva sylvestris* essential oil yield, chemical composition and biological activity in response to different environments across Southern Italy. *Industrial Crops and Products, 98*, 29–37. https://doi.org/10.1016/j.indcrop.2017.01.016

van Den Dool, H., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography A, 11*, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X

Dionísio, A. P., Molina, G., Souza de Carvalho, D., dos Santos, R., Bicas, J. L., & Pastore, G. M. (2012). 11 - natural flavourings from biotechnology for foods and beverages. In *Natural food additives, ingredients and flavourings* (pp. 231–259). Woodhead Publishing Series in Food Science, Technology and Nutrition. https://doi.org/10.1533/9780857095725.1.231, 9781845698119.

Dowek, S., Fallah, S., Basheer-Salimia, R., Jazzar, M., & Qawasmeh, A. (2020). Antibacterial, antioxidant and phytochemical screening of Palestinian mallow, malva sylvestris L. *International Journal of Pharmacy and Pharmaceutical Sciences, 12*, 12–16. https://doi.org/10.22159/ijpps.2020v12i10.39053

Dudareva, N., Klempien, A., Muhlemann, J. K., & Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist, 198*, 16–32. https://doi.org/10.1111/nph.12145

El Hadi, M., Zhang, F.-J., Wu, F.-F., Zhou, C.-H., & Tao, J. (2013). Advances in fruit aroma volatile research. *Molecules, 18*, 8200–8229. https://doi.org/10.3390/molecules18078200

Elkady, W. M., & Ayoub, I. M. (2018). Chemical profiling and antiproliferative effect of essential oils of two Araucaria species cultivated in Egypt. *Industrial Crops and Products, 118*, 188–195. https://doi.org/10.1016/j.indcrop.2018.03.051

Fathy, S. M., El-Dash, H. A., & Said, N. I. (2021). Neuroprotective effects of pomegranate (*Punica granatum* L.) juice and seed extract in paraquat-induced mouse model of Parkinson's disease. *BMC Complementary Medicine and Therapies, 21*, 130. https://doi.org/10.1186/s12906-021-03298-y

Figueira, J., Câmara, H., Pereira, J., & Câmara, J. S. (2014). Evaluation of volatile metabolites as markers in *Lycopersicon esculentum* L. cultivars discrimination by multivariate analysis of headspace solid phase microextraction and mass spectrometry data. *Food Chemistry, 145*, 653–663. https://doi.org/10.1016/j.foodchem.2013.08.061

González-Barrio, R., Periago, M. J., Luna-Recio, C., García-Alonso, F. J., & Navarro-González, I. (2018). Chemical composition of the edible flowers, pansy (*Viola wittrockiana*) and snapdragon (*Antirrhinum majus*) as new sources of bioactive compounds. *Food Chemistry, 252*, 373–380. https://doi.org/10.1016/j.foodchem.2018.01.102

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules, 24*, 2471. https://doi.org/10.3390/molecules24132471

Hou, T., Sana, S. S., Li, H., Xing, Y., Nanda, A., Netala, V. R., & Zhang, Z. (2022). Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience, 47*, Article 101716. https://doi.org/10.1016/j.fbio.2022.101716

Jakubczyk, K., Janda, K., Watychowicz, K., Łukasiak, J., & Wolska, J. (2018). Garden nasturtium (Tropaeolum majus L.) - a source of mineral elements and bioactive compounds. *Roczniki Panstwowego Zakladu Higieny, 69*, 119–126.

Jurenka, J. S. (2008). Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Alternative Medicine Review, 13*, 128–144.

Kim, T., Song, B., Cho, K. S., & Lee, I. S. (2020). Therapeutic potential of volatile terpenes and terpenoids from forests for inflammatory diseases. *International Journal of Molecular Sciences, 21*, 2187. https://doi.org/10.3390/ijms21062187

Koike, A., Barreira, J. C. M., Barros, L., Santos-Buelga, C., Villavicencio, A. L. C. H., & Ferreira, I. C. F. R. (2015). Irradiation as a novel approach to improve quality of *Tropaeolum majus* L. flowers: Benefits in phenolic profiles and antioxidant activity. *Innovative Food Science & Emerging Technologies, 30*, 138–144. https://doi.org/10.1016/j.ifset.2015.04.009

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E., & Etherton, T. D. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*, 71–88. https://doi.org/10.1016/S0002-9343(01)00995-0

Ku, C. M., & Lin, J. Y. (2013). Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. *Food Chemistry, 141*, 1104–1113. https://doi.org/10.1016/j.foodchem.2013.04.044

Li, H., Ge, Y., Luo, Z., Zhou, Y., Zhang, X., Zhang, J., & Fu, Q. (2017). Evaluation of the chemical composition, antioxidant and anti-inflammatory activities of distillate and residue fractions of sweet basil essential oil. *Journal of Food Science & Technology, 54*, 1882–1890. https://doi.org/10.1007/s13197-017-2620-x

Lin, J.-T., Chang, Y.-Y., Chen, Y.-C., Hu, C.-C., Chang, Y.-P., Hsu, S.-H., & Yang, D.-J. (2015). Induction of apoptotic death of human hepatocellular carcinoma (HepG2) cells by ethanolic extract from litchi (*Litchi chinensis* Sonn.) flower. *Journal of Functional Foods, 19*, 100–109. https://doi.org/10.1016/j.jff.2015.08.023

Lingesh, A., Paul, D., Naidu, V., & Satheeshkumar, N. (2019). AMPK activating and anti adipogenic potential of *Hibiscus rosa sinensis* flower in 3T3-L1 cells. *Journal of Ethnopharmacology, 233*, 123–130. https://doi.org/10.1016/j.jep.2018.12.039

Lo, M.-M., Benfodda, Z., Bénimélis, D., Fontaine, J.-X., Molinié, R., & Meffre, P. (2021). Extraction and identification of volatile organic compounds emitted by fragrant flowers of three *Tillandsia* species by HS-SPME/GC-MS. *Metabolites, 11*, 594. https://doi.org/10.3390/metabo11090594

Lubinska-Szczygieł, M., Różańska, A., Namieśnik, J., Dymerski, T., Shafreen, R. B., Weisz, M., Ezra, A., & Gorinstein, S. (2018). Quality of limes juices based on the aroma and antioxidant properties. *Food Control, 89*, 270–279. https://doi.org/10.1016/j.foodcont.2018.02.005

Mahomoodally, M. F., Mollica, A., Stefanucci, A., Aumeeruddy, M. Z., Poorneeka, R., & Zengin, G. (2018). Volatile components, pharmacological profile, and computational studies of essential oil from aegle marmelos (bael) leaves: A functional approach. *Industrial Crops and Products, 126*, 13–21. https://doi.org/10.1016/j.indcrop.2018.09.054

Martins, C. A. F., Weffort-Santos, A. M., Gasparetto, J. C., Trindade, A. C. L. B., Otuki, M. F., & Pontarolo, R. (2014). *Malva sylvestris* L. Extract suppresses desferrioxamine-induced PGE_2 and PGD_2 release in differentiated U937 cells: The development and validation of an LC-MS/MS method for prostaglandin quantification. *Biomedical Chromatography, 28*, 986–993. https://doi.org/10.1002/bmc.3106

Mousavi, S. M., Hashemi, S. A., Behbudi, G., Mazraedoost, S., Omidifar, N., Gholami, A., Chiang, W.-H., Babapoor, A., & Pynadathu Rumjit, N. (2021). A Review on health benefits of Malva sylvestris L. nutritional compounds for metabolites, antioxidants, and anti-inflammatory, anticancer, and antimicrobial applications. *Evidence-based Complementary and Alternative Medicine*, 2021. https://doi.org/10.1155/2021/5548404

Nade, V. S., Kawale, L. A., Dwivedi, S., & Yadav, A. V. (2010). Neuroprotective effect of *Hibiscus rosa sinensis* in an oxidative stress model of cerebral post-ischemic reperfusion injury in rats. *Pharmaceutical Biology, 48*, 822–827. https://doi.org/10.3109/13880200903283699

Najar, B., Marchioni, I., Ruffoni, B., Copetta, A., Pistelli, L., & Pistelli, L. (2019). Volatilomic analysis of four edible flowers from *Agastache* genus. *Molecules, 24*, 4480. https://doi.org/10.3390/molecules24244480

Nazaruk, J., & Borzym-Kluczyk, M. (2015). The role of triterpenes in the management of diabetes mellitus and its complications. *Phytochemistry Reviews, 14*, 675–690. https://doi.org/10.1007/s11101-014-9369-x

Neves, M., Antunes, M., Fernandes, W., Campos, M. J., Azevedo, Z. M., Freitas, V., Rocha, J. M., & Tecelão, C. (2021). Physicochemical and nutritional profile of leaves, flowers, and fruits of the edible halophyte chorão-da-praia (*Carpobrotus edulis*) on Portuguese west shores. *Food Bioscience, 43*, Article 101288. https://doi.org/10.1016/j.fbio.2021.101288

Ngan, L. T. M., Tan, M. T., Hoang, N. V. M., Thanh, D. T., Linh, N. T. T., Hoa, T. T. H., Nuong, N. T. M., & Hieu, T. T. (2021). Antibacterial activity of *Hibiscus rosa-sinensis* L. red flower against antibiotic-resistant strains of *Helicobacter pylori* and identification of the flower constituents. *Brazilian Journal of Medical and Biological Research, 54*, Article e10889. https://doi.org/10.1590/1414-431X2020e10889

Nicastro, H. L., Ross, S. A., & Milner, J. A. (2015). Garlic and onions: Their cancer prevention properties. *Cancer Prevention Research, 8*, 181–189. https://doi.org/10.1158/1940-6207.CAPR-14-0172

Ozkan, E. E., Seyhan, M. F., Sirin, O. K., Yilmaz-Ozden, T., Ersoy, E., Cakmar, S. D. H., Goren, A. C., Aydogan, H. Y., & Ozturk, O. (2021). Antiproliferative effects of Turkish pomegranate (*Punica granatum* L.) extracts on MCF-7 human breast cancer cell lines with focus on antioxidant potential and bioactive compounds analyzed by LC-MS/MS. *Journal of Food Biochemistry, 45*, Article e13904. https://doi.org/10.1111/jfbc.13904

Pacheco-Palencia, L. A., Noratto, G., Hingorani, L., Talcott, S. T., & Mertens-Talcott, S. U. (2008). Protective effects of standardized pomegranate (*Punica granatum* L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts. *Journal of Agricultural and Food Chemistry, 56*, 8434–8441. https://doi.org/10.1021/jf8005307

Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: Substances useful in human healthcare. *Archivum Immunologiae et Therapiae Experimentalis, 55*, 315–327. https://doi.org/10.1007/s00005-007-0039-1

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., Gauthier, C., Jacques, P.-E., Li, S., & Xia, J. (2021). MetaboAnalyst 5.0: Narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research, 49*, W388–W396. https://doi.org/10.1093/nar/gkab382

Pashazadeh, H., Özdemir, N., annou, O., & Koca, I. (2021). Antioxidant capacity, phytochemical compounds, and volatile compounds related to aromatic property of vinegar produced from black rosehip (Rosa pimpinellifolia L.) juice. *Food Bioscience, 44*, Article 101318. https://doi.org/10.1016/j.fbio.2021.101318, 2021.

Perestrelo, R., Silva, C., & Câmara, J. S. (2019). Madeira wine volatile profile. A platform to establish Madeira wine aroma descriptors. *Molecules, 24*, 3028. https://doi.org/10.3390/molecules24173028

Pintão, A. M., Pais, M. S. S., Coley, H., Kelland, L. R., & Judson, I. R. (1995). In vitro and in vivo antitumor activity of benzyl isothiocyanate: A natural product from *Tropaeolum majus*. *Planta Medica, 61*, 233–236. https://doi.org/10.1055/s-2006-958062

Putnik, P., Gabrić, D., Roohinejad, S., Barba, F. J., Granato, D., Lorenzo, J. M., & Kovacević, D. B. (2019). Bioavailability and food production of organosulfur compounds from edible allium species. In *Innovative thermal and non-thermal processing, bioaccessibility and bioavailability of nutrients and bioactive compounds* (pp. 293–308). Cambridge, United Kingdom: Elsevier. https://doi.org/10.1016/B978-0-12-814174-8.00010-X, 9780128141748.

Ramya, M., Jang, S., An, H.-R., Lee, S.-Y., Park, P.-M., & Park, P. H. (2020). Volatile organic compounds from orchids: From synthesis and function to gene regulation. *International Journal of Molecular Sciences, 21*, 1160. https://doi.org/10.3390/ijms21031160

Rojanathammanee, L., Puig, K. L., & Combs, C. K. (2013). Pomegranate polyphenols and extract inhibit nuclear factor of activated T-cell activity and microglial activation in vitro and in a transgenic mouse model of Alzheimer disease. *Journal of Nutrition, 143*, 597–605. https://doi.org/10.3945/jn.112.169516

Salib, J. Y., Daniel, E. N., Hifnawy, M. S., Azzam, S. M., Shaheed, I. B., & Abdel-Latif, S. M. (2014). Polyphenolic compounds from flowers of *Hibiscus rosa-sinensis* Linn. and their inhibitory effect on alkaline phosphatase enzyme activity in vitro. *Zeitschrift für Naturforschung C, 66*, 453–459. https://doi.org/10.1515/znc-2011-9-1003

Sieniawska, E., Sawicki, R., Swatko-Ossor, M., Napiorkowska, A., Przekora, A., Ginalska, G., Swatko-Ossor, M., & Augustynowicz-Kopec, E. (2018). The effect of combining natural terpenes and antituberculous agents against reference and clinical *Mycobacterium tuberculosis* strains. *Molecules, 23*, 176. https://doi.org/10.3390/molecules23010176

da Silva, V. P., Alves, C. C. F., Miranda, M. L. D., Bretanha, L. C., Balleste, M. P., Micke, G. A., Silveira, E. V., Martins, C. H. G., Ambrosio, M. A. L. V., de Souza Silva, T., Tavares, D. C., Magalhães, L. G., Silva, F. G., & Egea, M. B. (2018). Chemical composition and in vitro leishmanicidal, antibacterial and cytotoxic activities of essential oils of the Myrtaceae family occurring in the Cerrado biome. *Industrial Crops and Products, 123*, 638–645. https://doi.org/10.1016/j.indcrop.2018.07.033

Sofrata, A., Santangelo, E. M., Azeem, M., Borg-Karlson, A. K., Gustafsson, A., & Pütsep, K. (2011). Benzyl isothiocyanate, a major component from the roots of *Salvadora persica* is highly active against Gram-negative bacteria. *PLoS One, 6*, Article e23045. https://doi.org/10.1371/journal.pone.0023045

Spínola, V., Perestrelo, R., Câmara, J. S., & Castilho, P. C. (2015). Establishment of *Monstera deliciosa* fruit volatile metabolomic profile at different ripening stages using solid-phase microextraction combined with gas chromatography–mass spectrometry.

Food Research International, 67, 409–417. https://doi.org/10.1016/j.foodres.2014.11.055

Vučić, V., Grabež, M., Trchounian, A., & Arsić, A. (2019). Composition and potential health benefits of pomegranate: A review. *Current Pharmaceutical Design, 25*, 1817–1827. https://doi.org/10.2174/1381612825666190708183941

Wang, D., Özen, C., Abu-Reidah, I. M., Chigurupati, S., Patra, J. K., Horbanczuk, J. O., Jóźwik, A., Tzvetkov, N. T., Uhrin, P., & Atanasov, A. G. (2018). Vasculoprotective effects of pomegranate (*Punica granatum* L.). *Frontiers in Pharmacology, 9*, 544. https://doi.org/10.3389/fphar.2018.00544

Wu, S., & Tian, L. (2019). A new flavone glucoside together with known ellagitannins and flavones with anti-diabetic and anti-obesity activities from the flowers of pomegranate (*Punica granatum*). *Natural Product Research, 33*, 252–257. https://doi.org/10.1080/14786419.2018.1446009

Xiang, Z., Xia, C., Feng, S., Chen, T., Zhou, L., Liu, L., Kong, Q., Yang, H., & Ding, C. (2022). Assessment of free and bound phenolics in the flowers and floral organs of two Camellia species flower and their antioxidant activities. *Food Bioscience, 49*, Article 101905. https://doi.org/10.1016/j.fbio.2022.101905

Xiao, Y., Oumarou, D. B., Wang, S., & Liu, Y. (2020). Circular RNA involved in the protective effect of *Malva sylvestris* L. on myocardial ischemic/re-perfused injury. *Frontiers in Pharmacology, 11*, Article 520486. https://doi.org/10.3389/fphar.2020.520486

Xie, B., Nagalingam, A., Kuppusamy, P., Muniraj, N., Langford, P., Győrffy, B., Saxena, N. K., & Sharma, D. (2017). Benzyl Isothiocyanate potentiates p53 signaling and antitumor effects against breast cancer through activation of p53-LKB1 and p73-LKB1 axes. *Scientific Reports, 7*, Article 40070. https://doi.org/10.1038/srep40070

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in pharmacological activities of terpenoids. *Natural Product Communications, 15*, 1–13. https://doi.org/10.1177/1934578X20903555

Zuo, H., Li, Y., Cui, Y., & An, Y. (2017). Cardioprotective effect of *Malva sylvestris* L. in myocardial ischemic/reprefused rats. *Biomedicine & Pharmacotherapy, 95*, 679–684. https://doi.org/10.1016/j.biopha.2017.08.111