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REVIEW ARTICLE

New insights into *Citrus* genus: From ancient fruits to new hybrids

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Abstract

Citrus fruits are among the most ancient known and have always attracted the interest of humans, from both a nutritional and a health point of view. Even though there is substantial scientific literature regarding the *Citrus* genus and associated isolated bioactive compounds, there are still few studies on the botanical features, the phytochemical profiles, and the biological activities for some ancient *Citrus* fruits as well as for new hybrids that have become part of the international market. We have witnessed both the rediscovery and revaluation of some cultivated ancient fruits in some limited areas and the introduction of new hybrids destined to the food, ornamental, and food design market. This is the first review that sheds light on the botanical and chemical features as well as on the biological properties of *Citrus* fruits that are under-investigated, localizing them in an international context. The manuscript focuses in particular on five under-investigated species: *Citrus australasica* F. Muell. (Finger lime), *Citrus medica* L. var. *sarcodactylis* Swingle (Buddha's hand), *Citrus junos* Sieb ex Tan. (Yuzu), *Citrus limon* (L.) Osbeck var. *pompia* Camarda (Pompia), and *Citrus lumia* Risso (Lumia).

KEYWORDS
ancient *Citrus* fruit, biological activity, botanical features, *Citrus* genus, new *Citrus* hybrids, phytochemical profile

Abbreviations: AD-C, Alzheimer disease control group; AD-Y, Alzheimer disease yuzu group; ALP, alkaline phosphatase; BHE, Buddha's hand water extract; CA, *Citrus aurantifolia* (Christm.) Swingle; CL, *Citrus limon* (L.) Burm. f.; CPT1, carnitine palmitoyltransferase I; CR, chrysoeriol-7-*O*-rutinoside; CSEE, yuzu seed ethanol extract; CSEH, yuzu seed n-hexane extract; CSEW, yuzu seed water extract; DE, Durham's Emerald; DPPH, α,α-diphenyl-β-picrylhydrazyl; DSS, dextran sulfate sodium; DW, dry weight; EO, essential oil; FGF-2, fibroblast growth factor; FRAP, ferric reducing antioxidant power; FW, fresh weight; GAE, gallic acid equivalents; GC-FID, gas chromatography–flame ionization detector; GC-MS, gas chromatography–mass spectrometry; HC, high-cholesterol diet mice; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; HPLC-DAD, high-performance liquid chromatography coupled with a diode-array detection; HRV, heart rate variability; IL-1β, interleukin-1β; IL-6, interleukin-6; IκBα, inhibitor of nuclear factor kappa B; JAK2, Janus kinase 2; LPS, lipopolysaccharide; LSK, LiSiKe; MBC, minimum bactericidal concentration; MBD, mean bursting duration; MBR, mean bursting rate; MFR, mean firing rate; MIC, minimum inhibitory concentration; MISI_B, inter-spike intervals within a burst; NF-κB, nuclear factor-κB; NMR, nuclear magnetic resonance; NO, nitric oxide; ORAC, oxygen radical absorbance capacity; OVX, ovariectomized rats treated with vehicle; OVX/E2, ovariectomized rats treated with β-estradiol; OVX/YPEE, ovariectomized rats treated with Yuzu peel 70% ethanol extract; PEO-CP, peel essential oils obtained by cold pressing; PEO-HD, peel essential oils obtained by hydrodistillation; PEOs, peel essential oils; PG-PEVs, propylene glycol penetration-enhancer-vesicles; PI, pompia intrea; PMS, premenstrual symptoms; QRG, quercetin-3-rutinoside-7-glucoside; SEM, scanning electron microscopy; STAT3, signal transducer and activator of transcription 3; t-BOOH, tert-butyl hydroperoxide; TE, trolox equivalents; TEAC, trolox equivalent antioxidant capacity; TEM, transmission electron microscopy; TLR, toll-like receptor; TNF-α, tumor necrosis factor α; XB, XiangBin; YCAE, yuzu callus aqueous extract; YE, yuzu peel 70% ethanol extract; YP-01, yuzu peel paste; YPEE, yuzu peel 70% ethanol extract

1 | INTRODUCTION

The *Citrus* genus, belonging to the Rutaceae family, is one of the most diffused fruit tree crop worldwide, growing all over the world in more than 73 countries, with about 1.4 million hectares harvested and about 14 million tons of fruits produced in 2018 (FAO, 2020). Asia (55.9%) and Africa (36.7%) are the world's leading producing continents, followed by Americas (7.1%), Europe (0.2%), and Oceania (0.1%) (FAO, 2020). Among countries, China and Nigeria are the major producers with about 5.8 and more than 4 million tons, respectively (FAO, 2020). This wide spread of *Citrus* crop is certainly due to its wide application in food, cosmetics, and pharmaceutical industry (González, Domínguez, Moreno, & García, 2010).

Citrus fruits have been used in traditional medicine in several Asian countries, such as China, Japan, and Korea. The Chinese Pharmacopoeia reports nine traditional remedies by six *Citrus* species (Committee, 2010): *Citrus reticulata* Blanco, *Citrus medica* L. var. *sarcodactylis* Swingle, *C. medica* L., *Citrus wilsonii* Tanaka, *Citrus aurantium* L., and *Citrus sinensis* Osbeck. Peels or whole mature or immature fruits belonging to the above *Citrus* species have been used to treat digestive problems, cough, skin inflammation, muscle pain, and ringworm infections and as hypotensive agents (Lv et al., 2015).

Generally, *Citrus* fruits are consumed fresh, but they are also used to produce jams, juices, beverage, and canned products, whereas their essential oils (EOs) are employed in perfumes and cosmetics as well as in several pharmaceutical formulation as antimicrobial and antifungal agents (Dosoky & Setzer, 2018; Lv et al., 2015).

Citrus fruits are rich sources of nutrient and nonnutrient molecules. Among the first group, macronutrients, such as sugars and dietary fiber, and micronutrients, such as potassium, folate, calcium, thiamin, niacin, vitamin B6, vitamin C, phosphorus, magnesium, copper, riboflavin, and pantothenic acid, are the most abundant ones (Lv et al., 2015).

However, secondary metabolites such as flavonoids, alkaloids, coumarins, limonoids, carotenoids, and phenol acids, and volatile compounds such as terpenes and alcohols are the main bioactive compounds and the most investigated for their well-known health benefits, such as antioxidant, radical scavenging, anti-inflammatory, and cardioprotective effects (Barreca et al., 2017, 2020; Cappello et al., 2016; Parhiz, Roohbakhsh, Soltani, Rezaee, & Iranshahi, 2015).

Considering this, *Citrus* species have been widely investigated as whole fruits or waste products (pericarp, endocarp, residual membranes, and seeds) to discover new biofunctional components useful for nutraceutical applications (Barros, Ferreira, & Genovese, 2012; Zema et al., 2018).

Nowadays, the grafting of different *Citrus* species improves the availability of some rare varieties, thus making it possible to investigate their scarcely known phytochemical and phytotherapeutic features.

Some ancient species are also poorly known, probably because they are almost exclusively present in their zones of origin or in botanical gardens.

In light of this, the aim of this review is to collect available data about five uncommon and less investigated *Citrus* species in order to shed light on the botanical and chemical features as well as on their biological properties. The species selected for this screening include *Citrus australasica* F. Muell. (Finger lime), *C. medica* L. var. *sarcodactylis* Swingle (Buddha's hand), *Citrus junos* Sieb ex Tan. (Yuzu), *Citrus limon* (L.) Osbeck var. *pompia* Camarda (Pompia), and *Citrus lumia* Risso (Lumia) (Fig. 1).

2 | BOTANICAL FEATURES

2.1 | *Citrus limon* (L.) Osbeck var. *pompia* Camarda

Citrus limon (L.) Osbeck var. *pompia* Camarda is an ancient native cultivar growing in warm areas of the Northeast of Sardinia (Italy) (Fig. 2). The species has probably hybrid origin and, due to the limited availability of cultivation, there is little information about it (Camarda et al., 2013).

A multivariate analysis of the volatile fraction of flavedo samples from eight different *Citrus* species has highlighted the close association of pompia with bitter orange (*Citrus* × *aurantium* L.), chinotto (*Citrus* × *myrtifolia* Raf.), sweet orange (*Citrus* × *sinensis* L. Osbeck), and grapefruit (*Citrus* × *paradise* Macfad.). Conversely, lemon (*Citrus* × *limon* L. Osbeck) and cedar (*C. medica* L.) (Orrù et al., 2016; Petretto et al., 2016) have been found to differ significantly from this species, unlike previously stated by Mignani et al. (2015). However, genetic analysis has confirmed that pompia is a variety of lemon, thus leading to the attribution of the name *C. limon* var. *pompia* (Camarda et al., 2013).

Pompia is a medium-sized tree, up to 3-m tall, vigorous and upright, with thorns on branches. Leaves are ovate, with entire margin. Flowers are borne alone or grouped in inflorescences, with large, purple-white petals.

Fruits are medium-large, oblate, with a deep yellow hesperidium at full ripeness, larger in diameter than in length, up to 500–700 g; peduncle is resistant, calyx is small, and base is wrinkled. The big, thick mesocarp, as well as the coarse and irregular pericarp, is a fruit peculiarity, which earned it the rejected botanical classification of "*Citrus medica* var. *monstruosa*" (Camarda et al., 2013) (Figure 1a). Nowadays, the fruit's mesocarp is used in confectionary industries, or consumed as candies, dessert, or liqueur (Flamini et al., 2019).

2.2 | *Citrus australasica* F. Muell

Citrus australasica F. Muell., commonly known as finger lime, is widespread throughout the East coast of Australia, mainly from the South-East of Queensland to the North-East of New South Wales, growing mainly in tropical to subtropical rainforest (Fig. 2). It easily grows in temperate areas, with a slightly acid soil rich in nutrients, and high levels of organic matter (Delort & Jaquier, 2009).

Citrus australasica F. Muell. is a tree up to 6-m tall, with spines up to 25-mm long. Leaves are small, glabrous, obovate to elliptic, 1–5 cm long, and 3–25 mm wide, with notched apex and cuneate base; crenate margins are rich in aromatic oil glands. Flowers are white or pale pink in color, bisexual, with short peduncles 1–3 mm long, six to

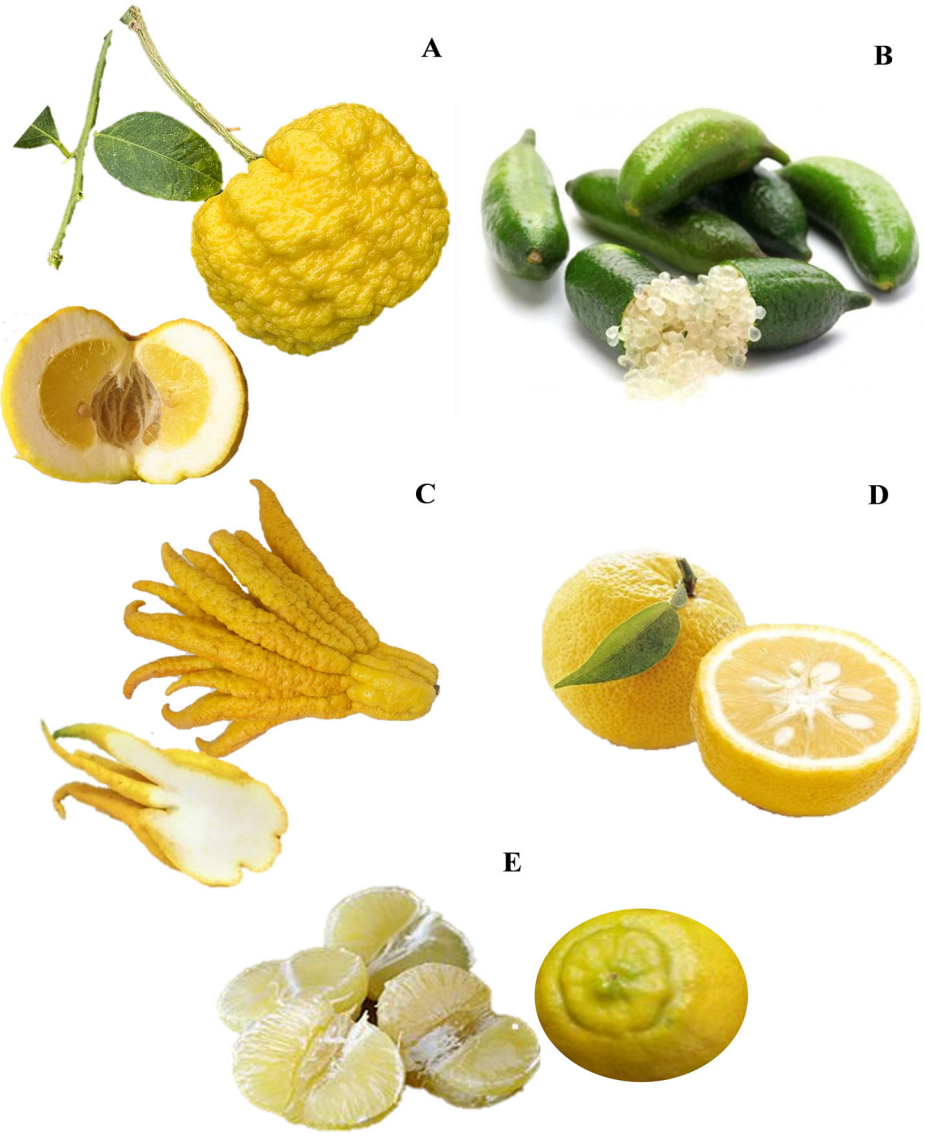

FIGURE 1 Pictures of the entire fruit and section details of the five *Citrus* species investigated: (a) *Citrus limon* (L.) Osbeck var. *pompia* Camarda (Pompia); (b) *Citrus australasica* F. Muell. (Finger lime); (c) *Citrus medica* L. var. *sarcodactylis* Swingle (Buddha's hand); (d) *Citrus junos* Sieb ex Tan. (Yuzu); and (e) *Citrus lumia* Risso (Lumia)

nine white oblong petals, 1.5-mm long, free concave sepals, numerous stamens with white filaments and yellow anthers, and a stout ovary with five to seven locules, which contain eight to 16 ovules. Fruits are unique in the *Citrus* family; indeed, as suggested by the common name, they have a finger-shaped form with a length up to 10 cm (Figure 1b) (Hawkeswood, 2017; Lim, 2012). The skin is usually green-black to purple, thin, and durable. The species is also called "lemon caviar" due to juice vesicles, tiny and round, which tend to come out of the fruit. The fruit skin is commonly green, but some varieties show red, purple, black, or yellow colors (Hawkeswood, 2017; Lim, 2012). Currently, five finger lime cultivars are registered: *C. australasica* "Alstonville" (A), *C. australasica* "Blunobia Pink Crystal", *C. australasica* "Durham's Emerald" (DE), *C. australasica* "Judy's Everbearing", and *C. australasica* "Pink Ice". Usually, *C. australasica* F. Muell. fruits are eaten fresh in fruit salad, or used as a garnish of flavor components in culinary. Moreover, due to the sour taste, it is also used in jam, sauces, and drinks (ANBG, 2020).

2.3 | *Citrus medica* L. var. *sarcodactylis* Swingle

Citrus medica L. var. *sarcodactylis* Swingle is native to North-Western India and generally distributed in South and South-East Asia, such as Sri Lanka, Thailand, Vietnam, China, Japan, and Taiwan (Chan, Hwang, Kuo, Hung, & Wu, 2017) (Fig. 2). It grows well in sandy, well-drained soils in full sun, with a consistent and regular moisture. *Citrus medica* L. var. *sarcodactylis* Swingle grows as a shrub or small tree, with purplish branches, leaves, and flowers when young. Branches are thorny

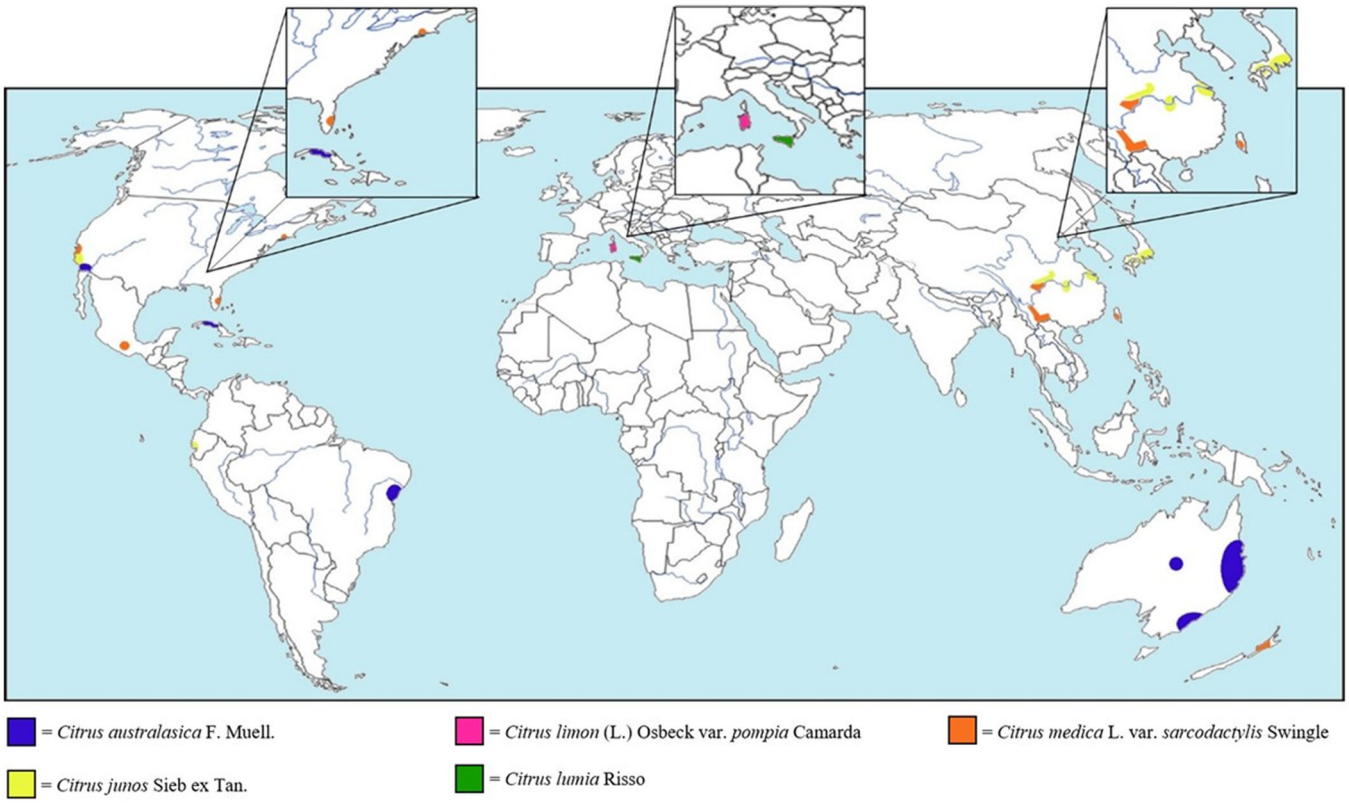

FIGURE 2 Geographical distribution of the five selected *Citrus* species (Camarda et al., 2013; Raimondo et al., 2015; Global Biodiversity Information Facility [https://www.gbif.org/])

and cladded by simple or unifoliolate evergreen leaves. The petiole is short, not winged, whereas the leaf is elliptic to ovate-elliptic, with serrate margins and rounded, obtuse, or mucronate apex. Inflorescences are axillary, with about 12 flowers consisting of five petals, 1.5–2 cm in length (Zhang, 2015). Fruit is unique, with finger-like projections as it ripens (Figure 1c). The elliptic fruit has a coarse surface, which can be white or pale yellow. The pericarp is soft, thick, and seedless in the center, and surrounds the sarcocarp, which contains 10–15 pellucid to pale, milky yellow segments. The sarcocarp is pale, milky yellow, with acidic to slightly sweet taste. Historically, *C. medica* L. var. *sarcodactylis* Swingle has been used as temple offering in the Buddhist religion (indeed its common name is Buddha's hand), mainly because each fruit purportedly resembles a closed hand in prayer. Actually, fruits are used in culinary candied or for salad dressing, marinades, cooked foods, or drinks, as well as jams and marmalades (MBG, 2020).

2.4 | *Citrus junos* Sieb ex Tan

Citrus junos Sieb ex Tan. is a natural cross of *C. reticulata* Blanco and *Citrus ichangensis*, which is widely cultivated in Korea, China, and Japan (Fig. 2). The slow growth (generally requires 10 years to fructify) needs sunny areas and rich, noncalcareous, light, and well-drained soils. It is relatively frost-hardy, resisting to temperature up to −12°C. It is a shrub or semi-vigorous thorny tree, with spear-shaped, pointed on the tip, and serrated margin leaves. The petiole can be as long as one third of the leaf, with small wings. Flowers are fragrant, with five petals. Fruits are medium sized, resembling a small orange or tangerine, with yellow rough skin, and scented rind and flesh (Figure 1d) (EFP, 2020). *Citrus junos* Sieb ex Tan. is commonly used in culinary, drinks, and marmalades; moreover, it has been used in traditional Chinese medicine to improve blood circulation and prevent colds (Hirota et al., 2010), whereas in traditional Japanese medicine it promotes mind and body health (Matsumoto, Kimura, & Hayashi, 2017).

2.5 | *Citrus lumia* Risso

Citrus lumia Risso is an ancient lime commonly called "Lumia," cultivated in Sicily, Mediterranean Europe, and North Africa, where it is also known as "Mediterranean lime". This small tree has unarmed branches, obovate, obtuse leaves with serrate margins, and slightly winged petioles. The medium-sized flowers have reddish petals, whereas fruits are globose and umbonate, with depressed apex and base (Figure 1e). The fruit peel is aromatic, with a characteristic sweet and floral smell, yellow, and smooth; the mesocarp is thin and bitter, whereas the endocarp has nine to 11 logs, full of seeds.

Citrus lumia Risso was historically used in essence preparation, as eupeptic, and in food industry, whereas nowadays its cultivation is amateur, which allows its preservation (Raimondo, Cornara, Mazzola, & Smeriglio, 2015).

3 | PHYTOCHEMICAL PROFILE

3.1 | *Citrus limon* (L.) Osbeck var. *pompia*

Among *Citrus* fruits, *C. limon* (L.) Osbeck var. *pompia* (Pompia) is certainly an unexplored one. Indeed, even the origin and taxonomy of pompia is still unknown (Orrù et al., 2016).

Characterization of its chemical constituents remains the first and critical step in order to investigate the biological properties and health benefits of this uncommon fruit.

Manconi et al. (2016) carried out a high-performance liquid chromatography (HPLC) coupled with diode-array detection (DAD), and a liquid chromatography–tandem mass spectrometry analysis to investigate the rind ethanol extract. The analysis showed the presence of 13 compounds: quinic acid, neoeriocitrin, neohesperidin, sinapic acid, naringin, rutin, robinin, ferulic acid, isorhamnetin-rutinoside, myricitrin, isoquerectin, eriocitrin, and phorizin, in order of abundance (Table 1). Among them, flavanones are highly represented in the extract with naringin, neoeriocitrin, and neohesperidin (23.77, 46.53, and 44.57 μg/mg of dry weight [DW], respectively), which probably are responsible for the fruit's bitter taste. In a subsequent analysis of pompia rind extract by Manconi et al. (2018), data showed a similar polyphenol composition with respect to naringin and neoeriocitrin content (28.0 and 42.5 μg/mg DW), whereas a highest content of neohesperidin was found (76.5% higher than the previous one). Furthermore, new compounds such as gallic acid, eriocitrin, hesperidin, and myricetin-3-galactoside (128.3, 40.4, 16.9, and 29.3 μg/mg DW) were also detected (Table 1).

Other than being eaten fresh, pompia is used traditionally in the preparation of the candied "Pompia intrea" (PI), which has been recognized as a traditional Italian product (MIPAF 2016). Deiana et al. (2019) analyzed, for the first time, the main components of PI to investigate the differences due to the presence of honey and heating treatments. Indeed, fresh fruits were transformed according to traditional procedure, adding multifloral honey in the second boiling step. Investigation of PI ethanol extract was carried out by headspace solid-phase microextraction followed by gas chromatography–flame ionization detector (GC–FID) and gas chromatography–mass spectrometry (GC–MS) analyses. Results showed a predominance of limonene (72.21–86.2%), which is one of the main compounds of pompia flavedo, as already found by Petretto et al. (2016). Moreover, β-myrcene, trans-β-ocimene, neryl acetate, and geranyl acetate were found in the PI extract (1.2–2.2%; 0.3–1.8%; 0.5–1.9%; and 0.4–1.4%, respectively) as previously reported (Petretto et al., 2016). Interestingly, few benzene derivatives such as benzaldehyde (0–0.1%) and phenyl-acetaldehyde (not defined to 0.7%) were found, probably due to honey addition (Jerković, 2013), whereas the presence of furfural (0.1–1.3%) is probably due to the heating process (Deiana et al., 2019).

Recently, Flamini et al. (2019) analyzed for the first time the phytochemical profile of peel essential oils (PEOs) obtained by hydrodistillation (PEO-HD) and cold pressing (PEO-CP) methods, and an EO obtained by hydrodistillation of leaves (LEO-HD), by using GC–MS analyses (Table 2).

Comparing PEO-HD and PEO-CP, although with some difference, monoterpene hydrocarbons remain the most abundant class (80% vs. 98.38%, respectively). Limonene and myrcene represent the main compounds with a relative abundance of 95.77% and 1.55%, respectively, in the PEO-CP and 75% and 2.12%, respectively, in PEO-HD.

Despite this, substantial differences have been found between the two PEOs.

Indeed, monoterpenes oxygenated represent the 16.44% in PEO-HD, whereas they are detected only in traces in the PEO-CP.

The LEO-HD showed also a similar composition, but with a markedly different relative abundance of the different classes of compounds. Indeed, also in this case, monoterpene hydrocarbons represent the main terpene class (42.01%), with limonene and myrcene as the most abundant compounds (28.64% and 0.91%, respectively). On the contrary, LEO-HD contain up to 53.5% of monoterpenes oxygenated and 3.04% of sesquiterpenes oxygenated, which are less abundant in PEO-HD (0.1%) and completely absent in PEO-CP.

In conclusion, pompia, from a phytochemical point of view, shows a peculiar composition both in EOs and in rind extract, which seems to indicate its individuality as a distinct species.

3.2 | *Citrus australasica* F. Muell

The attention on *C. australasica* F. Muell. (finger lime) started to increase, due to the fascinating and unique characteristics of the species. Despite this, studies on its phytochemical profile are few in comparison with other *Citrus* fruits. Recently, Delort et al. (2015), in order to identify and quantify the most abundant compounds and to compare their phytochemical profiles, analyzed the volatile fraction of peel extracts of three finger lime cultivars (A, JE and DE) by GC–MS analyses (Table 2).

Results showed that volatile compounds change among the three cultivars, with a unique chemotype for each one: limonene/sabinene for A (61.66%:20.55%); limonene/citronellal/isomenthone (64.38%:9.04%:7.29%) for JE; and limonene/citronellal/citronellol for DE (66.27%:9.26%:5.18%).

Up to date, these chemotypes have not been identified in other *Citrus* species (Delort et al., 2015).

Moreover, surprisingly, terpenes normally abundant in the *Citrus* genus, such as γ-terpinene (≤0.08%), α-pinene (≤0.98%), β-pinene (≤0.32%), and citral, generally the major constituents besides limonene in lime species, are lower or absent in all finger lime cultivars investigated (Delort et al., 2015).

A comparison among the phytochemical profiles of the three cultivars investigated showed that some volatile compounds could be used as markers of specific cultivars, namely, aliphatic and terpenyl acetates in cultivar A; citronellyl esters in cultivar JE; 4-methylnonanal, cumin aldehyde, and methyl thymol ether in cultivar DE (Delort et al., 2015).

TABLE 1 Polyphenol profile of the Citrus species selected. Results were obtained by HPLC/UV-Vis, HPLC/DAD, or LC/MS analyses

	Citrus junos Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus medica* L. var. *sarcodactylis* Swingle			*Citrus lumia* Risso	*Citrus australasica* F. Muell.	
			GFS-A	GFS-B	GFS-C			
Compound	WF[a]; S[b]; PU[c]; P[d]	R (μg/mg)	WF (μg/g)			A (mg/100 g)	P&F	References
(7S,8S)-4,7,9,9′-Tetrahydroxy-3,3′-dimethoxy-8–4′-oxyneolignan-9′-*O*-D-glucopyranoside							+	Wang et al., 2019
1-*O*-sinapoyl-*β*-*D*-glucose							+	
3,5,6-Trihydroxy-3′,4′,7-trimethoxyflavone					51.3			Chu et al., 2012
3-O-Methyl-5-pentylresorcinol-1-*O*-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside							+	Wang et al., 2019
5,7-Dimethoxycoumarin			897.7	779.0	1,206.6			Chu et al., 2012
6,7-Dimethoxycoumarin			100.5	35.8	16.0			
7-Hydroxycoumarin			7.5	13.4	12.7			
8a-(*O*-*β*-Gentiobiosyloxy)-7,8-(9,9-dimethylpyrano)							+	Wang et al., 2019
Apigenin						9.7		Smeriglio et al., 2019
Apigenin-7-*O*-glucoside	17.8 mg/100 g[a]							Yang et al., 2013
Apigenin 7-*O*-neohesperidoside							+	Wang et al., 2019
Bergapten			< LOQ	1.7	< LOQ			
Chlorogenic acid						151,512.0		Smeriglio et al., 2019
Chrysoeriol-7-O-rutinoside							+	Wang et al., 2019
Didymin							+	Wang et al., 2019
Diosmin			1,223.5	1,204.8	462.3	22.8	+	Chu et al., 2012; Smeriglio et al., 2019; Wang et al., 2019
Eriocitrin		40.4				1,012.4		Manconi et al., 2018; Smeriglio et al., 2019
		0.1						
Ferulic acid		1.0				51.0		Manconi et al., 2016; Smeriglio et al., 2019
Gallic acid		128.3						Manconi et al., 2018
Hesperidin	38.1 mg/100 g[a]	16.9	666.8	801.6	243.4	60.0		Chu et al., 2012; Kim, 2018; Manconi et al., 2018; Shim et al., 2019; Smeriglio et al., 2019; Yang et al., 2013
	36.3 mg/100g[c]							
	7.5%[b]							
Hydrocoumaric acid							+	Wang et al., 2019
Hyperoside						14.9		Smeriglio et al., 2019
Isoquercetin		0.4				4.3		Manconi et al., 2016; Smeriglio et al., 2019
Isorhamnetin 3,7-*O*-diglucose							+	Wang et al., 2019

(Continues)

TABLE 1 (Continued)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus medica* L. var. *sarcodactylis* Swingle			*Citrus lumia* Risso	*Citrus australasica* F. Muell.	References
			GFS-A	GFS-B	GFS-C			
	WF[a]; S[b]; PU[c]; P[d]	R (μg/mg)	WF (μg/g)			A (mg/100 g)	P&F	
Isorhamnetin-3-O-(6′-acetyl)-galactoside							+	
Isorhamnetin-3-*O*-glucoside							+	
Isorhamnetin-rutinoside		0.6						Manconi et al., 2016
Isoscopoletin			58.8	24.2	20.6			Chu et al., 2012
Kaempferol 3-*O*-sophoroside							+	Wang et al., 2019
Kaempferol-7-O-neohesperidoside						4.2		Smeriglio et al., 2019
Kaempferol-3-arabino-7-rhamnosid-permethylaether							+	Wang et al., 2019
Luteolin 7-*O*-rutinoside-4′-*O*-glucoside							+	
Lyoniresinol 2*α*-*O*-*β*-glucoside							+	
Lyoniresinol 9′-*O*-glucoside							+	
Methylnaringenin 7-*O*-rutinoside							+	
Methylnaringenin 7-*O*-neohesperidoside							+	
Myricetin-3-galactoside		29.3						Manconi et al., 2018
Myricitrin		0.5						Manconi et al., 2016
Naringin	13.1 mg/100 g[a]	28.8					+	Kim, 2018; Lv et al., 2015; Manconi et al., 2018; Shim et al., 2019; Yang et al., 2013
	11.6 mg/100g[c]	23.8						
	0.6%[d]							
Narirutin	29.2 mg/100 g[a]						+	Wang et al., 2019; Yang et al., 2013
Neodiosmin						3.3	+	Smeriglio et al., 2019; Wang et al., 2019
Neoeriocitrin		42.5				4.0		Manconi et al., 2016, 2018; Smeriglio et al., 2019
		46.5						
Neohesperidin		76.5					+	Manconi et al., 2016; Manconi et al., 2018; Wang et al., 2019
		44.6						
p-Coumaroyl glucose							+	Wang et al., 2019
Phlorizin		219.7						Manconi et al., 2016
Poncirin							+	Wang et al., 2019
Quercetin	1.9 mg/100 g[a]							Kim, 2018; Yang et al., 2013
	1.7 mg/100g[c]							

(Continues)

TABLE 1 (Continued)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus medica* L. var. *sarcodactylis* Swingle			*Citrus lumia* Risso	*Citrus australasica* F. Muell.	References
			GFS-A	GFS-B	GFS-C			
	WF[a]; S[b]; PU[c]; P[d]	R (μg/mg)	WF (μg/g)			A (mg/100 g)	P&F	
Quercetin 3,4′-di-*O*-*β*-*D*-glucose							+	Wang et al., 2019
Quercetin 3-*O*-sinapoyl-sophoroside							+	
Quercetin 3-rutinoside-7-glucoside							+	
Quinic acid		219.7						Manconi et al., 2016
Robinin		1.1						Manconi et al., 2016
Rhoifolin						2.6		Smeriglio et al., 2019
Rutin	4.9 mg/100 g[a]	8.6				1,092.0	+	Kim, 2018; Manconi et al., 2016; Smeriglio et al., 2019; Wang et al., 2019; Yang et al., 2013
	2.7 mg/100g[c]							
Rutin hydrate	4.2 mg/100 g[a]							Yang et al., 2013
Scopoletin			12.7	15.5	36.8			Chu et al., 2012
Sinapic acid		30.1						Manconi et al., 2016
Syringeti-3-*O*-glucoside							+	Wang et al., 2019
Tangeretin	0.6 mg/100 g[a]					23.4		Kim, 2018; Smeriglio et al., 2019; Yang et al., 2013
	0.7 mg/100g[c]							
Vanillic acid						1.8		Smeriglio et al., 2019
Vicenin-2							+	Wang et al., 2019
α-Glucosyl hesperidin							+	Wang et al., 2019

Abbreviations: GFS-A, Puning area; GFS-B, Guangzhou area; GFS-C, Guangdong area; WF, Whole fruit; R, Rind; S, Seed; PU, Pulp; P, Peel; A, Albedo; P&F, Pulp and flash; + found but not quantified; <LOQ, beyond limit of quantification; superscript letters (a-d) refer to polyphenols identified into WF, S, PU and P extracts, respectively.

In particular, cultivar A showed traces of aliphatic (heptyl acetate and octyl acetate) and terpenic acetates (linalyl acetate, *trans*- and *cis*-sabinene acetate, and bornyl acetate) as well as the presence of eucalyptol, absent in JE and DE. A high amount of citronellal, citronellol, and isomenthone was found in JE, whereas the DE profile appeared similar to JE with an increase in citronellal and citronellol, and the presence of *α*-phellandrene, which was absent in the JE chemotype (Delort et al., 2015).

Moreover, six new molecules were reported for the first time in a *Citrus* extract: 6-methyloctyl acetate, citronellyl citronellate, 1,2:5,6-diepoxy-p-menthane 4 (isomers), 2,3-epoxy-p-menthan-6-one, *cis* and *trans*-p-menth-1-en-3-ol-6-one, and 1,2-epoxy-p-methan-5-one. The first one was specific for cultivar A, citronellyl citronellate for cultivar JE and DE, whereas cis and trans-p-menth-1-en-3-ol-6-one for cultivar JE and DE (Delort et al., 2015).

Recently, Wang et al. (2019) described the polyphenol profile of peel and flesh extracts of two different cultivars of finger lime, Xiang-Bin (XB) and LiSiKe (LSK), native from Hainan Province (China). In this work, 33 compounds were identified by ultra-performance liquid chromatography–high-resolution mass spectrometry and ultra-performance liquid chromatography–DAD analyses, including 25 flavonoids, three coumarin derivatives, two resin phenol derivatives, one phenol derivative, one neolignan glycoside, and one secoiridoid derivative. Peels of both chemotypes showed neohesperidin as the most abundant compound (17.22% and 14.87% in LSK and XB, respectively), followed by alpha-glucosyl hesperidin (10.47% and 13.17% % in LSK and XB, respectively). On the contrary, quercetin-3-rutinoside-7-glucoside (QRG) and chrysoeriol-7-O-rutinoside (CR) were the main compounds in the flesh. In particular, 6.93% and 8.90% of QRG and 5.10% and 4.97% of CR were found in LSK and XB peel, respectively.

These differences are due probably to the regional distribution and pedoclimatic conditions of the investigated cultivars.

Wang et al. (2019) studied also the acid and sugar content. Citric, malic, and quinic acids are the most abundant ones in both LSK

TABLE 2 Phytochemical profile of volatile fraction isolated by *Citrus* species selected. Results, obtained by GC-FID and GC-MS analyses, were expressed as area percentages (%)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus lumia* Risso	*Citrus medica* L. var. *sarcodactylis* Swingle	*Citrus australasica* F. Muell.*		
					A	JE	DE
(+)-4-Carene	1.23[a]	–	0.27	–	–	–	–
(E)-β-Ocimene	2.13[b]	–	0.71	1.37	0.21	0.08	0.19
(E)-Limonene oxide	0.83[b]	–	–	–	–	–	–
(E)-Myroxide	0.73[b]	–	–	–	–	–	–
(E)-*p*-Mentha-2,8-dienol	0.10[b]						
(Z)-β-Farnesene	0.15[a]	–	0.01	–	–	–	–
(Z)-β-Ocimene	–	–	0.37	5.9	0.15	0.35	0.73
(Z)-Farnesol	0.24[b]	–	–	–	–	–	–
(Z)-Sabinene hydrate	0.56[b]	–	–	–	–	–	–
(Z)-Limonene oxide	1.07[b]	–	–	–	–	–	–
(Z)-*p*-Mentha-2,8-dienol	0.58[b]	–	–	–	–	–	–
(Z,E)-α-Farnesene	0.08[a]	–	–	–	–	–	–
10-Methylnonadecane	–	–	0.02	–	–	–	–
1-β-Bisabolene	–	–	0.44	0.51	–	–	–
2,3,3-Trimethyl-1,4-pentadiene	–	–	0.01	–	–	–	–
3,5,24-Trimethyltetracontane	–	–	0.01	–	–	–	–
4-Terpineol	0.95[b]	–	0.48	–	0.16	0.03	0.03
6-Methyl-5-hepten-2-one	0.06[b]	<0.1	–	–	–	–	–
2,3-Epoxygeranial	0.20[b]	–	–	–	–	–	–
6,7-Epoxymyrcene	0.07[b]	–	–	–	–	–	–
allo-Ocimene	–	–	–	0.34	–	–	–
Bicyclogermacrene	–	0.45	–	0.11	3.09	0.84	1.80
Bornylene	–	–	0.16	–	–	–	–
Camphene	0.11[b]	–	0.03	–	–	–	–
Camphor	0.13[b]						
Cariophyllene	–	–	0.18	–	–	–	–
Cariophyllene oxide	0.14[b]	0.73	–	–	–	–	–
Carvone	0.10[b]						
cis- Sabinene acetate	–	–	–	–	0.25	–	–
cis-α-Bergamotene	–	–	–	0.02	–	–	–
cis-α-Bisabolene	–	–	0.02	0.04	–	–	–
cis-β-Ocimene	0.89[a]	0.46	–	–	–	–	–
cis-β-Terpineol	0.13[a]	–	–	–	–	–	–
Citral	0.03[a]	–	1.06	–	–	–	–
Citronellal	–	1.27	0.04	0.05	–	9.04	9.26
Citronellol	0.04[a]	–	–	–	0.43	–	–
Citronellyl acetate	–	<0.1	–	–	–	0.04	0.04
Cyclopropane, pentyl-	–	–	0.10	–	–	–	–
Decanal	0.52[a]	<0.1	–	0.01	–	–	–
	0.38[b]						
Elericin	–	–	0.04	–	–	–	–

(Continues)

TABLE 2 (Continued)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus lumia* Risso	*Citrus medica* L. var. *sarcodactylis* Swingle	*Citrus australasica* F. Muell.*		
					A	JE	DE
epi-Globulol	0.23[b]	–	–	–	–	–	–
epi-α-Bisabolol	–	<0.1	–	–	–	–	–
epi-α-Cadinol	–	0.21	–	–	–	–	–
epi-β-Bisabolol	0.1[b]	–	–	–	–	–	–
epoxy-Alloaromadendrene	–	<0.1	–	–	–	–	–
Geranial	–	24.44	–	0.94	–	–	–
Geraniol	1.12[b]	0.57	–	0.09	–	–	–
Geranyl acetate	–	3.94	–	0.02	–	–	–
Germacrene-B	0.73[b]	–	–	–	–	<0.01	0.12
Germacrene-D	0.55[a]	–	–	0.52	0.20	0.37	0.90
Germacrene-D-4-ol	–	<0.1	–	–	–	–	–
Hexadecyloxirane	–	–	0.02	–	–	–	–
Humulene epoxide	0.28[b]	–	–	–	–	–	–
Isobornyl formate	–	–	0.04	–	–	–	–
Isogeranial	–	0.44	–	–	–	–	–
Isomenthone	–	–	–	–	–	7.29	0.03
Isoneral	–	0.37	–	–	–	–	–
Isoterpinolene	–	<0.1	–	–	–	–	–
L-Phellandrene	2.12[b]	–	–	–	–	–	–
Limonene	60.60[a]	28.64	48.91	47.59	61.66	64.38	66.27
	22.9[b]						
Limonene epoxide	0.38[b]	–	–	–	–	–	–
Linalool	7.43[b]	0.56	18.25	0.11	0.03	1.94	0.21
Linalyl anthranilate	–	–	10.96	–	–	–	–
Methyleugenol	–	–	0.01	–	–	–	–
Myrcene	–	0.91	–	–	–	1.52	1.54
Neoisomenthol	–	–	–	–	–	0.72	
Neophytadiene	–	–	–	–	<0.01	0.22	0.23
Neral	–	18.84	–	0.70	–	–	–
Nerol	0.12[b]	1.49	0.83	0.12	–	–	–
Nerol acetate	–	–	1.80	–	–	–	–
Neryl acetate	0.35[b]	1.48	–	0.02	–	–	–
Neryl propionate	–	–	1.10	–	–	–	–
Octanal	0.27[a]	–	0.13	–	–	–	–
Octyl acetate	0.79[b]	–	–	–	0.10	–	–
o-Ocimene	4.96[a]	–	–	–		–	–
Oxypeucedanin	–	–	–	–	5.67	0.16	–
p-Cymen-2-ol	1.23[a]	–	–	–	–	–	–
p-Cymen-8-ol	0.62[b]	–	–	–	–	–	–

(Continues)

TABLE 2 (Continued)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus lumia* Risso	*Citrus medica* L. var. *sarcodactylis* Swingle	*Citrus australasica* F. Muell.*		
					A	JE	DE
p-Cymene	0.67[b]	<0.1	–	–	<0.01	0.16	0.32
Piperitone	–	–	–	–	<0.01	0.96	<0.01
p-Mentha-1,4(8)-diene	2.33[a]	–	–	–	–	–	–
Sabinene	2.21[a]	0.28	–	0.143	20.55	0,16	0.58
	1.80[b]						
Spathulenol	1.36[b]	1.22	–	–	0.36	0.13	0.20
Terpinen-4-ol	1.02[a]	–	–	0.01	–	–	–
trans-Nerolidol	–	0.40	–	–	–	–	–
trans-Sabinene acetate	–	–	–	–	0.43	–	–
trans-α-Bergamotene	–	<0.1	–	0.45	–	–	–
trans-β-Caryophyllene	–		–	–	–	–	–
trans-β-Ocimene	0.57[a]	10.50	–	–	<0.01	0.38	<0.01
Valencene	–	<0.1	–	–	–	–	–
α-Bergamotene	–	–	0.28	–	–	–	–
α-Bisabolol	0.11[b]	–	–	–	–	–	–
α-Cadinol	–	0.39	–	–	–	–	–
α-Costol	0.81[b]	–	–	–	–	–	–
α-Cubebene	–	–	–	0.01	–	–	–
α-Humulene	–	0.11	–	0.04	0.09	0.60	<0.01
α-Phellandrene	0.37[a]	–	–	0.26	0.01	0.25	2.0
α-Pinene	3.97[a]	<0.1	0.62	3.317	0.55	0.31	0.98
	3.91[b]						
α-Terpenyl acetate	1.98[b]	–	–	–	0.66	<0.01	<0.01
α-Terpinene	0.66[b]	–	–	0.68	–	–	–
α-Terpineol	0.85[a]	0.14	5.22	0.09	–	0.21	<0.01
	1.18[b]						
α-Terpinolene	2.11[b]	0.16	–	1.65	0.02	0.03	0.10
α-Thujene	1.71[a]	–	0.02	0.649	0.12	<0.01	0.01
	1.87[b]						
β-Bisabolene	–	0.13	–	–	–	–	–
β-Bisabolol	–	0.10	–	–	–	–	–
β-Caryophyllene	–	0.76	–	0.5	–	–	–
β-Citral	0.04[a]	–	–	–	–	–	–
β-Citronellal	0.05[a]	–	0.86	–	–	–	–
β-Cubebene	–	–	–	<0.01	–	–	–
β-Cymenene	–	–	–	1.65	–	–	–
β-Elemene	–	–	–	0.01	–	–	–
β-Linalool	7.95[a]	–	–	–	–	–	–

(Continues)

TABLE 2 (Continued)

Compound	*Citrus junos* Sieb ex Tan.	*Citrus limon* (L.) Osbeck var. *pompia* Camarda	*Citrus lumia* Risso	*Citrus medica* L. var. *sarcodactylis* Swingle	*Citrus australasica* F. Muell.*		
					A	JE	DE
β-Myrcene	1.55[a]	–	–	1.55	–	–	–
	4.83[b]						
β-Oplopenone	–	<0.1	–	–	–	–	–
β-Phellandrene	0.30[b]	–	–	–	0.18	1.79	4.74
β-Pinene	3.96[a]	0.19	6.89	1.88	0.32	<0.01	0.03
	3.36[b]						
β-Santalene	–	–	0.01	–	–	–	–
γ-Terpinene	46.74[a]	–	0.15	29.45	0.08	0.01	0.01
	19.3[b]						
δ-3-Carene	–	0.90	–	–	0.09	0.19	0.36
δ-Cadinene	–	<0.1	–	0.02	–	–	–
δ-Elemene	0.31[b]	–	–	0.02	0.61	0.58	0.16
References	Hong et al., 2017; Liu et al., 2014	Flamini et al., 2019	Smeriglio et al., 2019	Deng et al., 2017	Delort et al., 2015		

*Volatile profile of a peel extract (peel oil) obtained by several *C. australasica* cultivars: A, Alstonville; JE, Judy's Everbearing; DE, Durham's Emerald.
[a]Yuzu essential oil.
[b]Volatile profile of yuzu whole fruit extract.

and XB fleshes (71.50, 4.80 and 1.13, and 73.49, 4.08 and 0.58 mg/g fresh weight [FW], respectively), whereas the order changes in LSK and XB peels, with citric acid as the main component followed by quinic and malic acids (8.26, 5.48 and 1.35, and 20.75, 4.39 and 2.23 mg/g FW, respectively). Among sugars, sucrose, glucose, and fructose are predominant. Indeed, sucrose is the main component of LSK peel (2.27 mg/g FW), whereas glucose and fructose are highest in XB (2.08 and 2.56 mg/g FW, respectively). A similar behavior was observed in the flesh, with 4.16 and 4.39 mg/g FW of fructose in LSK and XB, respectively, followed by glucose (3.15 and 3.34 mg/g FW, respectively) and sorbitol (2.77 and 2.25 mg/g, respectively).

The high amount in acids and the low content in sugars reflect the sour taste of finger lime fruit (Wang et al., 2019).

3.3 | *Citrus medica* L. var. *sarcodactylis* Swingle

With the modernization of traditional Chinese herbs, several species of *Citrus* started to be attractive sources of bioactive compounds. Among them, *C. medica* L. var. *sarcodactylis* Swingle (Buddha's hand), well known in folk medicine for its antiasthmatic, anti-inflammatory, hypoglycemic, hypolipemic, and antimicrobial properties, has been evaluated (Committee, 2010).

The phytochemical profile of the EO and of whole fruit extracts of *C. medica* L. var. *sarcodactylis* has been investigated recently.

Deng et al. (2017) analyzed the influence of different isolation methods (vacuum hydrodistillation and ultrafiltration in comparison with traditional hydrodistillation) on the phytochemical composition of the EOs obtained from fruit peel, showing significant differences in the phytochemical composition. Overall, they identified, by GC-MS analysis, 55 compounds belonging to different classes: cyclic and acyclic monoterpene hydrocarbons, cyclic and acyclic oxygenated monoterpenoids, aldehydes, sesquiterpene hydrocarbons, oxygenated sesquiterpenoids, and coumarins (Table 2).

However, focusing on the most abundant compounds (limonene, γ-terpinene, and *p*-cymene), Buddha's hand EOs showed a higher content of limonene by ultrafiltration (64.7%), followed by hydrodistillation and vacuum distillation (60.1% and 47.6%, respectively). On the contrary, an opposite behavior for γ-terpinene and *p*-cymene was highlighted. The first one increased substantially passing from ultrafiltration to hydrodistillation and vacuum distillation (0.1%, 6.1%, and 29.5%, respectively), whereas a dizzying decrease was recorded for *p*-cymene (26.1%, 17.6%, and 0%, respectively). These significant differences are due to the extraction conditions (pressure, temperature, and time). Anyway, vacuum distillation maintained most of the distinctiveness of this *Citrus* fruit, due to its high content of characteristic flavor components and low content of oxygenated compounds. Indeed, these compounds, especially monoterpenes and sesquiterpenes oxygenated, originate from prolonged exposure of EO to air, light, or high temperature, giving unpleasant odors to

the plant complex, which loses its native chemical and organoleptic features.

Chu, Li, Yin, Ye, and Zhang (2012) investigated the phytochemical profile of *C. medica* L. var. *sarcodactylis* fruits collected in three different areas of China: GFS-A, GFS-B ,and GFS-C (Puning, Guangzhou, and Guangdong areas, respectively).

Whole fruit extracts, obtained by pressurized liquid extraction, were analyzed by HPLC-DAD analysis. Eleven compounds, belonging to several classes, were identified and quantified simultaneously: six coumarins (isoscopoletin, scopoletin, 7-hydroxycoumarin, 6,7-dimethoxycoumarin, 5,7-dimethoxycoumarin, and bergapten), three flavonoids (diosmin, hesperidin, and 3,5,6-trihydroxy-3′,4′,7-trimethoxyflavone) (Table 1), and two limonoids (limonin and nomilin).

In addition to those already identified by Chu et al. (2012), reported in Table 1, Chan et al. (2017) identified other compounds belonging to several classes in the fruit methanol extract. They identified five coumarins (xanthyletin, skimmin, haploperoside A, leptodactylone, and 7-methoxycoumarin), one furanocoumarin (oxypeucedanin hydrate), one pyranocoumarin (nordentatin), one peptide (citrusin), one triterpenoid (obacunone), four acids (3-(2-O-D-glucopyranosyl-4-methoxyphenyl-propanoic acid, (E)-6-hydroxy-2,6-dimethylocta-2,7-dienoic acid, 1,2,3,4-tetrahydro-carboline-3-carboxylic acid, and citrylidene malonic acid), one phenolic acid (*cis*-p-coumaric acid), four esters (methyl vanillate, methyl benzoate, methyl paraben, and methyl-4-hydroxycinnamate), two alcohol (4-hydroxy-phenethyl alcohol and coniferin), one eleutheroside (syringin), three sterols (stigmasterol, β-sitosterol, and β-sitosterol-glucoside), and one flavonoid (chrysoeriol-8-C-glucoside).

Moreover, a new sesquiterpene was isolated, named Citrumedin-C, whose structure was established by comparison of two-dimensional nuclear magnetic resonance (NMR) spectroscopy and MS with literature data (Chan et al., 2017).

Citrus medica L. var. *sarcodactylis* Swingle has been investigated also for its polysaccharide content. Four water-soluble polysaccharides (FCp-1, FCp-2, FCp-3, and FCp-4) were isolated by hot-water extraction and ethanol precipitation, followed by separation procedure. Acid hydrolysis and methylation followed by infrared, GC–MS, and NMR analyses showed that only galacturonic acid was present in FCp-2 and FCp-4. Glucose was found to be present in FCp-3, whereas FCp-1 was a heteropolysaccharide composed by arabinose, galactose, glucose, rhamnose, and xylose (molar ratio 3.0:7.0:4.1:1.0:1.5) (He, Liang, Zhang, & Pan, 2014).

3.4 | *Citrus junos* Sieb. Ex Tanaka

Citrus junos Sieb. Ex Tanaka (yuzu) is a famous fruit widely used in China, Japan, and Korea for culinary, cosmetics, perfumes, aromatherapy, and medicine. Recently, Kim (2018) examined the chemical composition of crude polysaccharides from yuzu seeds, obtained by water extraction and ethanol precipitation. Results obtained by GC–MS analysis showed 57.60% of proteins, 27.70% of neutral sugar, and 14.70% of uronic acid. Among monosaccharides, galacturonic acid and glucuronic acid are the most abundant compounds (14.70 mol %), followed by arabinose, glucose, galactose, mannose, fucose, and rhamnose (12.85, 4.54, 4.32, 2.70, 1.68, and 1.61 mol %, respectively).

Hong et al. (2017) compared the volatile flavor compounds of this extremely sour fruit, with *C. limon* (L.) Burm. f. (CL) and *Citrus aurantifolia* (Christm.) Swingle (CA).

In this study, 67 compounds were identified, including alcohols (15.43%), aldehydes (1.08%), esters (4.26%), ketones (0.16%) oxygenated compounds (4.06%), and hydrocarbons as the most abundant functional groups (75.01%). This composition is similar to those of CL and CA, where hydrocarbons are the main class, with 76.18% and 63.86% of the total amount percentage, respectively. Among them, limonene, γ-terpinene, linalool, β-myrcene, *trans*-β-farnesene, α-pinene, β-pinene, and *trans*-β-ocimene were recorded as the most abundant compounds (Table 2). Taking into consideration major compounds as markers, limonene represents the most abundant one in all species investigated (22.79%, 31.33%, and 18.44% in yuzu, CL, and CA, respectively).

On the contrary, γ-terpinene (19.30%), linalool (7.43%), β-farnesene (4.24%), α-pinene (2.93%), and *trans*-β-ocimene (2.13%) are higher in yuzu than in the other species (13.7%, 1.25%, 0.31%, 2.93%, and 0.29% for CL and 11.26%, 1.69%, 0.6%, 2.33%, and 0.33% for CA, respectively). Comparing to yuzu, a similar amount of β-myrcene was observed in CL (4.83% and 5.67%), whereas an approximately double amount was found in CA (2.46%). Conversely, the lowest content of β-pinene was found in yuzu (3.36% vs. 8.14% and 9.3%, into CL and CA, respectively). This phytochemical profile is superimposable with that observed previously by Liu et al. (2014), who investigated the yuzu EO (Liu et al., 2014).

These results have shown that, despite some common organoleptic characteristics, the phytochemical analysis often allows to distinguish different *Citrus* species, because the presence of certain compounds as well as their relative abundance depend substantially on the taxonomic differences, climate and growth conditions of different species.

3.5 | *Citrus lumia* Risso

Citrus lumia Risso (lumia) cultivation is currently limited, as well as information about the chemical composition and health properties of this species.

However, recently two papers investigated for the first time the phytochemical profile of the EO as well as the polyphenols content of an albedo extract of lumia (Smeriglio et al., 2018, 2019).

The EO characterization, carried out by GC-FID and GC-MS analyses, showed the presence of 35 volatile compounds. Among them, limonene, linalool, linalyl anthranilate, β-pinene, and α-terpineol were the most abundant compounds (48.91%, 18.25%, 10.96%, 6.89%, and 5.22%, respectively) (Table 2). These results are in accordance with other *Citrus* EOs, presenting limonene as major constituent (32–98%), and resemble the EO of bergamot fruit, which contains 32-45% of limonene. However, linalool concentration is significantly higher in

comparison with other *Citrus* EOs, including bergamot one (10.23%). The EO composition highlighted a predominance of terpenic compounds, which include monoterpenic hydrocarbons (58.12%), oxygenated terpenes (26.73%), monoterpene derivatives (13.90%), and sesquiterpenes (0.93%). Once again, authors highlight how this profile is similar to that of bergamot. Indeed, lumia fragrance reminds the bergamot fruit probably due to this similarity in composition, underlining the hypothesis that lumia could be a progenitor of bergamot, although the taste is distinctly different, being aromatic with a tendence to sweet and floral. The same research group investigated the phytochemical profile of an albedo methanol extract of lumia, by reversed-phase liquid chromatography–diode array–fluorescence analysis (Smeriglio et al., 2019).

In this study, a similar behavior with other *Citrus* species was highlighted. Indeed, lumia albedo extract showed a high content in polyphenols (1,917.33 mg/100 g of FW), of which 89.34% are flavonoids and 10.66% are phenolic acids (Table 1). Among flavonoids, flavanones were the main class (98%), followed by flavonols (2%).

However, *Citrus* species such as *C. medica* and *C. limetta* Risso, with which the lumia is often confused, are usually characterized by a higher amount of hesperidin and naringin, whereas *C. reticulata* (Blanco) contains neohesperidin as major flavonoid.

From this point of view, lumia possesses an unusual profile, with eriocitrin and hesperidin as the most abundant compounds (1,012.41 and 600.38 mg/100 g FW, respectively), which alone represent the 52.81% and 31.31%, respectively, of flavonoids, together with the absence of naringin.

These results were corroborated also by micromorphological investigations, which showed clusters of blue-green, needle-shaped crystals of hesperidin, between flavedo and albedo layers, highlighted by toluidine blue O staining. These results were confirmed also by scanning electron microscopy (SEM) that, coupled with X-ray energy dispersive system, showed the organic composition of these crystals, clearly distinguishable from inorganic crystals such as those of calcium oxalate, also abundantly present in lumia (Smeriglio et al., 2019).

This study opens new perspectives for the studies of *Citrus*, by highlighting the importance of multidisciplinary analyses to identify correctly and thoroughly the chemical and micromorphological features of this genus.

4 | BIOLOGICAL ACTIVITY

4.1 | *Citrus limon* (L.) Osbeck var. *pompia* Camarda

Recently, Flamini et al. (2019), besides characterizing the phytochemical profile of pompia EO, as reported above, carried out also a preliminary study on its antioxidant and antibacterial activity (Table 3).

The antioxidant and free-radical scavenging power was investigated by ferric reducing antioxidant power (FRAP) and α,α-diphenyl-β-picrylhydrazyl (DPPH) assays (electron and hydrogen transfer-based in vitro assays, respectively), expressing results as half-inhibitory concentration (IC_{50}; mg/L). Pompia EO showed the strongest antioxidant activity into FRAP assay (1.87 mg/L), whereas a lower activity was observed into DPPH assay (39.33 mg/L).

The weak antioxidant activity found is probably due to the lack of γ-terpinene and terpinolene that were identified by Choi, Song, Ukeda, and Sawamura (2000) as the main antioxidant compounds present in *Citrus* EO, whereas limonene, despite being the most abundant terpene in Citrus EOs, does not play a pivotal role in conferring antioxidant activity to the phytocomplex.

Moreover, the EO did not show any antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus pseudintermedius*, neither against dermatophytes and toxigenic fungi (Flamini et al., 2019).

The inactivity toward the Gram-negative *E. coli* is consistent with previous results obtained with pompia leaf EO by Fancello et al. (2016), as well as with other studies, in which *Citrus* EOs exerted the strongest antibacterial activity against Gram-positive strains (Fancello et al., 2016; Ruiz & Flotats, 2014).

This could be explained not only by the resistance of *E. coli* to limonene (Chubukov et al., 2015) but also by the lesser presence of oxygenated monoterpenes, which seem to be the main agents responsible for antimicrobial activity (Fancello et al., 2016; Settanni et al., 2012).

Pekmezovic et al. (2016) evaluated the efficacy of this EO on mixed microbial infections caused by *Pseudomonas aeruginosa* and pathogenic fungi, which are common in chronic infections (Table 3). In this context, the EO showed a minimum inhibitory concentration (MIC) value of 50, 100, and 250 mg/L on fungal growth of *Candida albicans*, *Candida parapsilosis*, and *Aspergillus terreus*, respectively, suggesting a higher susceptibility of *C. albicans*. On the contrary, no activity on *Pseudomonas aeruginosa* was observed (MIC > 500 mg/mL). Moreover, a synergistic activity of pompia EO with itraconazole was found against planktonic *C. albicans*. Indeed, a sub-inhibitory concentration of itraconazole (MIC/4) with pompia EO (12.5 mg/L) inhibited fungal growth of 80% with promising research perspectives in the field of microbial resistance.

In addition to the EO, various extracts as tale or loaded into nanoformulations have been tested.

Manconi et al. (2018) investigated the incorporation of pompia rind, freeze-dried ethanol extract into vesicular systems such as liposomes, glycerosomes, and penetration-enhancer-containing vesicles prepared with propylene glycol (PG-PEVs) to enhance the antioxidant and antibacterial activity, with specific application for oral cavity protection (Table 3). The capability of these pharmaceutical formulations to improve the efficacy of pompia extract in counteracting oxidative stress induced by hydrogen peroxide was evaluated on keratinocytes, whereas the antimicrobial activity was investigated against planktonic cultures of the cariogenic bacteria *Streptococcus mutans* and *Lactobacillus acidophilus*, as well as on commensal *Streptococcus sanguinis*, by measuring the diameter of the bacterial growth inhibition zone.

Results showed that already an aqueous dispersion of pompia extract increased the keratinocyte viability of 25% with respect to positive control (hydrogen peroxide), which decreased the cell viability to 50%. Moreover, the incorporation in liposomes provided a further statistically significant increase in cell viability of 40%, reaching a value

TABLE 3 Biological effects of different extracts, essential oils (EOs), and formulations of *Citrus* species selected, investigated by *in vitro*, animal, and human studies

Citrus species	Phytocomplex	Model	Biological activity	Dose	References
Citrus limon (L.) Osbeck var. *pompia* Camarda	Peel EO	Colorimetric assay	Free-radical scavenging	FRAP: 1.87 mg/L	Flamini et al., 2019
				DPPH: 39.33 mg/ml	
	Leaf EO	*C. albicans*	Antifungal	100 mg/L	Pekmezovic et al., 2016
				12.5 mg/L + Itraconazole 4 g/L	
	Rind freeze-dried EE	3T3/keratinocytes	Cytoprotective	100 μg/ml[a]	Manconi et al., 2018
	Rind EE PG-PEV				
	Rind EE glycerosome	*S. mutans*	Bactericidal	<0.078 mg/ml	Pinna et al., 2019
	Rind EE PG-PEV				
	Rind raw EWE	*C. albicans*	Fungicidal	0.625 mg/ml	
Citrus australasica F. Muell. var. *XiangBin*	Peel ME	Colorimetric assay	Free-radical scavenging	DPPH: 1.60 TE/g FW	Wang et al., 2019
				FRAP: 1.46 TE/g FW	
				TEAC: 3.60 TE/g FW	
				ORAC: 14.7 TE/g FW	
		BV-2 cells	Anti-inflammatory	NO: 200–800 μg/ml[a]	
				IL-1β, IL-6, and TNF-α: 800 μg/ml[a]	
	Flesh ME	Colorimetric assay	Free-radical scavenging	DPPH: 0.84 TE/g FW	
				FRAP: 0.92 TE/g FW	
				TEAC:1.24 TE/g FW	
				ORAC 14.3 TE/g FW	
		BV-2 cells	Anti-inflammatory	NO: 200–800 μg/ml[a]	
				IL-1β, IL-6, and TNF-α: 800 μg/ml[a]	
Citrus australasica F. Muell. var. *LiSiKe*	Peel ME	Colorimetric assay	Free-radical scavenging	DPPH: 1.25 TE/g FW	
				FRAP: 1.13 TE/g FW	
				TEAC: 2.82 TE/g FW	
				ORAC: 16.36 TE/g FW	
		BV-2 cells	Anti-inflammatory	NO: 200–800 μg/ml[a]	
				IL-1β, IL-6, and TNF-α: 800 μg/ml[a]	
	Flesh ME	Colorimetric assay	Free-radical scavenging	DPPH: 0.92 TE/g FW	
				FRAP: 1.08 TE/g FW	
				TEAC: 1.57 TE/g FW	
				ORAC: 13.63 TE/g FW	
		BV-2 cells	Anti-inflammatory	NO: 200–800 μg/ml[a]	
				IL-1β, IL-6, and TNF-α: 800 μg/ml[a]	
Citrus medica L. var. *sarcodactylis* Swingle	Whole fruit WE	RSC96 Shwann cells	Neuroprotective	1.7-8.5 μg/ml[b]	Huang et al., 2014
	Peel EO	*S. aureus*	Antibacterial	0.625 mg/ml	Li et al., 2019

(Continues)

TABLE 3 (Continued)

Citrus species	Phytocomplex	Model	Biological activity	Dose	References
				1.25 mg/ml	
				19.2 mg/ml	
		B. subtilis	Antibacterial	0.625 mg/ml	
				1.25 mg/ml	
				19.2 mm	
		M. luteus	Antibacterial	1.25 mg/ml	
				5 mg/ml	
				16.1 mm	
		E. Coli	Antibacterial	2.5 mg/ml	
				1.25 mg/ml	
Citrus junos Sieb ex Tan	Peel 80% EE	Colorimetric assay	Antioxidant	Folin: 25.44 mg GAE/g DW	Shim et al., 2019
			Free-radical scavenging	11.2 mg/ml DPPH: 1,042.37 μg/ml	
				RP: 24.99 μg AAE/100 μg DW	
	Peel 100% EE		Antihyperuricemic	XO:1 mg/ml[a]	
	Peel 20% EE				
			Antiaging	E: 1 mg/ml[a]	
	Whole fruit 70% EE	Male Sprague-Dawley rats	Anticognitive dysfunction	Extract 3%[a]	Yang et al., 2013
			Hypoglycemic		
	Peel 70% EE	HepG2 cells	Hypolipidemic	100–200 μg/ml[b]	Shin et al., 2016
		Male C57BL/6 J mice	Hypocholesterolemic	Extract 1% and 5%[b]	
	Peel WE	Male BALB/c mice	Anticolitis symptoms	50 g/kg[b]	Abe et al., 2018
			Anti-inflammatory	Extract 0.5%[a]	
		SEAP-expressing RAW 264.7 cells	Anti-inflammatory	NF-κb: Extract 0.1–1%[b]	
	Peel 70% EE	OVX female Sprague-Dawley rats	Osteopenia prevention	Extract 5%[a]	Jeon et al., 2016
	Peel EO	Single blind randomized study	Antipremenstrual symptoms	5 min inhalation	Matsumoto et al., 2017
	Callus WE	Colorimetric assay	Antioxidant	Folin: 24.55 mg GAE/g DW	Adhikari et al., 2017
	Callus WE nanoliposome	B16F10 cells	Antiaging	500 μg/ml[a]	
	Seed WE	CCD-986sk cells	Antioxidant	Folin (10 mg/ml): 141.11 μg GAE/ml	Kim & Shin, 2013
			Free-radical scavenging	DPPH (10 mg/ml): 56.61%	
				TEAC (10 mg/ml): 40.02%	
		Sheep red blood cells	Immunostimolatory	1 mg/ml[b]	
	Seed EE	Colorimetric assay	Antioxidant	Folin (10 mg/ml): 188.71 μg GAE/ml	
			Free-radical scavenging	DPPH (10 mg/ml): 63.56%	
				TEAC (10 mg/ml): 45.53%	

(Continues)

TABLE 3 (Continued)

Citrus species	Phytocomplex	Model	Biological activity	Dose	References
		Sheep red blood cells	Immuno-stimolatory	1 mg/ml[b]	
	Seed HE	Colorimetric assay	Antioxidant	Folin (10 mg/ml): 26.19 μg GAE/ml	
			Free-radical scavenging	DPPH (10 mg/ml): 28.57%	
Citrus lumia Risso	Flavedo EO	Colorimetric assay	Antioxidant	β-Carotene: 22 μg/ml	Smeriglio et al., 2018
			Free-radical scavenging	ORAC: 46 μg/ml	
				DPPH: 104 μg/ml	
				Folin: 181 μg/ml	
				FRAP: 202 μg/ml	
				TEAC: 233 μg/ml	
			Anticognitive dysfunction	252.25 μg/ml	
		Embryonic rat cortical neuronal cells	Neurodepressive	MFR: 124.6 μg/ml	
				MBR: 32 μg/ml	
				%Spike_B: 21 μg/ml	
				MBD: 97.4 μg/ml	
				MISI_B: 190.9 μg/ml	
	Albedo MWE	Colorimetric assay	Antioxidant	ORAC: 2.57 μg/ml	Smeriglio et al., 2019
			Free-radical scavenging	FRAP: 5.22 μg/ml	
				Folin: 8.94 μg/ml	
				TEAC: 73.19 μg/ml	
				DPPH: 118.21 μg/ml	
				β-Carotene: 533.03 μg/ml	
		Human lymphocytes	Cytoprotective	15–50 μg/ml[b]	

Abbreviations: EE, ethanol extract; PG-PEV, propylene glycol penetration enhancer vesicle; EWE, ethanol–water extract; ME, methanol extract; WE, water extract; HE, hexane extract; MWE, methanol–water extract; TE, trolox equivalent; FW, fresh weight; GAE, gallic acid equivalents; DW, dry weight; AAE, ascorbic acid equivalents; IC_{50}, half-inhibitory concentration; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; DIZ, diameter of inhibition zone; FRAP, ferric reducing antioxidant power; DPPH, α,α-diphenyl-β-picrylhydrazyl; TEAC, trolox equivalent antioxidant capacity; ORAC, oxygen radicals absorbance capacity; RP, reducing power; NO, nitric oxide; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; XO, xanthine oxidase; E, Elastase; MBD, mean bursting duration; MBR, mean bursting rate; MFR, mean firing rate; MISI_B, inter spike intervals (s) within a burst; % Spikes_B, percentage of spikes in burst.

[a] Activity ≥ 50%.

[b] Activity ≤ 50%.

of 50% when the extract was loaded into glycerosomes and PG-PEVs, thus re-establishing physiological conditions (cell viability 100%).

Observing the inhibition halo of extract dispersed in water (reference) and vesicular systems, no significant difference was observed on the selected cariogenic bacteria, whereas an increase of inhibition halo zone (from ~8 to ~13 mm) was observed against *S. sanguinis* (Manconi et al., 2018).

Another recent work investigated the antimicrobial and fungicidal effects of a pompia raw extract as tale or incorporated into liposomes, glycerosomes, and PEVs, against *S. mutans* ad *Candida albicans*, respectively (Pinna et al., 2019) (Table 3).

Authors investigated these biological activities by using several approaches such as disc diffusion method, MIC and minimum bactericidal concentration (MBC), minimum fungicidal concentration, time-kill assay, and transmission electron microscopy (TEM).

Raw extract as well as vesicular systems did not show any cytotoxic activity against human gingival fibroblasts within the concentration range investigated (1–100 μg/ml).

Despite the raw extract did not show any antimicrobial activity, against neither *S. mutans* nor *C. albicans*, when delivered by nanovesicles, the antimicrobial activity against *S. mutans* was increased.

On the contrary, also in this case no antifungal effect was observed.

Raw extract reduced the *S. mutans* growth and viability, as demonstrated by MIC and time-kill assays, whereas it was not effective as fungicidal compound. These data in addition to TEM images suggested a fungistatic rather than a fungicidal effect against *C. albicans*.

Regarding vesicular systems, raw extract loaded into liposomes did not exert any antibacterial activity against *S. mutans* (MIC > 2.5 mg/mL), whereas when loaded into glycerosomes and PG-PEVs, no bacteria growth was detectable even at the lowest concentrations (MIC < 0.078 mg/mL). Regarding the antimicrobial activity against *C. albicans*, glycerosomes and PG-PEVs displayed a superimposable behavior with respect to the raw extract exerting a fungistatic activity and highlighting a greater resistance of the yeast membrane in comparison with bacteria (Pinna et al., 2019).

4.2 | *Citrus australasica* F. Muell

Finger lime possesses a distinctive phenotype, which can vary among different cultivars and fruit section. Information about the biological properties of this fascinating specie is poor, and therefore, its health benefit remains under-looked.

Recently, Wang et al. (2019) investigated the potential antioxidant activity of peel and flesh extracts of two Chinese cultivars, XB and LSK, by several in vitro colorimetric assays such as DPPH, FRAP, oxygen radical absorbance capacity (ORAC), and Trolox equivalent antioxidant capacity (TEAC). The observed data, expressed as mg of Trolox equivalents (TE)/g FW, showed that XB and LSK peels exert higher antioxidant activity than XB and LSK flesh. XB peel was the most active one (1.60, 1.46, and 3.60 mg TE/g FW in XB peel vs 1.25, 1.13, and 2.83 mg TE/g FW in LSK peel for DPPH, FRAP, and TEAC, respectively) (Table 3). These results can be due to the higher presence into finger lime peel with respect to flesh, of the most abundant flavonoids neohesperidin and α-glucosyl hesperidin, expressed as percentage on total polyphenols. In particular, the first one was the most abundant one into LSK peel (17.22% vs. 14.87% of XB), whereas α-glucosyl hesperidin was higher in XB peel (13.17%) in comparison with LSK peel (10.47%).

On the contrary, LSK flesh showed the best antioxidant activity with respect to XB flesh (0.92, 1.08, and 1.57 mg TE/g FW in LSK flesh vs. 0.84, 0.92, and 1.24 mg TE/g FW in XB flesh in DPPH, FRAP, and TEAC, respectively). Similarly, the two most abundant flavonoids in the flesh (QRG and CR) could confer a strong antioxidant activity to the extracts investigated.

Indeed, QRG accounts for 6.93% of total polyphenols in LSK flesh, and for 8.90% in XB flesh, whereas CR accounts for 5.10% of total polyphenols in LSK flesh, and for 4.97% in XB flesh.

Regarding the ORAC assay, LSK peel and flesh had a similar antioxidant behavior (14.74 and 14.32 mg TE/g FW, respectively), whereas XB peel showed a higher value (16.36 mg TE/g FW) than flesh (13.68 mg TE/g FW).

The complex of data indicates that the total antioxidant power depends on the specific antioxidant activities of the most abundant compounds, but also on synergistic effects that can occur between major and minor polyphenols, which confer greater activity to the plant phytocomplex.

Citrus are very popular for their anti-inflammatory activity (Lee et al., 2016); however, many fields are still unexplored; one of these is the protection of microglia from inflammatory agents.

Considering this, Wang et al. (2019) investigated the capability of the above four finger lime extracts to inhibit the nitric oxide (NO) release induced by lipopolysaccharide (LPS; 0.1 μg/mL) on microglia BV-2 cell line. Finger lime extracts, used at sub-cytotoxic concentrations (200–800 μg/mL), significantly prevent NO release, and inhibit the release of inflammatory markers, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), in a dose-dependent manner. Moreover, the genes that regulate main inflammatory pathways (Janus kinase 2 [JAK2]/signal transducer and activator of transcription 3 [STAT3], nuclear factor-κB [NF-κB]/inhibitor of nuclear factor kappa B [IκBα], and toll-like receptor [TLR]) were assessed by quantitative real-time reverse transcription polymerase chain reaction.

Finger lime extracts decrease the LPS-induced upregulation of inducible NO synthase, IL-6, JAK2, TNF-α, IL-1β, nuclear factor-κB (NF-κB), TLR2, TLR4, and LPS-induced down-regulation of the IκBα, whereas they did not affect the LPS-induced upregulation of TLR1 and TLR6. Among the finger lime extracts investigated, the peel showed the strongest regulation especially on STAT3, which may be explained by differences in flavonoid content (Wang et al., 2019).

4.3 | *Citrus medica* L. var. *sarcodactylis* Swingle

Citrus medica L. var. *sarcodactylis* Swingle (Buddha's hand) has been tested to investigate its potential on neuronal regeneration (Huang et al., 2014) (Table 3). Fibroblast growth factor (FGF-2) is one of the key modulators, which enhance the intrinsic growth potential of neurons, playing a pivotal role in the regeneration and repair of injured adult neurons by autocrine and paracrine signaling.

In particular, a linear correlation between FGF-2 expression and increasing expression of proteins involved into proliferation and migration events was found in RSC96 Schwann cells, primary supporting cells of the peripheral nerves.

In light of this, Huang et al. (2014) investigated the neural regenerative effect of Buddha's hand water extract (BHE) by analyzing the FGF-2 expression using a RSC96 Schwann cells model transfected with recombinant luciferase reporter plasmid containing an FGF-2 promoter sequence.

Results showed that BHE, within a concentration range of 1.7–85 μg/mL, enhanced luciferase expression in transformed Schwann cells, inducing molecular markers of cell migration such as FGF-2, urokinase-type plasminogen activator, and matrix metalloproteinase 9 and of cell proliferation such as the cell cycle-related proteins cyclin A and cyclin B1. Moreover, BHE increased, in a dose-dependent manner,

the expression of proliferating cell nuclear antigen, involved in DNA replication and repair, suggesting a potential role of BHE in repair and regeneration mechanisms of peripheral nerves (Huang et al., 2014).

Li, Cai, Liu, Sun, and Luo (2019) investigated the potential antibacterial activity of Buddha's hand EO against several common foodborne bacteria such as *E. coli*, *S. aureus*, *Bacillus subtilis*, and *Micrococcus luteus* (Table 3). Among them, EO showed a higher activity on *S. aureus* with an inhibition zone of 19.2 mm, followed by *B. subtilis* (16.3 mm), *M. luteus* (16.1 mm), and *E. coli* (11.2 mm). MIC and MBC value were also evaluated. Buddha's hand EO showed equal inhibitory effects on both *S. aureus* and *B. subtilis* (MIC = 0.625 mg/mL), and lower effects on *M. luteus* and *E. coli* (MIC = 1.25 and 2.5 mg/Ml, respectively). MBC resulted superimposable for *S. aureus*, *B. subtilis*, and *E. coli* (1.25 mg/mL), whereas a weak activity was observed on *M. luteus* (MIC = 5 mg/mL).

In order to investigate deeply the antibacterial mechanism, authors carried out SEM analyses.

A change of the bacteria morphology as well as a decrease of the permeability and integrity of cell membrane was observed, suggesting that the mechanism of action includes cell wall lysis with intracellular material leakage and subsequent cell death. Monoterpenes, alcohol and phenols are probably the main compounds implicated into the reported antibacterial activity, although a synergistic effect exerted by minor compounds cannot be excluded.

4.4 | *Citrus junos* Sieb ex Tan

Yuzu is certainly, among the *Citrus* fruit selected in this manuscript, the most investigated for its health benefits on human health and broad range of applications.

Recently, Shim, Chae, and Cho (2019) tried to optimize a standardized extraction procedure and titration method of yuzu peel to develop a natural remedy for cosmetic application (Table 3).

They tested several water and ethanol extracts at different percentages (20%, 40%, 60%, 80%, and 100% ethanol), which were analyzed by HPLC to titrate the main bioactive compounds identified (hesperidin and naringin). After that, the optimized extracts were evaluated for their antioxidant as well as xanthine oxidase and elastase inhibitory activities on in vitro cell-free model (Shim et al., 2019).

Xanthine oxidase is directly related to gout and hyperuricemia, due to its involvement in uric acid production, whereas elastase is a protease enzyme that degrades elastin leading to skin aging. Indeed, many elastase inhibitors possess anti-wrinkle and antiaging activity and could be used in the cosmetic field.

The 80% ethanol extract showed the highest total phenols content, 25.44 mg of gallic acid equivalents (GAE)/g of DW), as well as the greatest antioxidant activity, with an IC_{50} of 1,042.37 μg/mL in DPPH assay and a good reducing power on ferrous ions expressed as 24.99 μg of vitamin C equivalents/100 μg of dry extract. The 100% ethanol extract (1 mg/mL) had the greatest xanthine oxidase inhibitory activity (55.74%), whereas aqueous and 20% ethanol extracts showed the highest inhibitory activity on elastase (56.3% and 61.4%, respectively) (Shim et al., 2019).

The hesperidin and naringin contents were highest in the 100% ethanol extract. In light of this, authors postulated that the antioxidant and antiaging effects of yuzu peel extract could be attributed to phenolics such as flavonoids, suggesting a possible use of this extract for the treatment and prevention of metabolic disease and hyperuricemia. On the contrary, aqueous extract of *C. junos* could be used for its antiaging properties, although other studies will be needed to better investigate its phytochemical profile, in which polyphenols are likely to not represent the most abundant compounds (Shim et al., 2019).

Yang et al. (2013) investigated the possible therapeutic use of yuzu (lyophilized 70% ethanol extract) for cognitive decline in β-amyloid-treated rats (Table 3). Sprague–Dawley rats were divided into three groups, 20 rats each: Alzheimer disease control (AD-C), AD yuzu (AD-Y), and control (C), and fed with three different high-fat, polyphenol-free diets to induce insulin resistance. The AD-Y diet contained 3% lyophilized yuzu extract, whereas AD-C and C diets contained dextrin. On day 10, AD-C and AD-Y groups received hippocampal CA1 infusions of plaque-forming β-amyloid, whereas the C group received a reverse sequence of β-amyloid that does not accumulate in the brain and is not detected by immunohistochemistry (Yang et al., 2013). Yuzu treatment prevented β-amyloid accumulation in the AD-Y group, whereas beta-amyloid deposition was evident in the AD-C group. Moreover, the AD-C group showed a cognitive dysfunction that was not present in the AD-Y group. The AD-C group showed a higher serum glucose level with respect to C and AD-Y groups (7.7, 6.1, and 5.9 mmol/L, respectively), and a serum insulin value of 213 pmol/L, significantly higher in comparison with C and AD-Y groups (159 and 132 pmol/L, respectively). The AD-Y group showed a normal glucose tolerance, insulin sensitivity, and secretion, suggesting a yuzu preventive role on cognitive dysfunction, impaired energy, and glucose homeostasis induced by β-amyloid infusion (Yang et al., 2013).

Shin, Park, Sung, Chung, and Hwang (2016) recently investigated the potential role of yuzu peel 70% ethanol extract (YE) on hepatic lipid accumulation on an in vitro cell-based model (HepG2 cells), as well as on a mouse model (Table 3).

YE significantly inhibited, in dose-dependent manner up to 23%, the oleic acid-induced hepatic lipid accumulation in HepG2 cells, without showing any cytotoxicity within the concentration range investigated (100–400 μg/mL). Moreover, YE was able to upregulate, at 100 and 200 μg/mL, the expression levels of cholesterol metabolism-related proteins such as AMP-activated protein kinase, acetyl-CoA carboxylase, peroxisome proliferator-activated receptor alpha, and carnitine palmitoyltransferase 1 (CPT1) and to downregulate the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR).

This hypocholesterolemic effect was further confirmed in mice fed with a high-cholesterol diet. Compared to normal diet mice, high-cholesterol diet mice (HC) showed increased body weight, liver fat content, liver weight, total cholesterol content, and low-density lipoprotein cholesterol.

Both groups receiving the extract, HC + 1% YE and HC + 5% YE, showed a significantly decreased body weight, liver fat content, liver weight, total cholesterol, and low-density lipoprotein cholesterol compared with those of HC group. According to preliminary in vitro data, in

mice fed YE diet compared with HC diet PPAR-α and CPT1 expression were induced, whereas HMGCR expression was inhibited.

These results are in line with other studies (Kim et al., 2013; Sharma et al., 2019), which demonstrated a hypocholesterolemic effect of the yuzu extract (Table 3). Moreover, yuzu fruit extract seems to act additively with *Garcinia cambogia* extract in reducing reactive oxygen species, and to inhibit lipid accumulation as discovered by Sharma et al. (2019).

The juice-free, yuzu peel paste YP-0, as well as its aqueous extract, was also investigated for possible protection against ulcerative colitis, a well-known inflammatory bowel disease (Table 3) (Abe et al., 2018).

In this study, two mice groups were pretreated with 50 g/kg (w/w) YP-01 daily for 14 days, whereas two other groups were fed with standard diet. One group fed with YP-01 and one control group drank water with 4% (w/v) dextran sulfate sodium (DSS) for 5 days to induce colitis, whereas the other YP-01 and standard group continued with normal water (Abe et al., 2018). The results showed weight loss, colon shortening, diarrhea, and visible fecal blood on mice treated with DSS only, whereas these symptoms were counteracted by pretreatment with YP-01.

In order to understand the mechanism of action, anti-inflammatory and antioxidant effects of the yuzu aqueous extract were evaluated. The extract suppressed NF-κB activation in a murine macrophage cell line (RAW 264.7), and in addition it blocked TNF-α production in LPS-stimulated mice. Moreover, the yuzu aqueous extract showed a higher antioxidant activity with respect to the reference compounds hesperidin and ascorbic acid, suggesting a synergistic activity among its constituents. Considering these results, the effects of yuzu peel on DSS-induced colitis symptoms could be due to both anti-inflammatory and antioxidant in vivo effects (Abe et al., 2018).

Yuzu peel 70% ethanol extract (YPEE) was also tested on osteopenic ovariectomized female Sprague–Dawley rats, to study its potential effects on osteoblastogenesis (Jeon et al., 2016) (Table 3). Rats were divided into four groups: nonovariectomized rats, OVX (ovariectomized rats treated with vehicle), OVX/E2 (ovariectomized rats treated with β-estradiol, 25 μg/kg), and OVX/YPEE (ovariectomized rats treated with 5% YPEE). Results showed a weight gain in OVX rats; on the contrary, both OVX/E2 and OVX/YPEE groups did not show any significant weight alteration. As expected, the highest uterus weight was recorded in nonovariectomized rats group (1.80 g), whereas a decrease of about 91% was observed in the OVX group (0.16 g). From this point of view, despite a slight protection by YPEE already visible in OVX/YPEE group (0.24 g), the most evident result was recorded in OVX/E2 group (0.76 g) (Jeon et al., 2016).

An improvement of ovariectomy-induced dyslipidemia was recorded in the OVX/YPEE group, which showed also a higher concentration of high-density lipoprotein than the OVX group. Moreover, the OVX/YPEE and OVX/E2 groups showed a significantly higher femoral bone mineral density and bone mineral content with respect to the OVX group. Moreover, YPEE significantly enhanced bone volume over tissue volume, trabecular number, trabecular separation, and structure model index, suggesting the preservation of trabecular bone microarchitecture (Jeon et al., 2016).

To highlight the mechanism of action of YPEE and of its main flavanones (naringin, hesperidin, naringenin, and hesperetin) on osteoblastogenesis, these agents were also tested in vitro on the MC3T3-E1 subclone 4 and RAW 264.7 cells, followed by reverse transcription polymerase chain reaction and immunoblotting analyses (Jeon et al., 2016).

YPEE promotes alkaline phosphatase (ALP) activity, the expression of marker genes involved in osteoblast differentiation, ALP, osteocalcin, and runt-related transcription factor-2. Moreover, YPEE as well as its flavanones induces osteoblast differentiation by BMP-2-mediated p38 and Smad1/5/8 signaling pathways.

The YPEE activity can be probably ascribed to its most abundant bioactive compounds (flavanones). Interestingly, data showed that aglycones (hesperetin and naringenin) increased the ALP activity and mineralization more than flavanone glycosides (hesperidin and naringin), even at lower concentrations. Conversely, aglycones were more efficient than their corresponding glycosides in inducing osteoblastogenesis (Jeon et al., 2016).

In light of the results of in vivo and in vitro experiments, authors concluded that dietary intake of YPEE has a protective role on OVX-induced osteopenia and that YPEE and its main flavanones promote osteoblastogenesis providing the basis for new therapeutic applications (Jeon et al., 2016).

Recently, Matsumoto et al. (2017) investigated the therapeutic effects of yuzu EO on premenstrual symptoms (PMS), carrying out a single blind randomized crossover study (Table 3).

Common PMS includes anxiety, hostility, and fatigue, which can interfere with social, work, and academic performance. Due to the multifactorial etiology, any treatment is universally recognized as effective so many women recur to different approaches, including aromatherapy. In this study, 20 women with PMS symptoms were stimulated by inhalation with yuzu and lavender (as control) EOs for 10 min, following by heart rate variability (HRV) and autonomic nerve activity measurement, and profile of mood states assessment as psychological index.

Results showed that yuzu EO significantly decreased heart rate and increased high-frequency power of HRV, reflecting parasympathetic nerve activity in the luteal phase, as well as tension–anxiety, anger–hostility, fatigue, and total mood disturbance until 35 min after stimulation. These results are similar with those obtained by lavender EO stimulation, already widely used in this field as relaxing scent, confirming a possible use of yuzu EO in aromatherapy (Jeon et al., 2016).

Adhikari, Panthi, Pangeni, Kim, and Park (2017) investigated a yuzu callus aqueous extract (YCAE) as a source of bioactive compounds useful for antioxidant and antiaging applications (Table 3).

YCAE total phenolic content was 24.5 mg GAE/g DW. The biological activity of YCAE incorporated into nanoliposomes was assessed by measuring antityrosinase activity and melanogenesis on melanocytes as well as proliferation and procollagen synthesis on fibroblasts.

Results showed a significant decrease in melanin content in melanocytes (1.85-fold) compared with the positive control (arbutin) and an increase in procollagen synthesis (176%) and proliferation of fibroblasts (154%) at the concentration of 500 μg/mL. These findings suggest a potential use of YCAE nanoliposomes, which by the way

realize a flux through the human epidermis 17.67-folds higher than that of the callus extract alone, as antiaging cosmetic with skin-lightening effects.

Yuzu seeds are probably the less studied fruit components; however, recent studies highlighted their potential health benefits. Indeed, seeds are a good source of polyphenols, as discovered by Kim and Shin (2013) (Table 3).

They analyzed several yuzu seed extracts: water (CSEW), ethanol (CSEE), and *n*-hexane (CSEH), by evaluating total phenol content, DPPH and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical scavenging properties as well as anticomplementary activity. At equal concentration (10 mg/mL), the highest phenol content was observed in CSEE (188.71 μg/mL), followed by CSEW (141.11 μg/mL) and CSEH (26.19 μg/mL). According to previous results, CSEE and CSEW showed significantly higher antiradical activity in DPPH assay (63.56% and 56.61%, respectively) in comparison with CSEH (28.57%). A similar behavior was observed also in TEAC assay with CSEE, which showed the best antioxidant activity (45.53%) followed by CSEW (40.02%), whereas CSEH did not show $ABTS^{\bullet+}$ radical scavenging activity (Kim & Shin, 2013). Similarly, although at a highest concentration (1 mg/mL), anticomplement activity showed the same trend with CSEE, which showed the best inhibition (26.85%) followed by CSEW (7.84%). According to the observed biological activities, other than the highest amount of total phenols, CSEE possesses the highest content of limonin and nomilin (1.882% and 2.089%, respectively), followed by CSEW (0.327% and 0.139%, respectively) and CSEH (0.061% and 0.026%, respectively) (Kim & Shin, 2013).

In light of these results, among the yuzu seed extracts tested, CSEE is the most valuable source of dietary antioxidants with immunopotentiating activity.

4.5 | *Citrus Lumia* Risso

Lumia was used since ancient time for its intense and flourishing flavor as environmental scent, digestive properties, and for its soothing and relaxing properties (Smeriglio et al., 2018) (Table 3).

Smeriglio et al. (2018) recently investigated the phytochemical profile, antioxidant properties, and activity on the central nervous system of its EO. To this aim, they used different in vitro cell-free assays, based on several reaction environments and mechanisms, to evaluate its antioxidant and free-radical scavenging properties, as well as its iron-chelating capacity.

Lumia EO showed a strong and dose-dependent antioxidant and free-radical scavenging activities in all tests performed, with the following order of potency, expressed as IC_{50}: β-carotene (22 μg/mL), ORAC (46 μg/mL), DPPH (104 μg/mL), Folin–Ciocalteu (181 μg/mL), FRAP (202 μg/mL), and TEAC (233 μg/mL).

In light of this, lumia EO was able to neutralize different reactive species, especially those involved in hydrogen atom transfer-based assays such as β-carotene and ORAC and to reduce the availability of transition metals and consequently to counteract Fenton-like oxidative chain reactions.

Moreover, due to its strong antiradical activity, Lumia EO could preserve the integrity and functionality of biological membranes preventing oxidative damage. These properties are probably related to the abundance of monoterpene hydrocarbons, although a synergistic effect with other volatile compounds such as phenolic monoterpenes, allylic alcohols, aldehydes, and ketones cannot be excluded.

Several EOs are known worldwide for their positive effects on neuronal ailments (Xiang et al., 2017), opening the possibility of their application in neurological therapies. In this context, the same research group evaluated the role of lumia EO on the central nervous system by monitoring the acetylcholinesterase activity and the influence of lumia EO on the spontaneous electrical activity of a rat cortical neural network. Cholinergic disturbance seems to be the main cause of neurological impairments leading to chronic-degenerative disorders such as Alzheimer's disease.

Lumia EO showed a strong and concentration-dependent inhibition of AChE (IC_{50} = 258.25 μg/mL) about three times higher than *C. limon* EO (Aazza, Lyoussi, & Miguel, 2011). This strong activity may be related to terpenes, able to cross the blood–brain barrier modulating the brain functions. Several *Citrus* volatile compounds have been previously evaluated for their potential AChE inhibitory activity. However, the most abundant ones in lumia EO, limonene and linalool, possess only a weak AChE inhibitory activity (IC_{50} = 586.30 and ~300 μg/mL, respectively), suggesting that the strong activity of the whole lumia EO might depend on a synergistic effect.

However, the most interesting and innovative part of the work carried out by Smeriglio et al. (2018) is certainly the investigation of lumia EO effects on rat cortical neuronal networks grown on microelectrode arrays, to highlight its influence on spontaneous electrical activity. At this purpose, authors followed a multiparametric approach monitoring the mean firing rate (MFR; number of spikes/s) and the bursting behavior by four parameters: mean bursting rate (MBR; number of bursts/min), mean bursting duration (MBD; s), percentage of spikes in burst (% Spikes_B), and inter-spike intervals within a burst (MISI_B; s).

The exposure of cortical neuronal networks to increasing concentrations of lumia EO (5–400 μg/mL) induced a strong and dose-dependent inhibition of all parameters taken into account: MFR (IC_{50} = 124.6 μg/mL), MBR (IC_{50} = 32 μg/mL), %Spikes_B (IC_{50} = 21 μg/mL), MBD (IC_{50} = 97.4 μg/mL), and MISI_B (IC_{50} = 190.9 μg/mL). These results confirm the inhibitory activity of lumia EO on the spontaneous electrical activity of neurons, with an important effect on bursting behavior, which is extremely important for cortical neuronal network.

Smeriglio et al. (2019) investigated also the antioxidant and cytoprotective properties of a lumia albedo methanol/water (80:20, v/v) extract by several cell-free and cell-based assays (Table 3).

This albedo extract showed a strong and dose-dependent antioxidant and free-radical scavenging properties, with the following potency order expressed as IC_{50}: ORAC (2.57 μg/mL) > FRAP (5.22 μg/mL) > Folin–Ciocalteu (8.94 μg/mL) > TEAC (73.19 μg/mL) > β-carotene bleaching (118.21 μg/mL) > DPPH (533.03 μg/mL). According to previous literature data, eriocitrin should be the strongest antioxidant compound within lumia albedo extract. However, other compounds, such as rutin, or phenolic acids, such as chlorogenic and ferulic

acids, could contribute significantly to the antioxidant pattern of the extract.

The cytoprotective activity of lumia albedo extract was investigated on human lymphocytes exposed to tert-butyl hydroperoxide (t-BOOH). A significant cytoprotective activity within the noncytotoxic concentration range (5–50 μg/mL) was observed. The addition of increasing concentrations of lumia albedo extract (15–50 μg/mL) to lymphocytes exposed to t-BOOH resulted in a dose-dependent decrease of cell mortality, with a decrease of lactate dehydrogenase release and caspase 3 activity. Furthermore, protein carbonyl groups also decreased conspicuously following treatment with the albedo extract, further strengthening its antiperoxidase activity (Smeriglio et al., 2019).

Considering this, lumia represents certainly an antioxidant source, suggesting that albedo, currently considered only a waste product, could be used as a valuable raw material for the formulation of nutraceutical supplements preventing oxidative stress-related diseases.

5 | DISCUSSIONS

Analyzing the phytochemical features and the biological activities of the selected *Citrus* fruits investigated up to date, what emerges is that studies concerning these *Citrus* species are still quite incomplete. Among the revised species, the yuzu is certainly the most investigated one, both from the phytochemical and from the biological point of view.

The peel and pulp extracts, obtained by different extraction techniques and from fruits with different geographical origins, are certainly the most investigated, such as for Buddha's hand and finger lime (Chu et al., 2012; Deng et al., 2017; Wang et al., 2019). In some cases, extracts obtained from fruit transformation products have also been investigated (Deiana et al., 2019). Conversely, seeds are generally less investigated, with the exception of yuzu seeds that are a rich source of polyphenols and polysaccharides (Kim, 2018; Kim & Shin, 2013). Another highly investigated plant complex is the EO obtained from the fruit peel, although to date there is no study on the finger lime EO. In some cases, the authors also identified and isolated new compounds, such as for the fruit extract of the Buddha's hand. However, the authors focused on phytochemical aspects, neglecting the comparison between the biological activity of the plant complex and that of the most abundant compounds, thus failing to attribute the activity to a particular compound or to a synergistic effect of different compounds present in the extract (Deng et al., 2017). A very interesting aspect is the multidisciplinary approach used to study the lumia fruit, both from the micromorphological and from the phytochemical points of view (Smeriglio et al., 2018, 2019). These studies highlighted the micromorphological characteristics of the fruit by means of light and SEM, which had never been reported previously even for other much more investigated *Citrus* fruits.

As far as biological activity is concerned, yuzu is certainly the most investigated among the selected *Citrus* species. The fruit has been examined for its antiaging (Adhikari et al., 2017; Shim et al., 2019), neuroprotective (Yang et al., 2013), lipid- and cholesterol-lowering (Kim et al., 2013; Sharma et al., 2019; Shin et al., 2016), anti-inflammatory (Abe et al., 2018), and osteogenic activities (Jeon et al., 2016) both as a plant complex and as a source of promising isolated bioactive compounds. Moreover, it is the only fruit that has been clinically investigated, whereas the other ones have been studied mostly by in vitro cell-free and cell-based assays.

The EOs of the selected *Citrus* species have been investigated predominantly for their antimicrobial activity against GRAM+ and GRAM– bacteria, as well as against fungi. However, only one study highlighted the mechanism of action of the Buddha's hand EO through SEM (Li et al., 2019). Beyond the antimicrobial activity, few other activities of these *Citrus* EOs have been investigated. For example, despite the EOS easily cross the blood–brain barrier, few studies have focused on their neuroactive effects, with the exclusion of lumia and yuzu EOs (Matsumoto et al., 2017; Smeriglio et al., 2018).

Therefore, the fruits selected here require further in-depth studies, especially on animal models, because the results obtained in vitro are often difficult to be translate in vivo.

Moreover, a fundamental aspect that emerges from these studies is that some matrices such as seeds, juice, and albedo, which are potential sources of bioactive compounds, have not been investigated yet.

Finally, it would be interesting to compare the biological activity of new compounds isolated from the plant phytocomplexes to highlight possible structure–activity relationships possibly leading to pharmaceutical applications.

In conclusion, this review brings light on five Citrus species (*C. australasica* F. Muell., *C. medica* L. var. *sarcodactylis* Swingle, *C. junos* Sieb ex Tan., *C. limon* L. Osbeck var. *pompia* Camarda, and *C. lumia* Risso), including ancient fruits and new hybrids that are still little investigated. Although there are different reviews dedicated to the *Citrus* fruits and their most important bioactive compounds, none of these include the herein considered species. Therefore, this work fills a gap by allowing to locate geographically these species, and to compare their botanical features, which are often not readily accessible to the reader as they are published in noninternationally recognized languages. Moreover, this review also draws a clear picture of the chemical profile and biological properties of several plant complexes obtained from these five species, allowing the reader to grasp a broaden vision on the *Citrus* genus.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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