

## *Camellia japonica*: A phytochemical perspective and current applications facing its industrial exploitation

Antia G. Pereira<sup>a,1</sup>, Pascual Garcia-Perez<sup>a,e,1</sup>, Lucia Cassani<sup>a,c</sup>, Franklin Chamorro<sup>a</sup>, Hui Cao<sup>a</sup>, Francisco J. Barba<sup>d</sup>, Jesus Simal-Gandara<sup>a,f,\*</sup>, Miguel A. Prieto<sup>a,b,f,\*</sup>

<sup>a</sup> Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E-32004 Ourense, Spain

<sup>b</sup> Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolonia, 5300-253 Bragança, Portugal

<sup>c</sup> Instituto de Investigaciones en Ciencia y Tecnología de Materiales (INTEMA, CCT-CONICET), Colón 10850, Mar del Plata 7600, Argentina

<sup>d</sup> Nutrition and Food Science Area, Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine Department, Universitat de València, Faculty of Pharmacy, Avda. Vicent Andrés Estellés, s/n, Burjassot, 46100 València, Spain

<sup>e</sup> Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy

<sup>f</sup> Agrifood Research Group, Galicia Sur Health Research Institute (IIS Galicia Sur), SERGAS-UVIGO, Spain

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### ABSTRACT

In response to the increased popularity of medicinal plants, a number of conservation groups are recommending the investigation on poorly characterized and widely distributed species, as it is the case of camellias. In particular, *Camellia japonica* L. is a widespread species found in Galicia (NW Spain), where it has been largely exploited with ornamental purposes. Recent findings on its phytochemical characterization showed thousands of bioactive ingredients, mostly represented by phenolic compounds, together with terpenoids, and fatty acids. These molecules present associated biological activities, acting as antioxidant, antimicrobial, anti-inflammatory, and anticancer agents. This review is aimed at describing the main bioactive compounds of *C. japonica*, as well as the health-enhancing properties attributed to this medicinal plant. Novel strategies are needed to implement an efficient industrialization process for *C. japonica*, ranging from small-scale approaches to the establishment of large plantations, thus involving important sectors, such as the food, pharmaceutical and cosmetic industries.

### Introduction

The genus *Camellia* (Theaceae) includes more than 250 plants with an intricate taxonomy ("Integrated Taxonomic Information System (ITIS)", 2020), being *Camellia sinensis* L., *Camellia oleifera* Abel. and *Camellia japonica* L. the most outstanding species, traditionally used for multiple applications, including tea and essential oils production and ornamental purposes (Garcia-Jares, Sanchez-Nande, Lamas, & Lores, 2017). Its botanical origin is located in Eastern Asia, including the Himalayas, Japan, Southern China, and South-Eastern countries (Durrant, 1982), and it is currently distributed in tropical and subtropical regions worldwide.

In particular, Galicia, a North-Western region from Spain, constitutes one of the international benchmarks regarding the cultivation and production of *Camellia* spp., presenting almost 8,000 different varieties

(Xunta de Galicia, 2020), thanks to the edaphoclimatic conditions associated with this region, including humid climate, mild temperatures and fertile and acidic soils. However, the introduction of first camellias specimens to this region remains uncertain, although it is estimated to occur at late XVIII century (Salinero, Vela, De Ron, & Jesús, 2016). The most abundant species in Galicia is *C. japonica*, from which several types or varieties can be distinguished, due to the anatomical traits attributed to the flowers (Fig. 1), accounting for up to 3,000 different varieties, mostly known for their extended use in gardening (Kim, Cho, Yang, & Kim, 2017). In this sense, important Galician nurseries have gained specialization in the production of *C. japonica* and derived products, making heavy investments, especially for the mechanization and automation of production processes, obtaining a continuous production with stable quality of 1.5 million plants per year, for a value of around 40 million euros, which supposes about the 9% of the total Galician

\* Corresponding authors at: Universidade de Vigo, Nutrition and Bromatology Group, Department of Analytical Chemistry and Food Science, Faculty of Science, E-32004 Ourense, Spain.

E-mail addresses: [jsimal@uvigo.es](mailto:jsimal@uvigo.es) (J. Simal-Gandara), [mprieto@uvigo.es](mailto:mprieto@uvigo.es) (M.A. Prieto).

<sup>1</sup> Both authors contributed equally to this work.

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agricultural production (Salinero et al., 2016). In parallel, the importance of *C. japonica* as an industrially relevant plant was mostly reported in China, where more than 3,660,000 ha were dedicated to this crop, whose annual production reaches 645,000 tons of seeds, from which 164,000 tons of oil were obtained (Jiyin, Parks, & Yueqiang, 2005).

Despite the popularity of *C. japonica* as an ornamental plant, little attention has been paid to the characterization of its health-enhancing properties, and its effectiveness as a source of bioactive compounds. In this sense, the first references about the use of *C. japonica* with medicinal purposes dates back to 1613, when the use of *C. japonica* seeds and flowers was reported by the doctor of oriental medicine Heo Jun (Song, Won, & Kim, 2016). Thanks to the production of bioactive compounds, such as triterpenes, flavonoids, and essential fatty acids, *C. japonica* is considered as a valuable medicinal plant (Majumder, Ghosh, & Bhat-tacharya, 2020). As a result, *C. japonica* and its derived products present several pharmacological properties, acting as gastrointestinal modulators, anticancer, antimicrobial, antioxidant, neuroprotective, hypolipidemic, anti-obesity, and anti-inflammatory agents (Guo, Tong, Ren, Tu, & Li, 2018). In this sense, different *C. japonica*-derived matrices have been extensively used as relevant sources of bioactive compounds, including seeds and their derived oil, flowers, fruits and leaves (García-Jares et al., 2017). Keeping all this in mind, increasing interest is being observed in the last decades for the industrial exploitation of *C. japonica*. This review focuses on the phytochemistry of *C. japonica*, combining the description of its most prevalent bioactive compounds with their associated biological activities, with the aim of providing a current perspective about its potential as a medicinal plant to be exploited with nutraceutical purposes. This is a systematic review in which search engines with a multidisciplinary scope were employed, including PubMed, Web of Science, and Google Scholar. As well, the information regarding official data was directly obtained from original sources, as it was the case of the governmental sources Xunta de Galicia, Deputación de Pontevedra (both ascribed to Spanish government), and official organisms, such as the National Center for Biotechnology Information and the Integrated Taxonomy Information System (both ascribed to U.S. government). The inclusion and exclusion criteria were selected according

to Preferred Reported Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

### Bioactive compounds isolated from *C. japonica*

The great diversity of varieties of *C. japonica* implies that the study of its chemical composition is complex. In a generic way, its main compounds are phenolic compounds, terpenoids, fatty acids and a series of minor compounds that include pigments and biosugars (Meng et al., 2019). Such compounds are biosynthesized as the result of secondary metabolism, involved in the adaptative and defensive responses of plants against environmental conditions and threats (García-Pérez, Miras-Moreno, Lucini, & Gallego, 2021; Panghal, Shaji, Nain, Garg, & Chhikara, 2021). In this sense, the concentration of bioactive compounds are highly influenced by a set of both edaphoclimatic and physical factors, including geographical area, climatic conditions, soil quality, seasonal variations, and the exposure to several stresses, as it is the case of drought, heavy rains, or overexposure to ultraviolet light (Abe, Miura, & Motonaga, 2021; Venthodika et al., 2021; Wendel & Parks, 1985). Furthermore, different components have been detected in an organ and tissue-dependent manner, as observed for different camellias, thus suggesting the high complexity of the phytochemical composition of these species. In this section, an overview of the most prevalent chemical constituents of *C. japonica* is provided (Fig. 2), with a special focus on those compounds with associated biological activities, the so-called bioactive compounds.

### Phenolic compounds

Phenolic compounds constitute the largest family of secondary metabolites, with tens of thousands different compounds isolated to date (García-Pérez, Lozano-Milo, Landin, & Gallego, 2020), ubiquitously found among all the species belonging to the plant kingdom, including camellias. The main types of phenolic compounds present in *C. japonica* are simple phenolic compounds, phenolic acids, flavonoids and tannins (Table 1), whose concentration depends on the part of the plant

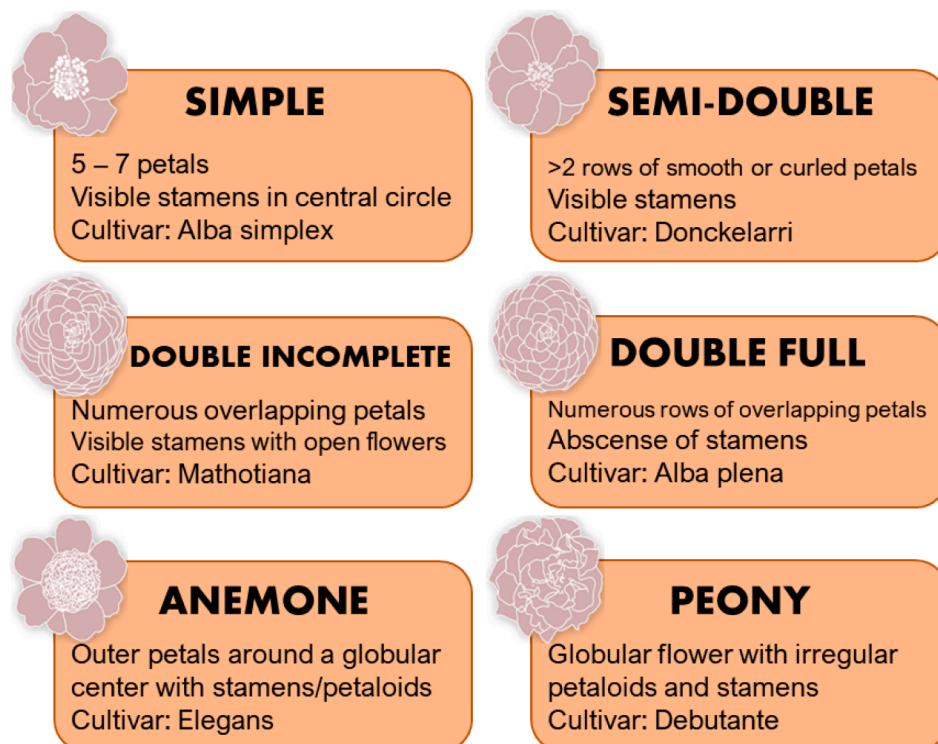


Fig. 1. Classification of *C. japonica* according to flower shape (Deputación de Pontevedra, 1980; Salinero et al., 2016; Xunta de Galicia, 2020).

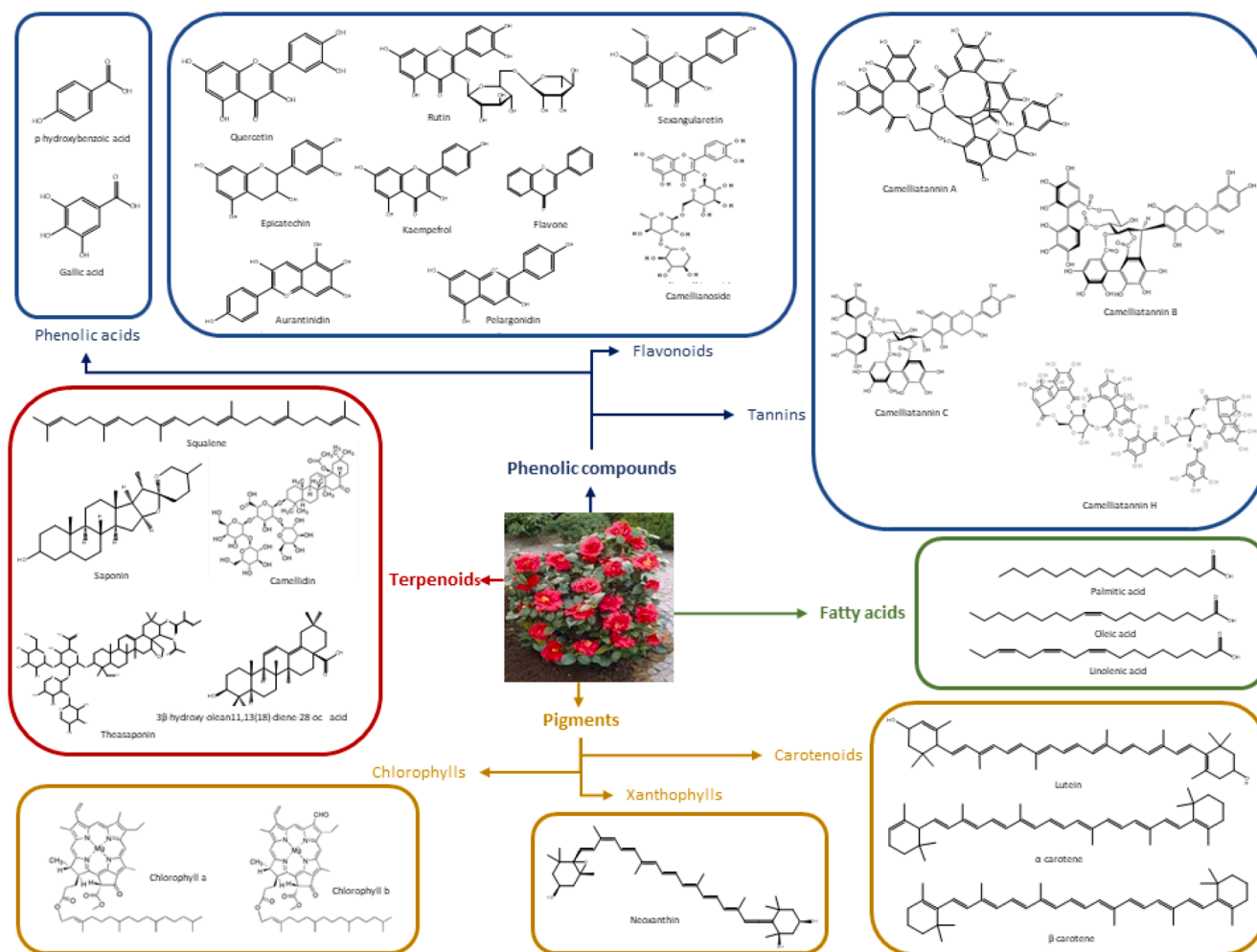


Fig. 2. Chemical structures of the main molecules present in *C. japonica*.

analyzed, being more abundant in the flowers and leaves (Stewart & Stewart, 2008). The concentration of phenolic compounds (Fig. 2) also displays a strong dependence on climatic factors, the state of maturity and seasonality. That is why the concentration of phenolic compounds in *C. japonica* is higher in young leaves, with a total phenol content of 74.62 mg per 100 g of dry weight, than in flower buds, with 65.02 mg per 100 g of dry weight, and flowers 62.42 mg per 100 g of dry weight (Lee et al., 2005).

The predominant phenolic acids of *C. japonica* are gallic acid and *p*-hydroxybenzoic acid, being both compounds present in the flowers. Gallic acid can also be isolated from leaves and fruits (Kanth, Lee, & Lee, 2014; Kim et al., 2018). It is also possible to identify and quantify other phenolic compounds in lower concentrations. These molecules include 3,4,5-trihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,3-digalloyl-*O*- $\alpha$ -*D*-glucopyranoside, and 2,3-digalloyl-*O*- $\beta$ -*D*-glucopyranoside (Hee, Hye, Hyang, Ok, & Mi, 2010). The main flavonoids are quercetin, kaempferol, sexangularetin, camellianoside, epicatechin (Jeganathan, Punyasiri, Kottawa-Arachchi, Ranatunga, Abeysinghe, Gunasekare, & Bandara, 2016; Onodera, Hanashiro, & Yasumoto, 2006), quercetin 3-*O*- $\beta$ -*D*-galactopyranoside, quercetin 3-*O*- $\beta$ -*D*-glucopyranoside, kaempferol 3-*O*- $\beta$ -*D*-galactopyranoside, and kaempferol 3-*O*- $\beta$ -*D*-glucopyranoside (Hee et al., 2010), present in both leaves and flowers of *C. japonica* (Yoon et al., 2017) or quercetin-7-glucoside, which is the most important yellow pigment in *Camellia chrysantha* (Hwang, Yoshikawa, Miyajima, & Okubo, 1992). Many of these flavonoids are responsible for the coloration of the flowers. For example, red *C. japonica* extracts have flavonoid epicatechin (Zhang,

Yin, Kong, & Jiang, 2011). These red and pink varieties are also rich in anthocyanins, being the most abundant cyanidin 3-*O*-(6-*O*-(*E*)-*p*-coumaroyl)- $\beta$ -glucopyranoside, cyanidin 3-*O*-(6-*O*-(*E*)-*p*-coumaroyl)- $\beta$ -galactopyranoside, cyanidin 3-*O*-(6-*O*-(*E*)-caffeoyl)- $\beta$ -glucopyranoside, cyanidin 3-*O*-(6-*O*-(*E*)-caffeoyl)- $\beta$ -galactopyranoside (Li, Hashimoto, Shimizu, & Sakata, 2009). This type of anthocyanins was not detected in *C. japonica* cultivars with white-petal flowers due to the absence of color (Li, Wang, Yin, Fan, & Li, 2019), as they present other pigments like leuco-anthocyanins (Endo, 1958). Other flavonoids have been described in seeds, including eight kaempferol oligosaccharides, namely tsubakiosides A-D, camelliaside B, and analogs (Ko, Rho, & Yoon, 2020). It is important to note that camellianoside constitutes an exclusive flavonoid of the genus *Camellia*, being a flavonol glucoside (quercetin-3-*O*- $\beta$ -*D*-xylopyranosyl-(1  $\rightarrow$  3)-*O*- $\alpha$ -*L*-rhamnopyranosyl-(1  $\rightarrow$  6)-*O*- $\beta$ -*D*-glucopyranoside) present in significant concentrations in the leaves. This compound is characterized by having an antioxidant capacity superior to that of reference antioxidants such as L-cysteine and L-ascorbic acid (Onodera et al., 2006).

Considering other subfamilies, tannins have been also isolated from *C. japonica*, represented by camelliatannins. The major molecules in this group are camelliatannins A, B, C, D, F, G and H (Han, Hatano, Yoshida, & Okuda, 1994; Hatano et al., 1995; Hatano, Shida, Han, & Okuda, 1991), all of them being constituted by an epicatechin core linked to a C-glucosyl ellagitannin moiety (Han et al., 1994). Furthermore, camellians A and B represent another tannins from the genus *Camellia*, which have been detected in the flower buds of *C. japonica* (Yoshida, Chou, Maruyama, & Okuda, 1990).

**Table 1**Phenolic compounds found in *C. japonica* extracts, identification from different plant parts and associated bioactivities (Center, 2020).

Compounds	Identification	Part	Associated bioactivities	Refs.
Phenolic acids				
Gallic acid	GC-MS	L, P, F	Antimicrobial, anti-inflammatory, antioxidant, anticancer	(Kanth et al., 2014; Lee et al., 2005, 2016; Moon & Kim, 2018)
<i>p</i> -hydroxybenzoic acid	HPLC	F	Antioxidant, antimicrobial	(Nakajima, Itokawa, & Ikuta, 1984)
3,4,5-trihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,3-digalloyl- <i>O</i> - $\alpha$ - <i>D</i> -glucopyranoside, and 2,3-digalloyl- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside	NMR, HPLC	F	Antioxidant	(Lee, Cho, Moon, & Park, 2011)
Flavonoids				
Quercetin	GC-MS	L, F	Antimicrobial, anti-inflammatory, anticancer, antioxidant	(Kim, Jeong, & Shim, 2010; Lee et al., 2016; Moon & Kim, 2018; Piao et al., 2011)
Quercetin 3- <i>O</i> - $\beta$ - <i>D</i> -galactopyranoside, quercetin 3- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside	NMR, GC-MS	F, L	Antioxidant, anti-hyperuricemia	(Lee et al., 2011; Yoon et al., 2017)
Camelliaside	HPLC	L	Antioxidant, antiviral	(Onodera et al., 2006; Park et al., 2002)
Epicatechin	GC-MS	L, F	Antimicrobial, anti-inflammatory, anticancer, antioxidant	(Kim et al., 2010; Lee et al., 2016; Moon & Kim, 2018; Piao et al., 2011)
Kaempferol	HPLC	F	Antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, anti-diabetes	(Nakajima et al., 1984)
Kaempferol 3- <i>O</i> - $\beta$ - <i>D</i> -galactopyranoside and kaempferol 3- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside	NMR, GC-MS	F, L	Antioxidant, anti-hyperuricemia	(Lee et al., 2011; Yoon et al., 2017)
Kaempferol-3- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosyl(1 $\rightarrow$ 3)- $\alpha$ - <i>L</i> -rhamnopyranosyl(1 $\rightarrow$ 6)- <i>O</i> - $\beta$ - <i>D</i> -galactopyranoside, kaempferol-3- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosyl(1 $\rightarrow$ 6)- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosyl(1 $\rightarrow$ 2)- <i>O</i> - $\beta$ - <i>D</i> -galactopyranoside, kaempferol-3- <i>O</i> - $\beta$ - <i>D</i> -xylopyranosyl(1 $\rightarrow$ 3)- <i>O</i> - $\alpha$ - <i>L</i> -rhamnopyranosyl(1 $\rightarrow$ 6)- <i>O</i> - $\beta$ - <i>D</i> -glucopyranosyl(1 $\rightarrow$ 2)- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside, tsubakiosides A-D, camelliaside B	HPLC-PDA	S	Antioxidant, anti-aging	(Ko et al., 2020)
Sexangularetin	HPLC	F	Antioxidant, anti-inflammatory, antimicrobial, anticancer	(Nakajima et al., 1984)
Anthocyanidins: cyanidin 3- <i>O</i> -(6- <i>O</i> -( <i>E</i> )- <i>p</i> -coumaroyl)- $\beta$ -glucopyranoside, cyanidin 3- <i>O</i> -(6- <i>O</i> -( <i>E</i> )- <i>p</i> -coumaroyl)- $\beta$ -galactopyranoside, cyanidin 3- <i>O</i> -(6- <i>O</i> -( <i>E</i> )-caffeoyl)- $\beta$ -glucopyranoside, cyanidin 3- <i>O</i> -(6- <i>O</i> -( <i>E</i> )-caffeoyl)- $\beta$ -galactopyranoside	HPLC	F	Antioxidant	(Li et al., 2009)
Tannins				
Camelliatannins A, B, C, H	NMR	L	Antiviral	(Yang et al., 2015)
Camelliatannins D, F, G	NMR	L	Antiviral	(Han et al., 1994; Hatano et al., 1991, 1995)
Camelliins A and B	NMR	F	Antiviral	(Yoshida et al., 1990)

Description. GC-MS: gas chromatography-mass spectrometry analysis; HPLC: high-performance liquid chromatography; NMR: nuclear magnetic resonance spectroscopy; TLC: thin-layer chromatography; HPLC-PDA: high-performance liquid chromatography coupled to photodiode array detector; // L: leaves; F: flowers; S: seeds; P: fruit peels.

### Terpenoids

Together with phenolic compounds, terpenoids constitute another important family of secondary metabolites, closely related to plant adaptation and defense, with associated biological activities. The major terpenoids in *C. japonica* are saponins (Table 2), including camellidins as relevant saponin exclusive of the genus *Camellia*. Camellidins are composed of 3 $\beta$ -hydroxy-18 $\beta$ -acetoxy-28-norolean-12-en-16-one (camellidin I) or 3 $\beta$ , 8 $\beta$ -dihydroxy-28-norolean-12-en-16-one (camellidin II) as possible aglycon moieties, and *D*-glucuronic acid, *D*-glucose and two moles of *D*-galactose as the sugar moieties (Chakraborty, Chakraborty, & Saha, 2018). Other saponins present in *C. japonica* are camerosides, camelliasaponins (A1, A2, B1, B2, C1, C2) (Yoshikawa et al., 1996) and camellioside D (Cui et al., 2018; Rho, Choi, Kil, Ko, & Yoon, 2019; Yoshikawa et al., 2007); other terpenoids present in *C. japonica* include squalene, oleanane triterpenes (Uddin et al., 2014; Yang et al., 2015), 3- $\beta$ -*O*-acetyl-16 $\beta$ -hydroxy-12-oxoolean, 3- $\beta$ -*O*-acetyl-16 $\beta$ -hydroxy-11-oxoolean-12-ene, 3- $\beta$ -*O*-acetyl-16 $\beta$ -hydroxyolean-12-ene, 3- $\alpha$ -hydroxy-1-oxofriedelan, friedelin, 3- $\beta$ -friedelanol, canophyllol, 3-oxofriedelan-1 (2)-ene,  $\beta$ -amyrin, camellenodiol, camelledionol (Thao et al., 2010), and two recently identified 3,4-dry-28-noroleanan-type triterpenoids (Cho et al., 2020).

### Fatty acids

Camellias have been traditionally used in the production of essential oils and edible oils, mainly containing oleic acid as the predominant fatty acid (Su, Shih, & Lin, 2014), which represents the 88.2% of the total lipids in *C. japonica*. Oleic acid, consequently, is the major fatty acid found as part of both glycolipids and phospholipids, being abundantly distributed in all positions within their structure in their esterified form (*sn*-1, 2 and 3) (Noh & Yoon, 2012). Other fatty acids found in minor concentrations are palmitic acid and linolenic acid (Table 2) (Zeng & Endo, 2019). Therefore, the essential oil of *C. japonica* has a similar composition to that of other edible oils, such as olive oil (Fang, Fei, Sun, & Jin, 2016), but with a lower content of saturated fatty acids (Salinero & Corral, 2008), thus suggesting a healthier profile motivated by the prevalence of polyunsaturated fatty acids, presenting meaningful beneficial properties to human health upon its consumption.

### Minor compounds

Besides polyphenols, terpenoids, and fatty acids, *C. japonica* is considered a natural source of other compounds, as well, being reported at much lower concentrations in these species, as it is the case of

**Table 2**Other bioactive compounds found in *C. japonica* extracts: identification from different plant parts and associated bioactivities (Center, 2020).

Family	Compounds	Identification	Part	Associated bioactivities	Refs.
Terpenoids					
Triterpenoids	Squalene	GC-MS	L, B	Anti-inflammatory, anticancer, antioxidant	(Lee et al., 2016; Majumder et al., 2020; Moon & Kim, 2018; Thao et al., 2010)
Saponins	Camoreoside B	HCCC, RP-HPLC	S	Anti-obesity	(Ochiai et al., 2018)
	Camoreoside C, G	HCCC, RP-HPLC	S	Anti-obesity	(Rho et al., 2019)
	Camelliasaponin C2	HPLC, NMR	S	Anti-obesity	(Cui et al., 2018)
	Camelliasaponins A1, A2, B1, B2, C1, C2	HPLC	S	Inhibition of alcohol absorption	(Yoshikawa et al., 1996)
	Theasaponin E1	HPLC, GC-MS, LC-MS, NMR	S	Anti-obesity	(Cui et al., 2018)
	Camellidin	NMR	L	Antimicrobial	(Nagata et al., 1985)
Oleanane triterpenes	Camelidionol, 3 $\beta$ -hydroxy-olean-11,13(18)-diene-28-oic acid, 3 $\beta$ -acetoxylean-12-ene-28-oic acid	HPLC, silica gel, RP-C <sub>18</sub>	P, F	Anti-obesity, anti-diabetes, antiviral	(Uddin et al., 2014; Yang et al., 2015)
	3- $\beta$ -O-acetyl-16 $\beta$ -hydroxy-12-oxoolean, 3- $\beta$ -O-acetyl-16 $\beta$ -hydroxy-11-oxoolean-12-ene, 3- $\beta$ -O-acetyl-16 $\beta$ -hydroxyolean-12-ene, 3- $\alpha$ -hydroxy-1-oxofriedelan, 3-oxofriedelan-1(2)-ene	NMR	S	Anticancer	(Thao et al., 2010)
	Camellioside D	NMR	F	Platelet aggregation control	(Yoshikawa et al., 2007)
Fatty acids					
Saturated	Palmitic acid	GLC	O	Antiviral, antioxidant, anti-inflammatory	(Zeng & Endo, 2019)
Unsaturated	Oleic acid, linolenic acid	GC	O	Antihypertensive, antioxidant, anti-inflammatory	(Guo et al., 2018)
Pigments					
Carotenoids	Lutein, $\alpha$ -carotene, $\beta$ -carotene	HPLC UV/VIS	L	Antimicrobial, antioxidant	(Taylor & McDowell, 1991)
Xanthophylls	Neoxanthin	TLC	F	Antioxidant	(Scogin, 1986)
Chlorophylls	Chlorophyll a, b	HPLC UV/VIS	L	-	(Taylor & McDowell, 1991)
Minor compounds					
Free sugars	Glucose, fructose, saccharose	HPLC RI	F	-	(Trinh et al., 2018)
Insoluble sugars	Arabinose, xylose	HPLC RI	F	-	(Trinh et al., 2018)
Vitamins	E	HPLC	L, S	-	(Saenjum et al., 2020)
Aminoacids	Aspartic acid, glutamic acid, histidine, alanine	FS	L	-	(Kim et al., 2005; Liu et al., 2020)
Minerals	Phosphorus, calcium, potassium, sodium, iron, manganese, zinc, aluminum, copper	FS	L	-	(Kim et al., 2005; Liu et al., 2020)

Description. GC-MS: gas chromatography-mass spectrometry analysis; HCCC: high-performance countercurrent chromatography; RP-HPLC: reversed-phase high-performance liquid chromatography; HPLC: high-performance liquid chromatography; NMR: nuclear magnetic resonance spectroscopy; LC-MS: liquid chromatography-mass spectrometry; RP-C<sub>18</sub>: reverse phase-C<sub>18</sub>; GLC: gas liquid chromatography; GC: gas chromatography; HPLC UV/VIS: high-performance liquid chromatography equipped with a ultraviolet/visible detector; HPLC RI: high-performance liquid chromatography equipped with a refractive index detector; FS: flame spectroscopy; // L: leaves; B: bark; S: seeds; P: fruit peels; F: flowers; O: oil.

pigments, major responsible for the photosynthetic activity and the colored nature of flowers together with anthocyanins, biosugars, vitamins and minerals. Most of the pigments of *C. japonica* are in the flowers, being one of the main characteristics to discriminate different varieties. These pigments vary from red to white, being possible to find yellow pigments. The interspecific hybrids of the variety 'Suzanne Withers' have as main pigments several carotenoids and two flavonoids (derived from kaempferol and quercetin). In some cases, it is possible to distinguish purple flowers in varieties of *C. japonica* of red flowers. This color modification may be due to the presence of aluminum ions, which were detected in concentrations 14–21 times higher than in red petals without modifications. Therefore, this color change may be due to the chelation of aluminum ions with anthocyanins (Tanikawa, Inoue, & Nakayama, 2016). In the case of leaves, the main pigments are chlorophylls (a and b) (Gogoi & Borua, 2017), as well as other chlorophyll-type compounds that were identified as chlorophyllides and pheoforbids (Taylor & McDowell, 1991).

*C. japonica* also has significant amounts of biosugars, mostly represented by hexoses and pentoses, being glucose, fructose, and sucrose the most prominent examples. This plant also presents insoluble sugars, as it is the case of xylose and arabinose (Trinh, Choi, & Bae, 2018). Other minor compounds present in *C. japonica*, principally in the leaves, include vitamin E, some amino acids, including essential amino acids,

and other biologically active compounds related to hyperuricemia (Yoon et al., 2017). Vitamin E is present in various forms, i.e.:  $\alpha$ -tocopherol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol,  $\alpha$ -tocotrienol,  $\gamma$ -tocotrienol, and  $\delta$ -tocotrienol, found in a great extent in the seeds of *C. japonica* (Saenjum, Pattananandecha, & Nakagawa, 2020). The most abundant amino acids in the whole plant are aspartic acid, glutamic acid, histidine and alanine, whose concentration decreases with the delay in the harvesting time. Camellias are also rich in minerals. The most abundant minerals are phosphorus, calcium, potassium, sodium, iron, manganese, zinc, aluminum and copper, which increase in concentration if the harvesting time is delayed or the plant is treated with aluminum solution (Kim, Choi, & Shim, 2005; Liu et al., 2020).

#### Biological properties: *C. japonica* as an underexplored medicinal plant

Although the study of its composition is to some extent limited, camellias have been traditionally used in the medicine and cosmetics of Eastern countries in a regular basis, motivated by the biologically active properties of the compounds present in this genus and their organoleptic characteristics. The most outstanding bioactivities associated with such compounds (Fig. 3) are antioxidant, antimicrobial, anti-inflammatory, anticancer, anti-obesity, antidiabetic, and antihypertensive, among others (Estevinho, Feás, Salinero, Mansilla, Vela, Saínez, Vázquez-Tato, &

Seijas, 2012). Table 3 includes an overview of the most relevant mechanisms of action associated with the different bioactivities attributed to *C. japonica* extracts. Altogether, these bioactivities contribute to the consideration of this species as a medicinal plant, showing a promising potential for *C. japonica* facing its exploitation with industrial purposes, especially those related with the food, pharmaceutical and cosmetic sectors.

#### Antioxidant activity

*Camellia* species are unanimously considered a natural source of antioxidant compounds, whose content depends on several factors like cultivar and environmental conditions. This antioxidant activity may be due to the high concentrations of phenolic compounds found in their constitutive tissues, as reported by the determination of total phenolic content, reaching values up to 21.75 mg/g (Jeong et al., 2010). Concerning the mechanisms of action, the antioxidant activity of *Camellia* spp. has been reported by free-radical scavenging activity. Accordingly, young *C. japonica* leaves exhibited higher antioxidant activities than mature leaves in terms of radical oxygen species scavenging, especially hydrogen peroxide and hydroxyl radicals (Mizutani & Masaki, 2014). Regarding the influence of solvents on the extraction of antioxidant compounds from leaves, methanolic and acetic extracts caused the highest rates of antioxidant activity, according to 2,2-diphenyl-1-picrylhydrazyl free-radical scavenging method (DPPH), with inhibitory concentration 50 (IC<sub>50</sub>) values ranging from 246.56 µg/mL for methanolic extracts to 320.17 µg/mL for acetic extracts, whereas the IC<sub>50</sub> values obtained by the β-carotene-linoleic acid test were 258.19 µg/mL and 396.88 µg/mL, respectively (Wang, 2012).

Besides leaves, flowers from *C. japonica* present antioxidant properties, as well, as their extracts exhibited DPPH and reactive oxygen species (ROS) scavenging activity in human HaCaT keratinocytes (immortalized human keratinocytes), and increased the expression of cellular antioxidant enzymes, such as superoxide dismutase, catalase

and glutathione peroxidase (Piao et al., 2011). High concentrations of antioxidants prevent the damage caused by free radicals which is associated with the development of cancer. At this respect, the petals of several varieties of *C. japonica* had a maximum IC<sub>50</sub> (evaluated with DPPH) value of 3.8 µg/mL in pink cultivars and 43.1 µg/mL in white cultivars, showing a direct correlation between antioxidant activity and phenolic content (Kanth et al., 2014). In the same way, *C. japonica* oil presents an antioxidant power comparable to gallic acid and α-tocopherol (Choi, Min, Oh, & Shin, 2013). All these antioxidant properties have prompted the use of camellias with cosmetic purposes due to their anti-aging and anti-pollution properties. However, the heterogeneity of phenomena associated with antioxidant activity should cover the development of different methodologies, in order to fully depict the total antioxidant capacity of camellia extracts, including *in vivo* models.

#### Antimicrobial activity

Extracts with antimicrobial properties from camellias can be obtained from leaves and flowers, being ethanol one of the best solvents that promote high rates of antimicrobial activity, in terms of antibacterial, antifungal and antiviral activity (Moon & Kim, 2018). A methanolic extract from petals of *C. japonica* has its best antimicrobial results in the acidic fraction, with an inhibitory zone of 14–19 mm diameter in a disk test against the pathogens *Salmonella* Typhimurium DT104, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus*. This bioactivity is suggested to be due to the presence of fumaric acid. As a result of this associated activity, these extracts were applied with satisfactory results in milk fortification, promoting an increase in shelf life (Kim, Davidson, & Chung, 2001).

In parallel, the methanolic extracts of young leaves and stems from *C. japonica* have been evaluated in terms of antimicrobial activity, showing a high efficiency against *S. aureus* (Choi et al., 2013). Moreover, the ethanolic extract of fermented leaves of *C. japonica* showed great antimicrobial properties against *Staphylococcus epidermidis*, *Bacillus*

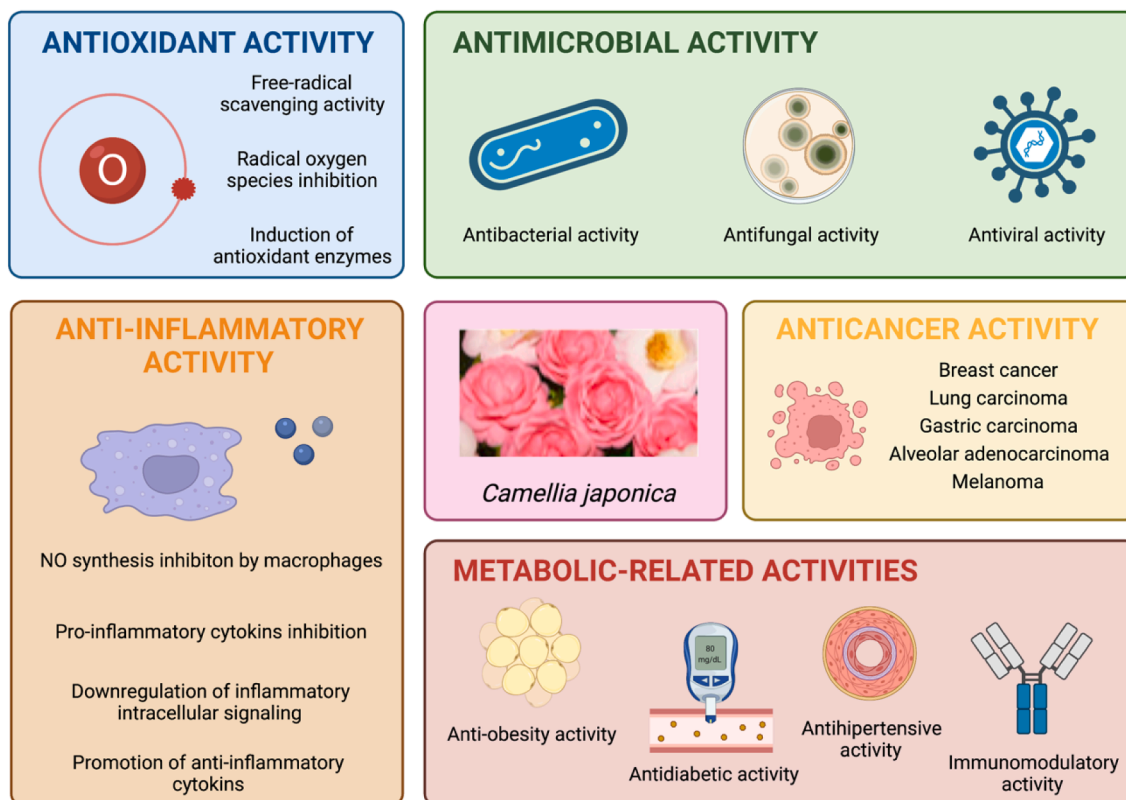


Fig. 3. Bioactivities associated with *C. japonica* extracts.

**Table 3**  
Mechanisms of action attributed to the bioactivities associated with *C. japonica* extracts

Bioactivities	Mechanisms of action	References
Antioxidant activity	Free-radical scavenging activity	(Kanth et al., 2014; Lee et al., 2011; Moon & Kim, 2018; Piao et al., 2011; Zhang et al., 2011)
	Metal chelation Reducing power ROS quenching	(Moon & Kim, 2018) (Moon & Kim, 2018) (Moon & Kim, 2018; Piao et al., 2011)
	Induction of antioxidant enzymes: SOD, CAT, GPx	(Piao et al., 2011)
Antimicrobial activity	Bacteriostatic activity against a wide range of foodborne bacteria and pathogens: <i>Enterobacter</i> spp., <i>S. epidermidis</i> , <i>B. subtilis</i> , <i>K. pneumoniae</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>S. Typhimurium</i> , <i>L. monocytogenes</i>	(Kharchenko, 2019; Kim et al., 2001; Lee et al., 2005; Moon & Kim, 2018)
	Antiviral activity against porcine epidemic diarrhea virus and HIV-1	(Park et al., 2002; Yang et al., 2015)
	Antifungal activity via inhibition of conidia germination	(Nagata et al., 1985)
Anti-inflammatory activity	Reduction of oxidative stress	(Akanda & Park, 2017; Lee et al., 2016)
	Reduction of LPS-induced NO production by RAW 264.7 macrophages	(Akanda & Park, 2017; Kim et al., 2012)
	Reduction of pro-inflammatory cytokines levels: PGE <sub>2</sub> , TNF- $\alpha$ , IL-1 $\beta$ , IkB $\alpha$	(Kim et al., 2012; Nam et al., 2021)
Anticancer activity	Reduction of pro-inflammatory enzymes: iNOS, COX-2	(Kim et al., 2012; Nam et al., 2021)
	Cytotoxicity assessed against different <i>in vitro</i> -cultured cancer cell lines: B16 melanoma 4A5 cells, MCF-7 human breast adenocarcinoma cells, Calu-6 human lung adenocarcinoma cells, SNU-601 gastric adenocarcinoma cells, LLC1 Lewis lung carcinoma cells, HL-60 human promyelocytic leukemia cells	(Hwang et al., 2006; Kim et al., 2010; Nakamura et al., 2012; Thao et al., 2010)
	Decrease of glucose blood levels	(Cheredy et al., 2011)
Antidiabetic activity	Inhibition of advanced glycation end-products synthesis	(Sato et al., 2017)
Anti-obesity activity	Cholesterol-lowering effects: decrease of serum TC, TAG and LDL-C levels and increase of serum HDL-C levels	(Lee et al., 2016)
	Decrease of body weight and regulation of hepatic lipid profiles	(Ochiai et al., 2018)
	Increase of fecal fat excretion	(Ochiai et al., 2018)

Abbreviations: CAT, catalase; COX-2, cyclooxygenase; GPx, glutathione peroxidase; HDL-C: high density lipoproteins-cholesterol; HIV-1, human immunodeficiency virus 1; IL-1 $\beta$ , interleukin 1 $\beta$ ; iNOS, inducible nitric oxide synthase; IkB $\alpha$ , nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha; LDL-C, low density lipoproteins-cholesterol LPS, lipopolysaccharide; NO, nitric oxide; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; SOD, superoxide dismutase; TAG, triglycerides; TC, total cholesterol; TNF- $\alpha$ : tumor necrosis factor alpha.

*subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli* (Moon & Kim, 2018). These antimicrobial properties are common for different varieties of *C. japonica*, such as Kramer's Supreme, C.M. Wilson, La Pace, Mrs. Lyman Clarke, Benikarako, and Fanny Bolis. Among them, the extracts from the variety Mrs. Lyman Clarke caused the highest antimicrobial activity against *Enterobacter cloacae* (Kharchenko, 2019). Therefore,

according to these previous results, it is suggested that *C. japonica* extracts show a greater effectiveness against Gram-positive bacteria, with only a slight activity reported for Gram-negative bacteria.

Furthermore, Sharma et al. recently reported that the antimicrobial activity of *C. japonica* leaf extracts can be improved through the use of gold nanoparticles, showing an increased antimicrobial activity against different microbial strains, such as *B. subtilis*, *S. aureus*, *Streptococcus faecalis*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *E. coli* and the fungus *Candida albicans* (Sharma, Selvakumar, Hwa, Sami, & Kumaresan, 2019). Additionally, the antiviral activity of *C. japonica* was assessed in flower extracts against the porcine epidemic diarrhea virus (PEDV) due to the presence of oleanane triterpenes (Yang et al., 2015), as well as the acetonetic leaf extracts were proven effective against human immunodeficiency virus (HIV), mostly due to camelliatannin H (Park et al., 2002). On these bases, a detailed study of such extracts is further needed to identify the secondary metabolites responsible for this activity and thus determining the potential of *C. japonica* as a source of natural antibiotics.

#### Anti-inflammatory activity

*C. japonica* oil has been proved to have an excellent anti-inflammatory activity, as reported by different methods. Regarding its mechanism of action, it was found that *C. japonica* oil inhibits lipopolysaccharide (LPS)-induced production of nitric oxide (NO) and reduced the levels of prostaglandin E (PGE), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in RAW264 macrophages, together with a decrease in the expression of pro-inflammatory enzymes, such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), activator protein 1 (AP-1), nuclear factor-kappa B (NF- $\kappa$ B), and a downregulation of the signaling pathways extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38) and c-Jun N-terminal kinase (JNK) (Kim et al., 2012).

Besides *C. japonica* oil, other derived extracts can also be used in the treatment of esophageal inflammation, as proved for the ethanolic extracts from *C. japonica* shoots via the reduction of ROS, NO, TNF- $\alpha$ , NF- $\kappa$ B, and interleukin-1 $\beta$  (IL-1 $\beta$ ), among other pro-inflammatory signaling pathways. As a result, such extract reduced the rate of esophageal tissue damage by attenuating different histological changes (Nam, Nan, & Choo, 2021). This evidence provides a scientific insight on the promising attributes of *C. japonica* as a biological producer of anti-inflammatory agents, that should be assigned to individual compounds in further studies.

#### Anticancer activity

Leaf and flower extracts of *C. japonica* have been tested against different cancer cell lines, including MCF-7 (human breast adenocarcinoma pleural effusion), Calu-6 (human lung carcinoma) and SNU-601 (human gastric carcinoma) cells, with leaf extracts promoting a strong growth inhibitory concentration below 100  $\mu$ g/mL (Hwang, Cha, Park, Lee, & Lee, 2006). Other cancer cell lines sensitive to *C. japonica* include A549 (adenocarcinoma human alveolar basal epithelial cells), LLC (Lewis lung carcinoma) and HL-60 (human promyelocytic leukemia cells), mostly due to the combination of different triterpenoid compounds (Thao et al., 2010). According to chromatographic studies, the triterpenoids found on *C. japonica* extracts are suggested to promote an intense antitumor activity. Flower extracts showed a high effectiveness against melanoma B16 (mouse melanoma) cell line, due to the presence of molecules such as camellioside (Nakamura et al., 2012) and triterpenic oligoglycosides (Fujimoto et al., 2012). Therefore, the development of extracts from camellia flowers could represent novel therapeutic targets against cancer, although there is an urgent need to perform the pertinent *in vivo* and interventional studies to satisfactorily assess the effectiveness of *C. japonica* extracts on human patients. Indeed, the reported antioxidant and anti-inflammatory activity of such

extracts may assist on the development of anticancer activity of *C. japonica* since both processes are closely related to the onset of carcinogenesis (García-Pérez, Barreal, Rojo-De Dios, Cameselle-Teijeiro, & Gallego, 2019).

#### Anti-obesity activity

Besides inflammatory and cancerous diseases, the extracts of *C. japonica* have been widely evaluated in terms of the prevention of other chronic diseases, as it is the case of obesity. In this regard, seed extracts of *C. japonica* can be used to treat obesity, as suggested by the inhibitory activity on pancreatic lipase and accumulation of body fat promoted by saponins. In parallel, the same *in vivo* study carried out with mice fed a high-fat diet supplemented with seed extracts increased the fecal fat excretion, reduced the body weight and improved both the plasmatic and hepatic lipid profile (Ochiai, Nozaki, Kato, & Ishihara, 2018).

In the same way, fruit extracts were subjected to a murine *in vivo* model to assess their cholesterol-lowering properties, reflecting a reduction in serum total cholesterol, triglycerides, and low-density lipoproteins, accompanied by an increase in serum high-density lipoproteins (Lee et al., 2016). Consequently, *C. japonica* can be considered as a natural remedy to avoid hypercholesterolemia, as referred by different patents. In one of them, flower aqueous extracts were able to suppress CCAAT/enhancer binding protein  $\alpha$  (C/EBP $\alpha$ ) and peroxisome proliferator-activated receptor  $\gamma$  (PPAR  $\gamma$ ) expression, proving their anti-obesity properties (Hee et al., 2010). Overall, these observations suggest that the incorporation of *C. japonica*-derived products forms a promising solution to counter the physiological implications associated with the high prevalence of obesity worldwide.

#### Antidiabetic activity

As seen for obesity, other currently prevalent diseases have been addressed, from a preventive point of view, using *C. japonica* extracts. Thus, camellias can be used in the prevention and treatment of diabetes mellitus, as reported by different studies (Salehi et al., 2019). Thus, the methanolic leaf extract of *C. japonica* reduced the blood glucose levels of alloxane-induced diabetic rats after one only oral administration (Cherreddy, Somasundaram, & Kandalam, 2011). This bioactivity has been suggested to be due to the presence of polyphenols, catechins and polysaccharides in the above-mentioned extracts, as observed in several interventional studies carried out with humans (Fu et al., 2017).

In the same way, *C. japonica* tea was shown to exert antidiabetic effects because of the high levels of theaflavins (Fu et al., 2017). In fact, different studies showed that regular tea consumption increases insulin activity up to 15 times *in vitro* in an epididymal fat cell assay. Chromatographic studies revealed that this property was due to the presence of epigallocatechin gallate, tannins, theaflavins and other undefined compounds (Anderson & Polansky, 2002). Nevertheless, despite these studies, further investigations should be focused on the effectiveness of *C. japonica* extracts as antidiabetic agents by the development of interventional studies.

#### Antihypertensive activity

Several authors valued the effectiveness of different parts of the camellia to reduce ACE angiotensin converting enzyme (ACE) activity. According to an optimization study, the extracts with the highest ACE activity are obtained from *C. japonica* seeds subjected to hydrolytic extraction at 50.98 °C and pH of 7.12, promoting a reduction in the diastolic blood pressure (Lim et al., 2017). Metabolomic studies have shown that this activity may be due to epigallocatechin 3-O-(3-O-methyl)gallate, which is characterized by having a high absorption rate and great stability in the blood (Kurita, Maeda-Yamamoto, Tachibana, & Kamei, 2010). As it was shown for the antidiabetic properties of

*C. japonica*, specific studies are required in order to assess its antihypertensive and cardioprotective effects, through both *in vivo* and clinical studies.

#### Other biological activities

Some bioactivities from *C. japonica* have not been characterized and studied properly, to date. Many of these activities are closely related to the antioxidant capacity of the extracts obtained from this plant. For instance, *C. japonica* seeds can be used in the prevention and treatment of tooth decay, due to the presence of polyphenols, which have shown great potential in the prevention of oral diseases also motivated by their antimicrobial properties (Ferrazzano et al., 2011). The stems and fruits-derived extracts of *C. japonica* show promising wound healing effects, improving the generation of induced pluripotent stem cells (iPSC), as recorded on *in vivo* mouse wound models (Jeon et al., 2018). The gastroprotective effect of methanolic flower extracts from *C. japonica* exhibit potent inhibitory activities on ethanol-induced gastric mucosa lesions in rats, driven by the presence of triterpenic oligoglycosides and camelliosides A, B, C and D (Yoshikawa et al., 2007). Finally, the hypouricemic properties of *C. japonica* leaf extracts were reported through the inhibition of xanthine oxidase activity (Yoon et al., 2017).

#### Prospects on the industrialization of camellias

Up to date, only a limited number of *C. japonica* varieties have been used with food purposes, instead of other *Camellia* species in the food industry that have been widely exploited to that aim. Some examples include *Camellia oleifera*, which is used to produce edible and essential oils, and the leaves of *Camellia sinensis* for the production of different varieties of tea (García-Jares et al., 2017). With respect to *C. japonica*, the seeds have been exploited for the production of the essential “tsubaki oil”, traditionally used in Eastern regions as an ingredient in cosmetic formulations for skin and hair protection and nutrition (Lim, 2014; Tanaka, 1976), as a result of its anti-inflammatory (Kim et al., 2012), anti-ageing and skin barrier stabilizer properties (Jung et al., 2007; Narayanaswamy & Ismail, 2015). In addition, *C. japonica* leaves show a potential application in tea production (Mizutani & Masaki, 2014), or as an edible vegetable in the food industry (Kunkel, 1984; Tanaka, 1976), together with the flowers (Way et al., 2009). Fig. 4 shows a proposed workflow about the large-scale production of *C. japonica* facing its industrialization with food, pharmaceutical and cosmetical purposes.

The exploitation of *C. japonica* will depend on the major purpose of application, thus deciding the choice of a convenient plant part and an efficient extraction procedure. The part of the plant used, according to its chemical composition, will be decisive to establish the collection and storage conditions of the raw material. Thus, concerning suppliers' quality standards, the bark is essentially stored under controlled conditions in terms of air and humidity, whereas leaves are air-dried, and flowers are normally lyophilized and ground state (Zhao et al., 2019). Moreover, the application of the raw material will depend on the extraction and purification technique of the bioactive compounds, aiming at developing an efficient procedure to ensure their production, without interfering with their stability and function (Sasidharan, Chen, Saravanan, Sundram, & Yoga Latha, 2011; Zhao et al., 2019).

Once collected and pre-treated, plant materials are subjected to extraction. Among different extraction methods, conventional procedures have been classically performed in the case of camellias, for instance Soxhlet extraction, maceration, and distillation. Nevertheless, due to the low efficiency and sustainability of these techniques (Azmir et al., 2013), multiple novel methods have been established in recent decades e.g.: ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field-assisted extraction, supercritical fluid extraction or pressurized liquid extraction, etc. (Pereira et al., 2020). After bioactive compounds extraction, plant extracts are often subjected to the determination of their bioactivities through



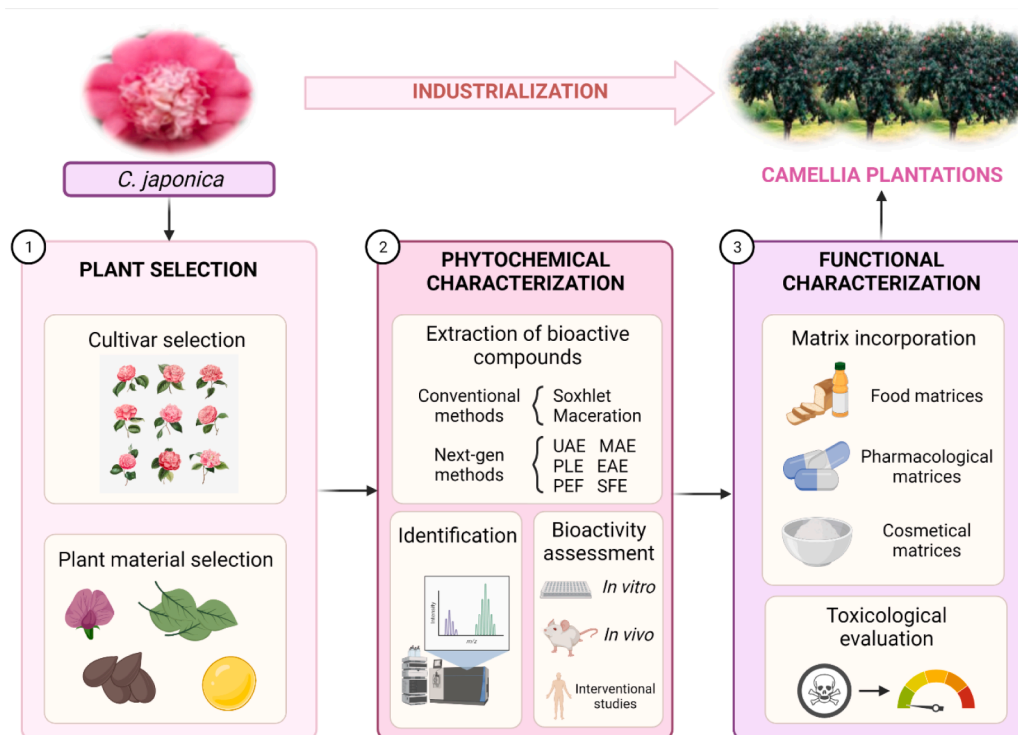


Fig. 4. Proposed workflow for *Camellia japonica* industrialization.

different *in vitro*, *in vivo*, and interventional studies (Fig. 3). Finally, once the bioactivity has been assessed, extracts are incorporated into different matrices ranging from food and cosmetic products to pharmaceutical preparations and further analyzed in terms of toxicology and risk assessment to ensure their efficacy and safety for human consumption.

Furthermore, the advanced stages of the industrialization process concern the cultivation of *C. japonica*, which requires the opening of new scenarios facing their large-scale production, based on the establishment of plantations. To boost camellia production on emerging areas, as it is the case of Galicia, the edaphoclimatic conditions of proposed locations should meet the optimal criteria to ensure a prominent growth of camellias, usually characterized by shady, humid climates with mild temperatures, acidic fertile soils, with preference to wind absence. In addition to geographical requirements, seasonality is another relevant factor facing the success of plantations, as sowing should avoid growing season (spring) and being preferentially performed in the shallow of convenient soils (Corral, Gesto, & Vázquez, 2000). In brief, a multifaceted approach from laboratory scale to open-field cultivation is required with the aim of promoting an efficient industrialization of *C. japonica*, contributing to its large-scale exploitation as a medicinal plant in different sectors, such as the food, cosmetic and pharmaceutical industries.

## Conclusions

Although *C. japonica* is used as an ornamental plant, it is a source of bioactive compounds, including phenolic compounds, terpenoids, fatty acids and other minor compounds. The numerous studies compiled in this review confirm that the extracts obtained from different parts of this plant possess multiple associated bioactivities that play a beneficial effect on human health, acting as antioxidants, antimicrobial, anti-inflammatory, anticancer, and anti-aging agents, that would justify their exploitation at an industrial level as a medicinal plant. Therefore, more studies are essential to reveal the phytochemical composition and the mechanisms of action of camellia bioactive compounds. This information is key to the future development of different camellia-based

preparations with commercial purposes, including drug design, food fortification, additives production, etc. Furthermore, the growing interest of the global population in consuming healthier foods with fewer artificial additives means that the development of this type of product has a potential good reception in the market, and the establishment of camellia plantations becomes a much-needed strategy to meet industrialization requirements, based on previous research conducted at a laboratory scale.

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## 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.

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