

Coffee Leaves: An Upcoming Novel Food?#

Authors

Andrea Montis^{1,2}, Florence Souard^{3,4}, Cédric Delporte^{1,2}, Piet Stoffelen⁵, Caroline Stévigny^{1*}, Pierre Van Antwerpen^{1,2*}

Affiliations

- 1 RD3 Department-Unit of Pharmacognosy, Bioanalysis and Drug Discovery, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium
- 2 APFP Analytical platform of the faculty of pharmacy, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium
- 3 Département de Pharmacochimie Moléculaire, UMR 5063 CNRS, Université Grenoble Alpes, Saint-Martin d'Hères, France
- 4 DPP Department – Unit of Pharmacology, Pharmacotherapy and Pharmaceutical care, Faculty of Pharmacy, Université libre de Bruxelles, Brussels, Belgium
- 5 Meise Botanic Garden, Domein van Bouchout, Meise, Belgium

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70469 Stuttgart, Germany

Correspondence

Andrea Montis

Ph.D. student/researcher

RD3 Department-Unit of Pharmacognosy,
Bioanalysis and Drug Discovery, Faculty of Pharmacy,
Université libre de Bruxelles

Campus Plaine, CP 205/09, 1050 Brussels, Belgium

Phone: + 32 (0) 26 50 52 79

andrea.montis@ulb.be

ABSTRACT

Unlike those of coffee beans, the healthy properties of coffee leaves have been overlooked for a long time, even if they are consumed as a beverage by local communities of several African countries. Due to the presence of xanthines, diterpenes, xanthenes, and several other polyphenol derivatives as main secondary metabolites, coffee leaves might be useful to prevent many daily disorders. At the same time, as for all bioactive molecules, careless use of coffee leaf infusions may be unsafe due to their adverse effects, such as the excessive stimulant effects on the central nervous system or their interactions with other concomitantly administered drugs. Moreover, the presence of some toxic diterpene derivatives requires careful analytical controls on manufactured products made with coffee leaves. Accordingly, knowledge about the properties of coffee leaves needs to be increased to know if they might be considered a good source for producing new supplements. The purpose of the present review is to highlight the biosynthesis, metabolism, and distribution of the 4 main classes of secondary metabolites present in coffee leaves, their main pharmacological and toxicological aspects, and their main roles *in planta*. Differences in coffee leaf chemical composition depending on the coffee species will also be carefully considered.

Introduction

Coffee seeds are used all over the world to make the known coffee beverage. The 2 main exported coffee species are *Coffea arabica* L. (ARA) and *Coffea canephora* Pierre ex A. Froehner (Rubiaceae, CAN), which correspond respectively to 56% and 44% of the

world's coffee production [1,2]. The share of CAN coffee, also named *Robusta* coffee, is increasing steadily. ARA is an allopolyploid species ($2n = 44$), mostly cultivated across the tropics and subtropics. Wild ARA populations can be found in the southwest of Ethiopia, in northern Kenya, and the eastern part of South Sudan. It is interesting to note that the second main exported cof-

* Equal contribution of these authors.

Dedicated to Professor Arnold Vlietinck on the occasion of his 80th birthday.

ABBREVIATIONS

ARA	<i>C. arabica</i>
Atr	atractyloside
CAN	<i>C. canephora</i>
CGAs	chlorogenic acids
CPK	creatine phosphokinase
ERK	extracellular signal-regulated kinase
HFD	high-fat diet
IRAK	interleukin-1 receptor-associated kinase
LDH	lactate dehydrogenase
MAPK	mitogen-activated protein kinase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mTORK2	mTOR complex 2
NF- κ B	nuclear factor κ B
PKC	protein kinase C
SAM	S-adenosylmethionine
TGF- β 1	transforming growth factor beta 1
TNBS	2,3,4-trinitrobenzene sulfonic acid

fee species, *CAN*, is genetically closely linked to *ARA*. Particularly, phylogenetic studies confirmed that *CAN* and *C. eugenioides* can be considered the *ARA*'s progenitor species, each providing half of *ARA*'s genome. It has been assumed that these species hybridized between 1.08 million and 543 thousand years ago and that the emergence of *ARA* could be related to the environmental changes in East Africa during glacial-interglacial cycles in the last 1 million years [3].

Although the chemical composition of coffee beans has been studied for many years, very limited studies have been performed on the coffee leaf properties and their potential use as a healthy food product.

In several countries, including Ethiopia, India, Indonesia, and Jamaica, sun-dried coffee leaves are currently used as a traditional tea substitute [4]. Moreover, coffee leaves are consumed by the communities of some countries for traditional medicinal purposes. For example, in Ethiopia, coffee leaf infusions are used as a laxative drug [5]. In Mexico, their infusions are used to treat fever, and in Nicaragua, their decoctions have been described as a useful herbal remedy for headaches and stomach pains [6]. In 2018, a notification was submitted to the European Food Safety Authority (EFSA) under Article 14 of the Novel Food Regulation (EU) 2015/2283 to place on the market an infusion from coffee leaves as a traditional food. The 2 relevant species are *ARA* and *CAN*. After a rigorous analysis of the request, the EFSA "did not raise safety objections to the placing on the market of the requested product". Coffee leaves could be marketed in the EU as a herbal infusion or as an ingredient for other beverages [7]. One of the main peculiarities of coffee leaf infusions is the higher antioxidant potential and the lower caffeine content: 5 times less compared to the traditional tea and 2 times less compared to mate leaves (*Ilex paraguariensis*, Aquifoliaceae) (► **Table 1**) [4]. Indeed, due to the high content of CGAs and xanthenes, coffee leaves could be used to prevent several diseases caused by the most common inflammatory processes.

Moreover, recent studies have shown a good efficacy of dietary polyphenols in preventing some severe pathologies. For example, they appear to be useful in preventing some forms of cancer [8] and treating diseases affecting the central nervous system [9].

In addition to the high content of polyphenols, coffee leaves are also a rich source of particular diterpenes and alkaloids. Among the main alkaloids, the most abundant one found in coffee leaves is caffeine [31]. This purine alkaloid is produced by the plant responding to stress (such as predation by insects or fungal infections) [32]. Beneficial effects of pure oral caffeine on oxidative stress have been demonstrated; caffeine could also protect from type 2 diabetes mellitus and its complications and other chronic disorders associated with increased oxidative damage in blood and tissues [33].

Regarding diterpene content, cafestol and kahweol are the 2 main diterpenes found in coffee plants and seeds. Both compounds showed good antidiabetic and anticarcinogenic activities. Particularly, they can inhibit tumor cell activity by inducing their apoptosis [34, 35].

These secondary metabolites can be affected considerably by the environment, the field conditions, the growing season and ecosystem, the plant variety, and age. Other parameters, such as the growth stage of the leaf, can also affect the content of metabolites in coffee leaves. The literature describes 3 different developmental stages for coffee leaves that are categorized as (a) buds and young leaves, (b) mature leaves, and (c) aged leaves [10, 36, 37]. Young leaves weigh approximately 25 mg (fresh weight) and are approximately 20 mm long and 7 mm wide. Mature leaves are fully expanded, weighing approximately 1.2 g, whereas aged leaves are dark green, they often show dark spots on their surface, and their weight is approximately 1.3 g [36]. However, some authors do not agree with these standard sizes, which can vary according to the crop location, and have assigned a specific value for coffee leaves used in their experiments. For example, Monteiro et al. [10] claimed that an *ARA* leaf 7 cm long and 3 cm wide could be considered a young leaf.

Moreover, they clearly showed that the size of coffee leaves varies according to each coffee species. Other authors prefer to identify the developmental stage of coffee leaves according to their color and texture. Rodrigues Salgado et al. [37] considered that young leaves are light green, smooth, and recently expanded, whereas mature leaves are intense green and less smooth.

Taking into account the healthy benefits of coffee leaf infusions and considering the emphasized consumption of food supplements in developed countries defined by The European Food Safety Authority as "concentrated sources of nutrients, or other substances with a nutritional or physiological effect marketed in 'dose' form and intended to correct nutritional deficiencies, maintain an adequate intake of certain nutrients, or to support specific physiological functions" [38], this review will gather the biochemical aspects of coffee leaf key metabolites, their possible nutritional or physiological uses, and their toxic aspects. Data will be summarized by grouping the main known pharmacological activities according to *in vitro*, *in vivo*, and human studies. A brief overview of the available studies on coffee leaf extracts and the biochemical differences depending on coffee species will also be given.

► **Table 1** Content of the main representative secondary metabolites in the green beans, roasted beans, and mature leaves of *Coffea arabica* (A) and *Coffea canephora* (C) and of *Camellia sinensis* (green tea) and *Ilex paraguariensis* leaves.

Metabolite	Coffee leaves		Green coffee beans		Roasted coffee beans		Tea leaves	Mate leaves	References
	(A)	(C)	(A)	(C)	(A)	(C)	<i>Camellia sinensis</i>	<i>Ilex paraguariensis</i>	
Caffeine	2.5–6.0	2.8–7.5	7.6–29.0	15.1–33.3	5.3–20.3	10.6–23.4	36.6–38.0	11.6–18.7	[10–19]
Trigonelline	< 3.0	4.2–6.0	8.8–27.6	7.5–34.2	< 2.5	< 2.5	–	–	[10, 13, 14, 16, 18, 20–22]
Theobromine	< 0.1	0.1–0.8	< 0.1	< 0.1	< 0.1	< 0.1	1.0–1.2	4.5–11.6	[10, 11, 13, 18, 19]
Theophylline	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	–	–	[17]
Chlorogenic acids*	19.2–39.6	19.3–24.5	57.0–80.3	86.2–102.0	< 3.0	< 1.0	< 14.0	28.8–97.6	[10, 12, 14, 16, 23–27]
Mangiferin	0.5–9.2	ND	ND	ND	ND	ND	–	–	[10, 12, 28, 29]
Cafestol	< 6.0	< 0.1	2.7–11.0	1.5–3.7	< 5.0	< 3.0	–	–	[18, 24, 30]
Kahweol	ND	ND	1.1–6.7	1.5–3.7	< 10.0	ND	–	–	[18, 24, 30]

Concentrations are expressed as mg/g of dry matter and consider the variability depending on the country of harvest and the climatic differences. *CGAs are expressed as mg of total CGAs/g of dry matter; ND = not detected; – = no available information

The experimental research has been conducted by consulting the following websites: Pubmed, ScienceDirect, Google Scholar, Scopus, SciFinder. The keywords searched were: I. coffee (*coffea*) II. caffeine, III. xanthines, IV. CGAs, V. xanthonones, VI. diterpenes, VII. biosynthesis, VIII. coffee leaves, IX. *in vitro* studies, and X. *in vivo* studies. The bibliographical research was carried out in English, French, and Italian.

Alkaloids

The main alkaloids found in coffee leaves are caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), and trigonelline.

The first 3 metabolites are classified as purine alkaloids, and among them, caffeine is the major alkaloid in coffee plants (► **Table 1**). The role of alkaloids in plants has not been clearly defined, but they do not seem to be used as a nitrogen source to increase the nitrogen pool in the plant organs. One of the main roles of these alkaloids might be defensive. Due to the “*chemical defense theory*”, caffeine production is significantly increased if the plant is subjected to stressful conditions such as nonideal climatic conditions [39]. Insect attacks may also cause caffeine increases in plant tissues to prevent some other infections. For example, it has been shown that when plants of the *Camellia* genus (Theaceae) are attacked by *Xyleborus fornicatus*, caffeine biosynthesis increases to prevent fungal infections by *Monacosporium ambrosium* [40]. *Theobroma cacao* (Sterculiaceae) plants may inhibit fungal infections by *Crinipellis pernicioso* by also producing more caffeine. The caffeine content in infected tissues is 7–8 times higher

than the content in healthy tissues, but no information concerning a potential increase of theobromine has been reported [32].

Alkaloids: metabolism and turnover in coffee leaves

The main caffeine biosynthetic pathway is a reaction including 4 steps: 3 methylations and a hydrolysis reaction. The first step of this pathway is the methylation of the xanthosine by a SAM-dependent *N*-methyltransferase. The product formed is 7-methylxanthosine. This compound is a substrate of an *N*-methylnucleosidase that can remove the ribose of the molecule by catalyzing the hydrolysis reaction.

The 2 last steps include 2 methylations, carried out by the theobromine synthase and caffeine synthase. While theobromine synthase may only catalyze the synthesis of the theobromine from 7-methylxanthine, caffeine synthase can use paraxanthine, theobromine, and 7-methylxanthine as a substrate to synthesize caffeine. Xanthosine, the initial substrate for purine alkaloid synthesis, is formed through 4 different pathways: (i) *de novo* purine biosynthesis, (ii) degradation pathways of adenine nucleotides (AMP route), (iii) guanine nucleotides (GMP route), and (iv) the SAM cycle (SAM route) [40,41].

In coffee plants, biosynthetic activity has been described in both leaves and fruits. The main biosynthetic activity has been found in young leaves of ARA, especially in the upper leaves. In the young expanding leaves of ARA plants, theobromine has also been detected but in lower concentrations than caffeine (► **Table 1**) [42]. Caffeine is present in cotyledons and leaves in ARA seedlings, but it was not found in the older part of shoots and roots [43].

Caffeine accumulates in mature leaves before it is catabolized into theophylline [44, 45]. Studies on leaf and bud extracts of *ARA* analyzed by LC-UV showed that caffeine content is greater in young leaves and lower in mature and aged leaves. Results also showed that in mature and aged leaves, the levels of theobromine were significantly reduced. Theophylline was not detected in the leaf extracts [36].

Trigonelline represents the second most abundant alkaloid in coffee leaves. This compound could be considered a reserve compound for nicotinamide adenine dinucleotide (NAD) biosynthesis, but this function is limited to the very early stages of germination of some specific tissues. Some other physiological functions of trigonelline have been proposed in several plants. Trigonelline can be produced in response to oxidative stress; in addition, trigonelline can act as a leaf-closing factor, a cell arrester in G2, and an inducer of the Nod gene (the Nod gene induction leads to nodule formation, a process facilitating a symbiotic relationship between organisms) [43].

Trigonelline is synthesized from nicotinamide formed during the NAD cycle. The first step of biosynthesis is the deamination of nicotinamide, leading to the formation of nicotinic acid. In coffee leaves, nicotinic acid is mostly converted into trigonelline due to the intervention of a SAM-dependent nicotinate *N*-methyltransferase. This alkaloid is finally catabolized to nicotinic acid and recycled for NAD synthesis. In tea leaves, though, nicotinic acid is completely converted into nicotinic acid glucoside [46].

Alkaloids: differences between coffee species

Few studies focused on the content of secondary metabolites in coffee leaves. Monteiro et al. [10] provided some information about the main differences between coffee species. It has been found that *ARA* had the highest content of caffeine in the leaves. Particularly, caffeine was detected in the leaves of *ARA* and *CAN* with concentrations about 15 mg/g and 12 mg/g respectively in young leaves, and 6 mg/g and 5 mg/g respectively in mature leaves (► **Table 1**). Results are expressed as mg/g of dry matter, and the data have been based on LC-UV analysis. Caffeine has not been found in the leaves of *C. eugenioides* and *C. racemosa*. This study aimed also to highlight the concentration of theobromine and trigonelline in the leaves of these same 4 coffee species. In *ARA* leaves, the concentration of theobromine was 0.8 mg/g in the young leaves and 0.04 mg/g in the mature leaves. In *CAN* the concentration of theobromine was within the range of 0.1–4 mg/g in the young leaves and 0.05–0.8 mg/g in the mature leaves. Theobromine was also found in 1 of 2 *C. racemosa* genotypes analyzed.

Trigonelline content was higher in the young and mature leaves of *C. eugenioides* and *C. racemosa*, approximately 12 mg/g and 9 mg/g in *C. eugenioides* leaves and 11 mg/g and 10 mg/g in *C. racemosa* leaves, respectively. In *ARA* and *CAN* leaves, trigonelline content was about 12 mg/g and 3 mg/g in young and mature leaves of *ARA* and 6 mg/g and 5 mg/g in young and mature leaves of *CAN*, respectively.

Another study, which focused on *ARA*, showed that trigonelline content in young and mature leaves of this species is around 4–7 mg/g [20].

Alkaloids: pharmacology and toxicity

The beneficial effects of coffee purine alkaloids have been largely demonstrated. *In vitro* and *in vivo* studies have been carried out to confirm the efficacy of caffeine in the prevention of some central nervous system diseases. Even if caffeine showed good efficacy, the beneficial effects could be observed only at doses much higher than those ingested with coffee leaf tea.

In vitro studies were performed to demonstrate how caffeine could be considered a regulator of Ca²⁺ levels in the cell due to its activity on ryanodine receptors and the inhibition of phosphodiesterases. Caffeine is a ryanodine receptor agonist. By activating ryanodine on the sarcoplasmic reticulum, caffeine at mM concentration can increase the amount of intracellular calcium [47, 48]. Since the metabolic pathways involving the modulation of phosphodiesterase and activation of ryanodine receptors can play a role in some neurodegenerative diseases, caffeine could be used to limit the Ca²⁺ level dysregulation. However, it seems to be difficult to obtain satisfactory effects by administering a nontoxic dose of caffeine.

It has also been shown how caffeine, as well as theobromine, might prevent the membrane damages caused by the lipid peroxidation induced by some reactive oxygen species (ROS) like hydroxyl radical ([•]OH), peroxy radical (ROO[•]), and singlet oxygen (¹O₂) [49]. In a study carried out by Devasagayam et al. [50], the antioxidant activity of caffeine was compared to that of ascorbic acid, and results obtained by using millimolar doses of caffeine showed that the percent of inhibition of lipid peroxidation induced by ROS in rat liver microsomes was 60.5% while ascorbic acid exerted only a 21.5% inhibition. Consequently, it could be concluded that the antioxidant ability of caffeine was significantly higher than ascorbic acid for lipid peroxidation.

An *in vitro* study has also been performed by Dulloo et al. [51] to investigate the synergistic effect of caffeine and polyphenols (especially catechin derivatives) of green tea extract on thermogenesis stimulation. This effect can be due to the inhibition of noradrenaline degradation exerted by polyphenols and the inhibition of transcellular phosphodiesterases exerted by caffeine. An experiment was carried out to test the effect of these metabolites on the respiration rate of interscapular brown adipose tissue from rats. It was observed that a combination of caffeine (100 μM) and catechins (200 μM) could significantly increase the respiration rate (2 times more if compared to basal values). In contrast, caffeine and polyphenols alone did not exert a significant effect.

Some *in vivo* studies allowed highlighting the beneficial effects of caffeine on central nervous system disorders. Protective effects in Parkinson's disease have been studied, and it was concluded that the neuroprotective effects of caffeine in the substantia nigra might be due to competitive caffeine inhibition with adenosine. Caffeine at 300 mg can affect all adenosine receptors, but A₁ and A_{2A} receptors are more susceptible [52]. By inhibiting the activity of A_{2A} receptors (A_{2A}R), caffeine decreases the activation of adenylyl cyclase. Consequently, protein kinase A is less activated, and intracellular calcium levels are decreased, leading to decreased glutamate release from the cells. Thus, caffeine can reduce neuroinflammation by attenuating excitotoxicity. A study carried out on mice showed that in animals treated with 10 mg/kg of caffeine 10 min before MPTP administration, the residual

level of dopamine in mice was 40% of the control (mice treated with saline). Caffeine at 20 mg/kg could almost fully inhibit the dopamine depletion caused by MPTP, a substance that strongly enhances oxidative stress and upregulates Bax expression and apoptosis of substantia nigra dopaminergic neurons. The effects of caffeine on A_{2A}R were also compared to those of some synthetic antagonists such as SCH 58261, 3,7-dimethyl-1-propargyl-xanthine (DMPX), and istradefylline (KW-6002), and it has been shown that in both cases, A_{2A}R antagonism could significantly reduce striatal dopamine depletion [52, 53]. It remains to be seen whether a coffee leaf extract containing a smaller dose of chronically administered caffeine could be useful to prevent Parkinson's disease in which an MPTP treatment does not strongly provoke neurological toxicity.

Furthermore, caffeine also showed good efficacy in an Alzheimer's disease mouse model. Indeed, caffeine might reduce the extracellular depositions of diffuse and neuritic plaques composed of the amyloid- β peptide. Particularly, the treatment of mice with an average daily dose of 1.5 mg of caffeine for 6 weeks resulted in a reduction of presenilin 1 and β -secretase, indicating lower hippocampal β -amyloid levels [54].

Human studies have been carried out to show how caffeine can enhance the effects of some drugs. For example, caffeine can enhance the analgesic effect of acetaminophen. Particularly, a study was designed to compare the analgesic effect of acetaminophen and caffeine alone to a combination of acetaminophen and caffeine in response to tonic and phasic pain stimulation. In this 4-way crossover, double-blind, placebo-controlled study, 24 subjects were treated orally with 1000 mg acetaminophen, 130 mg caffeine, and a combination of both. Results showed analgesic effects of acetaminophen and acetaminophen plus caffeine but not caffeine alone. Moreover, the best results were obtained with the association of acetaminophen and caffeine. In addition, the combination also demonstrated that caffeine accelerated acetaminophen absorption, indicated by a more quickly reached maximum level [55].

Caffeine could also be useful against diabetes. However, Dewar and Heuberger [56] reviewed the effect of caffeine intake on insulin sensitivity and glycemic control by concluding that there are still controversies on the potential utility of this metabolite in the treatment of diabetes. In a double-blinded, randomized, placebo-controlled study, the administration of 250 mg of caffeine or placebo in individuals with a type 1 diabetes diagnosis (T1DM) resulted in significantly decreased episodes of hypoglycemia overnight for the group treated with caffeine. In another randomized, double-blind study, Richardson et al. [57] administered 5 mg/kg of caffeine or a placebo to 12 men. Blood glucose and insulin levels were monitored. They showed that subjects who consumed caffeine had higher levels of insulin. However, even if the insulin was increased, the same individuals showed high blood glucose levels, indicating a decrease in insulin sensitivity [58]. In contrast with this last study, Krebs et al. [59] found that glucose levels were only slightly increased after caffeine intake and that no differences in insulin sensitivity could be detected.

However, overconsumption of caffeine may cause some adverse effects, which involve overstimulation of the sympathetic nervous system. Symptoms are anxiety, palpitations, insomnia,

hypertension, and muscle spasms [60]. One of the main daily negative consequences caused by the adverse effects of caffeine is insomnia. Drake et al. [61] compared the disruptive sleep effects of caffeine administered in a dose of 400 mg to a group of volunteers at 0, 3, and 6 hours before habitual bedtime. The study results suggested that 400 mg of caffeine taken 0 to 6 hours before bedtime may significantly disrupt sleep compared with the placebo group's sleep quality. Even at 6 hours before bedtime, caffeine reduced sleep by more than 1 hour. In another study performed by Bonnet et al. [62], the administration of 400 mg of caffeine 30 minutes before bedtime drastically reduced sleep quality in treated patients. In addition, significant changes in cardiac function were detected with the electrocardiograms of patients treated with caffeine due to an increase in cardiac sympathetic activity. Nevertheless, considering the content of caffeine in coffee leaves (► **Table 1**) and the weight of a traditional tea bag (2 g), there is no need to be worried about these side effects. However, the problem can arise from drinking "espresso coffee", one of the most common coffee beverages, due to its high caffeine content. A recent study found that the content of caffeine, trigonelline, and nicotinic acid could be strongly influenced by the coffee cultivars and espresso machines that are used. It was concluded that the combination of a temperature of 92°C and pressure at 7 or 9 bar is the ideal setting for the most efficient extraction of these compounds. The content of caffeine in a 25 mL cup of espresso coffee made with these extracting conditions is about 117–200 mg [63].

The second most abundant alkaloid in coffee leaves is trigonelline (► **Table 1**). Trigonelline is mainly known for its neuroprotective and hypoglycemic effects. It has been shown that trigonelline at a 50 mg/kg dose for 28 days may progressively decrease the blood glucose level in streptozotocin- and HFD-induced type 2 diabetes rats. Results showed a decrease from 423.8 mg/dL glucose in the diabetic control group to 125.4 mg/dL in diabetic rats treated with trigonelline [64]. The same treatment decreased serum insulin level from 53 mU/L in the diabetic control group to 41 mU/L in diabetic rats treated with trigonelline. Moreover, trigonelline has positive effects on diabetes complications such as auditory neuropathy. An improvement of the hearing threshold shift, a delayed latency of the auditory evoked potential, and improved sensory fiber loss in mice with auditory neuropathy induced by pyridoxine was observed after chronic administration of 10 mg/kg trigonelline [65].

Regarding its neuroprotective effects, trigonelline seemed to be also beneficial in Parkinson's disease. Mirzaie et al. [66] proved that a dose of 100 mg/kg of trigonelline exerts dose-dependent neuroprotective and anti-apoptotic effects in the case of 6-hydroxydopamine toxicity. Particularly, it has been observed that trigonelline prevented the reduction of the number of Nissl-stained neurons of the substantia nigra pars compacta.

To our knowledge, the only toxic effects linked to trigonelline have been observed at doses much higher than the content of trigonelline that could be reasonably ingested with coffee leaves. In rats, the oral and subcutaneous LD₅₀ doses of trigonelline are 5000 mg/kg. It has been shown that Sabra albino mice fed with 50 mg/kg trigonelline daily for 21 days did not show alterations in the weight of the thyroid, thymus, adrenals, kidney, liver, uterus,

us, or ovaries. Therefore, no data are available on acute exposure or potential reproductive or teratogenic effects [67].

Polyphenols: Acyl-quinic Acids and Xanthones

Phenolic compounds are one of the major healthy secondary metabolites present in coffee leaves. Due to their antioxidant properties, polyphenols may be useful to prevent several diseases caused by the oxidation of the main biomolecules. Polyphenols could be used to prevent inflammation and cell apoptosis and to prevent the rise of some forms of cancer [68]. Recently, dietary polyphenols were found useful to prevent prostate cancer [8]. The main known polyphenols of coffee leaves include several derivatives of benzoic, caffeic, *p*-coumaric, protocatechuic, sinapic, ferulic, and acyl-quinic acids [31]. Due to the importance of their presence in coffee tissues and their strong health benefits, this review focused principally on the description of the main acyl-quinic acids and xanthones present in the leaves. It is generally recognized that phenolic compounds are useful in response to biotic or abiotic stress. They could be involved in a possible adaptation of the plants to climate change [69], and they might make the plant more resistant to pests or diseases, such as those induced by insect infections [70, 71].

Acyl-quinic acids: metabolism and turnover in coffee leaves

Acyl-quinic acids are a group of plant-derived compounds produced principally through the esterification of hydroxycinnamic and quinic acid. Clifford et al. [72] found that the main acyl-quinic acid biosynthetic pathway uses phenylalanine as the main precursor. The two first steps of this pathway involve deamination of the phenylalanine, leading to the formation of the cinnamic acid, and then hydroxylation of the latter, leading to the formation of *p*-coumaric acid, catalyzed respectively by a phenylalanine ammonia-lyase and a cinnamate 4'-hydroxylase. The next step is the conversion of *p*-coumaric acid into *p*-coumaroyl-CoA catalyzed by a 4-cinnamoyl-CoA ligase. The *p*-coumaroyl-CoA may be a substrate for 2 different enzymes: a quinate hydroxycinnamoyl transferase and a *p*-coumaroyl-3'-hydroxylase. The first enzyme catalyzes the synthesis of 5-*O*-*p*-coumaroylquinic acid, while the second catalyzes the caffeoyl-CoA synthesis. The caffeoyl-CoA may be directly converted into 5-*O*-caffeoylquinic acid (5-CQA) or in feruloyl-CoA, thanks to a caffeoyl-CoA-3-*O*-methyltransferase. Feruloyl-CoA will be used to synthesize 5-*O*-feruloylquinic acid. Finally, 5-*O*-*p*-coumaroylquinic acid may be directly converted into 5-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid into 5-*O*-feruloylquinic acid.

For all these compounds, several isomeric forms exist. For example, 3 different isomers can occur for caffeoylquinic acid, namely chlorogenic acid (5-CQA), neochlorogenic acid (3-CQA), and cryptochlorogenic acid (4-CQA). Among these 3 isomers, the major one present in water leaf extracts is 5-CQA [73]. Moreover, several dimeric forms of these compounds are possible: some dicaffeoyl-quinic acids (diCQA), such as 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA, have been found in coffee leaves [74, 75].

► **Table 2** Coffee species with the highest caffeine, trigonelline, chlorogenic acids, and mangiferin in mature leaves.

Metabolite	Species		References
Caffeine	<i>C. arabica</i> 2.5–6.0	<i>C. canephora</i> 2.8–7.5	[10]
Trigonelline	<i>C. eugenioides</i> 7.8–11.2	<i>C. racemosa</i> 9.7–9.8	[10, 20]
Chlorogenic acids	<i>C. racemosa</i> 21.7–36.6	<i>C. eugenioides</i> 29.4–41.1	[10]
Mangiferin	<i>C. anthonyi</i> >100.0	<i>C. salvatrix</i> >150.0	[23]

Concentrations are expressed as mg/g of dry matter. Little information is available about the content of cafestol and kahweol in coffee species leaves other than ARA and CAN.

Acyl-quinic acids: differences between coffee species

The content of CGAs found in coffee leaves is greater than the CGA content of tea leaves but lower than that of mate leaves (► **Table 1**). Monteiro et al. [10] reviewed the content of CGAs and their dimers detected by LC-UV in the young and mature leaves of ARA, CAN, *C. eugenioides*, and *C. racemosa*. It has been found globally that CGA concentration was strongly higher in the young leaves of the four species. Considering the young leaves of these 4 coffee species, ARA showed the highest content of total CGAs with 74 mg/g of dry matter, while for mature leaves, *C. racemosa* showed the highest content of total CGAs with 62 mg/g of dry matter (► **Table 2**).

Campa et al. [23] described the content of acyl-quinic acids detected by LC-mass spectrometry (MS) in the mature leaves of several African coffee species grown in French tropical greenhouses and several coffee species from Madagascar. Among the 15 analyzed species from Africa, the concentration of acyl-quinic acids was found higher in ARA, *C. eugenioides*, *C. anthonyi*, *C. stenophylla*, *C. liberica*, and CAN. ARA var. *Laurina* was the species with the highest content of acyl-quinic acids in the leaves (40 mg/g of dry matter). Among the 9 analyzed species from Madagascar, *C. leroyi*, *C. ankaranensis*, and *C. vohemarensis* have been found to have the highest content of acyl-quinic acids. Particularly, *C. leroyi* contained higher levels of acyl-quinic acids in the leaves (20 mg/g of dry matter). Another study, conducted by Rodríguez-Gómez et al. [73], focused on the determination by LC-ECD and LC-MS of the 3 main caffeoylquinic acid isomers in the mature leaves of 7 different African coffee species and 1 subspecies grown in the tropical greenhouses of Meise Botanic Garden (Belgium). Results showed that species with the higher content of total CGAs were *C. liberica*, *C. liberica* var. *liberica*, and *C. anthonyi* with an amount of 203, 124, and 48 mg/L of total CGAs, respectively, in aqueous extracts made by suspending 15 mg of dried leaf powder into 1.5 mL of water.

Acyl-quinic acids: pharmacology and toxicity

CGAs are known as metabolites efficient in preventing some forms of cancer, diabetes, and obesity. If coffee leaves are used as food supplements, a possible application of these products

could be to prevent obesity. However, more studies on coffee leaf extracts must be performed to determine which extract concentration should be ingested to observe significant effects.

One of the most important health benefits of these compounds is anticancer activity. It is known that a wide variety of tumors are associated with a dysregulation of the MAPK/ERK signaling pathway. Several mechanisms that could justify the anticancer activity of these compounds have been proposed, and one of them includes the inactivation of the MAPK/ERK signaling pathway. One of the 3 MAPK subfamilies includes ERK [76,77]. Normally, the RAS protein, one of the main known oncogenes, can lead to the activation of MEK protein kinase, thus phosphorylating and activating ERK. ERK proteins can activate some transcription factors for genes involved in the regulation of cell survival and proliferation. Yan et al. [78] demonstrated that the combination of CGAs (250 $\mu\text{mol/L}$) and 5-fluorouracil (5-FU 20 $\mu\text{mol/L}$) significantly inhibited ERK protein phosphorylation in *in vitro* HepG2 and Hep3B cells. Consequently, significant inhibition of hepatocellular carcinoma cell proliferation could be observed through this mechanism.

Another pathway involved in cancer cell proliferation is associated with the mTORC2. Ritcor, an mTORC2 subunit, may be overexpressed in many gliomas. The activation of mTORC2 caused by the overexpression of Ritcor can enhance cancer cell proliferation and their capacity to invade other tissues [79,80]. Furthermore, the Ritcor-mTORC2 complex is important for the organization of the actin cytoskeleton structure [81].

Recently, Tan et al. [82] demonstrated that CGA treatment (5, 20, and 80 μM) might inhibit the migration and invasive ability of cancer cells by decreasing the phosphorylation of Akt, the expression of Ritcor and F-actin, which is involved in cell growth, and the organization of the actin cytoskeleton.

In vivo studies have been performed to highlight the antidiabetic and anti-obesity activity of CGAs [83]. In induced-obese mice fed with HFD supplemented with chlorogenic acid at 0.02% (w/w), a decrease of the blood glucose concentration in the circulation could be observed. CGAs are known to inhibit the activity of hepatic glucose-6-phosphatase. Consequently, insulin production is decreased. Moreover, by making glucose unavailable to produce energy, the main energy source is obtained from fat reserves [84].

Huang et al. [85] investigated the effect of 5-CQA on lipid metabolism by feeding Sprague-Dawley rats with an HFD. The rats were randomly assigned to (1) a normal control (NC) group, (2) an HFD control group, (3) an HFD with a low-dose CGA (20 mg/kg) group, and (4) an HFD with high-dose CGA (90 mg/kg). CGA was orally administered once per day for 3 months. In the group treated with the higher dose of CGA, hepatic total cholesterol and triacylglycerol levels were significantly lower than the HFD control group. The anti-obesity activity is still to be tested by chronically administering a modest dose of these compounds.

CGAs are also useful in the treatment of hypertension [86]. Recent studies showed that CGAs (1 μM) can relax rat vessels by stimulating the activity of the nitric oxide synthase [87].

Only a few papers dealt with the possible toxic effects of CGAs. Chaube and Swinard studied a possible teratogenic effect of CGAs by administering high doses of these compounds on rats [88]. Particularly, it has been shown that an administration of 5–

500 mg/kg of CGA during gestation did not cause fetal lethality. Concerning the possible carcinogenic effect, it has been found that rats fed with CGAs (2%) for more than 1 year developed papillomas and carcinomas of the digestive, respiratory, and urinary tract [89]. CGAs should also induce DNA strand breaks, especially when the oxygen radicals formation is enhanced by transition metals [90]. A possible immunotoxic effect has been reported by Kimura et al. [91]. The administration of chlorogenic acid and caffeic acid (2.5×10^{-5} M) could inhibit histamine release induced by concanavalin A plus phosphatidylserine in *in vitro* rat mast cells.

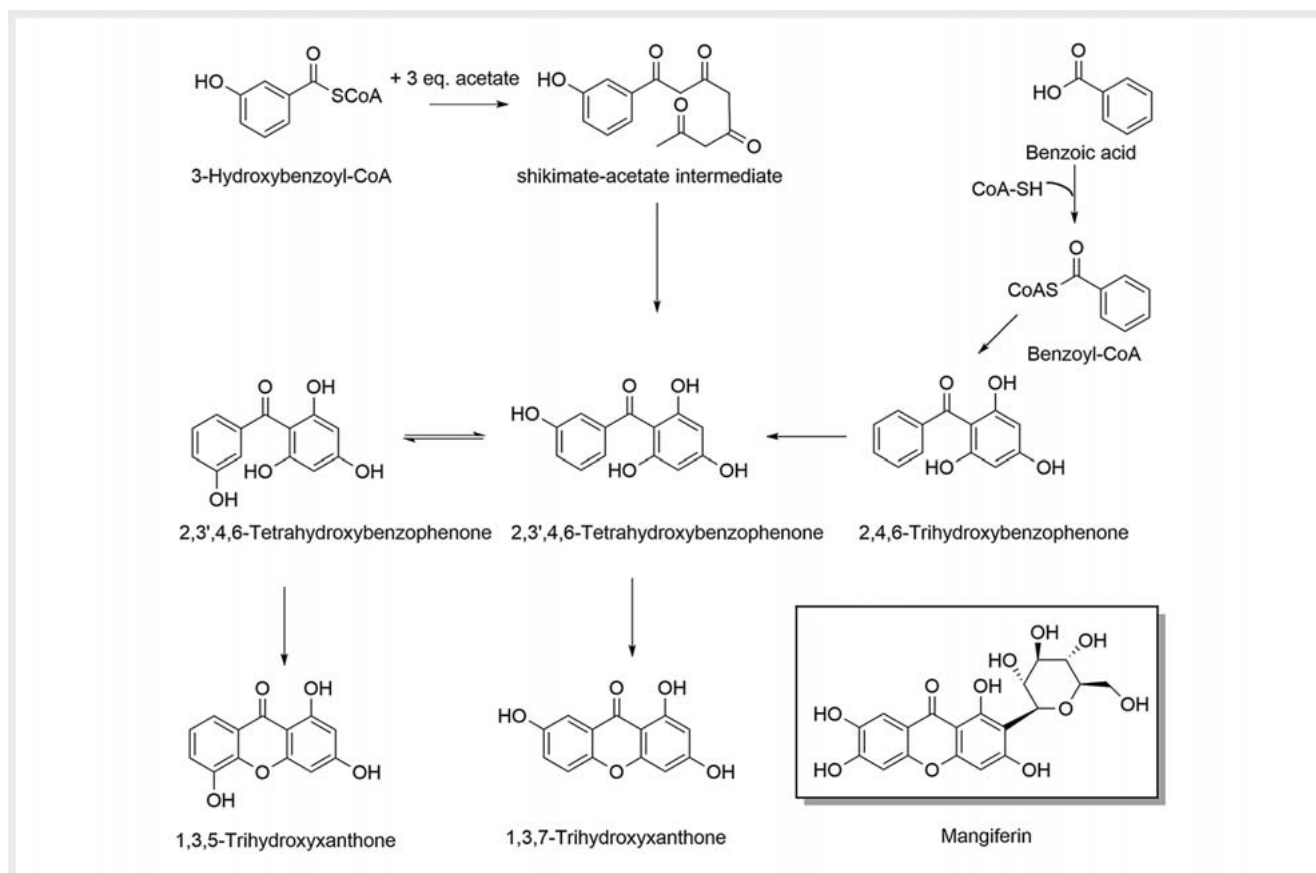
Xanthenes: Metabolism and Turnover in Coffee Leaves

Xanthenes are a vast family of compounds found in plants, fungi, and lichens [92]. Chemically speaking, xanthenes derive from benzoic acid derivatives and malonyl-CoA, and as shown in ► **Fig. 1**, the main precursor is 3-hydroxybenzoic acid. This compound may react with a molecule of CoA, leading to the formation of 3-hydroxybenzoyl CoA. 3-hydroxybenzoyl CoA reacts with 3 molecules of acetate by forming an unstable intermediate that could be defined as a shikimate-acetate intermediate and is used to synthesize a benzophenone. Consequently, it can be concluded that benzophenones represent the main precursors for the xanthone biosynthesis in plants. The 2 different tetrahydroxybenzophenone isomers that might be formed are then subjected to the ring-closure process, leading to 1,3,5-trihydroxyxanthone and 1,3,7-trihydroxyxanthone. Alternatively, benzoic acid is used as a precursor of biosynthesis. Benzoic acid could be converted in benzoyl-CoA, which may react with 3 molecules of malonyl-CoA, leading to the formation of a 2,4,6-trihydroxybenzophenone. Due to the intervention of a benzophenone-3'-hydroxylase, this intermediate can be transformed into a 2,3',4,6-tetrahydroxybenzophenone, which will be converted in 1,3,7-trihydroxyxanthone [93,94].

Xanthenes represent a vast class of compounds, often including xanthone glycosides, prenylated xanthenes, and xanthone-lignoids found in a dimeric or trimeric form in the plants [94,95]. It is interesting to note that these metabolites are present in the leaves of *ARA* while they are undetectable in the fruits (► **Table 1**). Xanthone biosynthesis has been found more active in the young leaves when compared with the mature leaves, where the content of xanthenes decreases progressively. One of the major xanthenes found in coffee leaves is mangiferin (► **Fig. 1**), which has been shown to decrease with aging. In fact, stage 3 and 2 leaves (leaves from the second and first node below the apex) seem to have 3-fold less mangiferin than the youngest leaves [23].

Xanthenes: differences between coffee species

Among plants studied by Campa et al. [23], the African coffee species that showed the higher mangiferin content were *C. salvatrix*, *C. anthonyi*, *C. eugenoides*, and *C. pseudozanguebariae*. *ARA* leaves showed a low mangiferin content, while this xanthone has not been detected in *CAN* leaves. None of the 9 Madagascan species studied contained mangiferin (► **Table 2**). The different repartition of mangiferin in the leaves of the African species may



► **Fig. 1** The major biosynthetic pathway of xanthenes and chemical structure of mangiferin. Adapted from Schmidt et al. [93]. [rerif]

be because they came from different regions. Current data show that species from East Central Africa and East Africa, such as *C. anthonyi*, *C. salvatrix*, and *C. pseudozanguebariae*, have the highest mangiferin content in the leaves. *C. eugenioides* leaves also contain high amounts of mangiferin, perhaps because this species and *C. anthonyi* may be related to *ARA*'s progenitor species. However, *C. anthonyi* and *C. eugenioides* do not grow in the same habitats, which means that the genetic closeness between these 2 species should be further investigated [96].

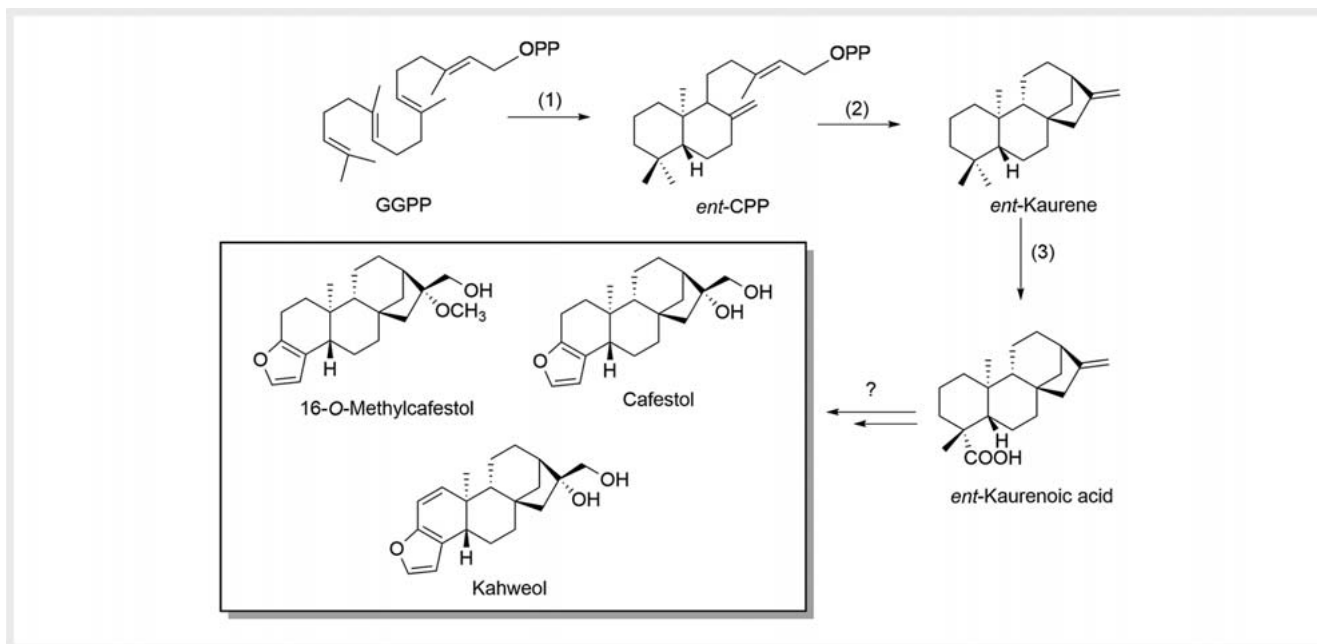
Another study aiming to quantify mangiferin and isomangiferin in *ARA* leaves from Brazil and Costa Rica by LC-MS showed that the ranges of total mangiferins from Brazil (0.6–5 mg/g) and Costa Rica (0.8–4 mg/g) were similar across the samples. However, the content of mangiferin is higher in the leaves of Brazilian plants [28]. Another work highlighted the xanthone composition of young and mature coffee leaves by LC-UV from *ARA*, *CAN*, *C. eugenioides*, and *C. racemosa*. It has been confirmed that young coffee leaves had more mangiferin and isomangiferin than mature ones. Furthermore, both xanthone derivatives have not been detected in *CAN* and *C. racemosa* leaves. Between *C. eugenioides* and *ARA*, *C. eugenioides* leaves showed the highest content of total xanthenes (mangiferin and isomangiferin) with a mean of 37 mg/g of dry matter [10]. A study carried out by De Almeida et al. using LC-MS [29] demonstrated that the contents of flavonoids, CGAs, and xanthenes in Brazilian *ARA* leaves differ according to the re-

gion of origin. The highest content, 41 mg/g, was detected in leaves collected in Minas Gerais, whereas a lower content of polyphenols was detected in leaves harvested in São Paulo (25 mg/g) and Ceará in the Northeast of Brazil (10 mg/g).

Xanthenes: pharmacology and toxicity

As mentioned above, mangiferin is the main xanthone found in some coffee species, so, consequently, most of the studies described below focus on the pharmacological activity attributed to this molecule. Mangiferin has been described as a molecule useful in preventing inflammatory diseases, such as colitis, and myocardial infarction. Some neuroprotective and antidiabetic effects can also be attributed to mangiferin, but in both cases, the doses necessary to exert a sufficient effect cannot be ingested with coffee leaf tea. Studies performed on the chronic consumption of concentrated extracts will be required.

In vivo studies focusing on its efficacy for myocardial infarction have shown that pretreatment with 10 mg/100 g body weight of mangiferin may decrease the serum levels of marker enzymes such as CPK and LDH and transaminases in isoproterenol myocardial infarcted rats [97]. The benefits of mangiferin could be due to its antioxidant and free radical quenching effects and the increased inhibition of lipid peroxidation [98,99]. Xanthenes are also useful as a neuroprotective agent. Kavitha et al. [100] have proven the efficacy of xanthenes in preventing Parkinson's dis-



► **Fig. 2** The major biosynthetic pathway of diterpenes cafestol and kahweol. (1) copalyl phosphate synthase; (2) *ent*-kaurene synthase; (3) *ent*-kaurene oxidase. Adapted from Pot et al. [110]. [rerif]

ease. The administration of mangiferin for 14 days (10–40 mg/kg) may prevent dopamine depletion and behavioral deficits in mice treated with MPTP.

In diabetes, mangiferin may significantly decrease plasma glucose levels and simultaneously decrease the risk of developing diabetes complications such as diabetic nephropathy by modulating the MAPK, PKC isoforms, TGF- β 1 pathways, and NF- κ B signaling cascades involved in this pathophysiology [101, 102]. It has been shown that the administration of 40 mg/kg bodyweight for 30 days in streptozotocin-induced diabetic rats can decrease glucose levels from 395 mg/dL to 214 mg/dL. Moreover, the same mangiferin treatment could revert the increase of some nephrotoxicity markers such as plasma creatinine, plasma uric acid, and urinary albumin induced by streptozotocin.

Mangiferin may also prevent other inflammatory disorders such as colitis. Mangiferin (10 μ M) inhibited the expression of TNF- α , IL-1 β , and IL-6 by 81%, 89%, and 88%, respectively, in lipopolysaccharide (LPS)-treated mice. Furthermore, mangiferin prevented colon shortening, macroscopic score, and colonic-myeloperoxidase-activity in TNBS-induced colitic mice. Mangiferin could also inhibit TNBS-induced IRAK1 phosphorylation and NF- κ B activation [103].

Due to their anti-inflammatory activity, xanthones could also play an important role in asthma by reducing eosinophils and prostaglandin D2 in bronchoalveolar lavage fluid by downregulating the levels of cytokines/chemokines and increasing serum Th1-related cytokine expression [104].

Though xanthones are considered safe compounds, some toxic effects at high doses have been reported. A subchronic toxicity test that administered 10, 50, and 100 mg/kg BW/day mangosteen pericarp hydro-extract rich in xanthones to 20 rats for

3 months showed that the kidney of 1 rat had a protruded nucleus in the proximal convoluted tubule. Furthermore, the subjects of the 100 mg/kg BW/day treated group showed torn glomeruli, but the reason for these disorders has not yet been clarified. However, results could suggest that the safe dosage of the mangosteen pericarp hydro-extract would be less than 100 mg/kg BW/day [105]. The content of xanthone derivatives in the aqueous extract is 2–7 mg/g, while the methanol extract contains about 500 mg xanthones/g of extract (or 185 mg/g of dry matter) [106]. The content of mangiferin in the leaves of some coffee species is also about 100 mg/g of dry matter (► **Table 2**).

Diterpenes

Among diterpene derivatives found in coffee leaves, the *ent*-kaurene diterpenoids merit particular attention. These compounds were only found in coffee plant tissues, and the major ones are cafestol and kahweol (► **Fig. 2**) [107]. The plant could use terpenic compounds to respond to biotic or abiotic stress because the compounds improve the plant's defense mechanisms by acting as phytoalexins [108]. It has also been observed that terpenes might be useful to deter herbivores. To our knowledge, no information is available regarding the specific role of the main diterpenes cafestol and kahweol found in coffee plants [109].

Diterpenes: metabolism and turnover in coffee leaves

Little information concerning the biosynthesis of these compounds is available. Ferreira et al. [111] proposed 1 possible biosynthetic pathway for synthesizing cafestol and kahweol in coffee tissues (► **Fig. 2**). According to this pathway, the main precursor should be a unit of geranylgeranyl diphosphate (GGDP). Due to

the action of a copalyl phosphate synthase, GGDP may be converted into an *ent*-copalyl diphosphate (CDP). Another enzyme, the *ent*-kaurene synthase, can thus convert the CDP derivative to *ent*-kaurene. The last step is oxidation catalyzed by the *ent*-kaurene oxidase, which leads to the formation of the *ent*-kaurenoic acid. The conversion of the *ent*-kaurenoic acid in cafestol, kahweol, and their derivatives has not yet been clarified.

Ent-kaurane diterpenoids have been found in both the fruits and leaves of coffee plants, and their presence in the leaves might be crucial to discriminate several coffee species [112]. No detailed information about the turnover of these diterpenes in coffee leaves is available at the moment.

Diterpenes: differences between coffee species

Dias et al. conducted a study that explored the content of diterpenes in coffee leaves of *CAN* and *ARA* grown in Brazilian and evaluated cafestol and kahweol in coffee tissues by LC-UV/vis [30]. The concentration of cafestol was around 0.5 mg/g in *ARA* leaves and around 10 times less in *CAN* leaves. A negligible amount of kahweol was found only in the leaves of *ARA*. The class of diterpene derivatives found as discriminant metabolites by Souard et al. [112] might also include some atractyl, diketoatractyligenine, hydroatractylitriol derivatives, etc. Among the 9 species studied by our laboratory, these derivatives have not been detected in the leaves of *ARA*, *CAN*, and *C. anthonyi* [112].

Diterpenes: pharmacology and toxicity

Several healthy effects of coffee consumption are associated with the presence of cafestol and kahweol. Particularly, these compounds could exert a good anticarcinogenic and antidiabetic activity. It is important to clarify that the efficacy in diabetes has only been observed by administering high doses of diterpenes in animals. Moreover, relevant toxic effects have been attributed to some *ent*-kaurane diterpenes.

The anticarcinogenic activity linked to these compounds might be explained by their capacity to induce apoptotic cell death. Studies on kahweol (1.0–10 μ M) demonstrated that this compound might activate the mitochondrial caspase-dependent apoptotic pathway, down-regulate the anti-apoptotic proteins, and alter signals linked to Jun N-terminal kinase (JNK) involved in apoptosis [113]. Other studies showed that kahweol at a dose of 20–40 μ M might also downregulate the STAT3 signaling pathway and the expression of anti-apoptotic Bcl-2 family proteins in human lung adenocarcinoma A549 cells [114,115]. Another proposed mechanism includes the possibility for coffee diterpenes to inhibit angiogenesis involved in cancer growth and metastasis. Moeenfarid et al. [116] compared the anti-angiogenic effects of cafestol palmitate and kahweol palmitate in an *in vitro* angiogenesis model to demonstrate that both compounds at a concentration of 50 mM might inhibit angiogenesis on human microvascular endothelial cells by decreasing the VEGFR2 phosphorylation when compared to control.

Furthermore, anticarcinogenic activity has been explored *in vivo* by several authors. A study reported that rats fed with cafestol or kahweol palmitate (60 mg) for 3, 2, and 1 day before 7,12-dimethylbenz[a]anthracene administration developed fewer

mammary tumors after 4 months when compared with the control group [117].

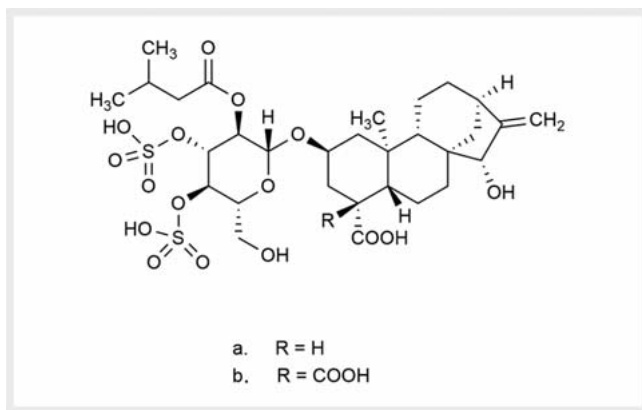
The antigenotoxic effect of these diterpenes has also been observed. Particularly, cafestol and kahweol may inhibit the covalent binding of aflatoxin B1 metabolites to DNA in microsomal subcellular fractions from livers of treated rats when they are fed with a 52.5:47.5 mixture of cafestol and kahweol (0–6200 ppm). Significant inhibition was detected at 2300 ppm, and maximal reduction of DNA adduct formation was detected with 6200 ppm of dietary cafestol and kahweol [118].

Cafestol can also exert an antidiabetic activity. Mellbye et al. [34] found that this compound can postpone the development of type-2 diabetes in mice. In this work, 47 male mice were randomly fed with food containing 1.1 (high), 0.4 (low), and 0 (control) mg of cafestol for 10 weeks. At the end of the experiment, it was found that fasting plasma glucose was about 30% lower in the cafestol groups than in the control group. Fasting glucagon was 20% lower, and insulin sensitivity improved by 42% in the high-cafestol group. Another study aimed to show how kahweol can improve glucose homeostasis *in vivo* [119]. Particularly, it was observed that upon administration of kahweol (100 mg/kg) in mice every 2 days, blood glucose clearance was faster than that of control mice. The same study showed that kahweol increases glucose uptake by activating AMPK (AMP-activated protein-kinase) as metformin.

Diterpenes present in the unsaponifiable fraction of coffee can also affect the levels of serum cholesterol. Weusten-Van der Wouw et al. [120] performed a human study with 15 volunteers who have consumed 0.75 g/day of coffee unsaponifiable fraction for 1 month. A cholesterol increase by 48 mg/dl was observed when compared to cholesterol levels of placebo. It was concluded that this effect was due to cafestol and kahweol because an administration of the oil deprived of these diterpenes had no effect. Nevertheless, the content of cafestol and kahweol of coffee leaves is very low (► **Table 1**). Consequently, ingestion of high amounts of coffee leaf extract would be necessary to observe these adverse effects. More studies would be necessary to conclude that a daily and chronic consumption of low amounts of coffee diterpenes does not represent a risk.

Among the *ent*-kaurane diterpenes, strong toxic effects have been highlighted for atractyl derivatives. One of the first studies reported fatal renal and hepatic necrosis cases in children after ingestion of herbal remedies containing these molecules [121]. Atr derivatives consist of 2 important chemical groups, an aglycone with a perhydrophenanthrene structure and a glycoside moiety (► **Fig. 3**). It has been found that the aglycone moiety (atractyligenin) of this class of compounds may have significant toxic effects on mitochondria. More specifically, they are considered a competitive inhibitor of the adenine nucleotide translocase, which is responsible for exchanging ADP and ATP across the mitochondrial inner membrane. The alteration of this pump results in a blockage of oxidative phosphorylation [122,123].

Atr can inhibit gluconeogenesis and fatty acid oxidation, but it can accelerate glycolysis and glycogenolysis. The result is a first acute hyperglycemic phase due to the acceleration of glycogenolysis followed by a marked hypoglycemic phase leading to depression of respiration, tissue hypoxia, convulsion, coma, and in severe



► **Fig. 3** Structures of atractyloside (a) and carboxyatractyloside (b). Adapted from Lang et al. [123]. [rerif]

cases, death. This class of compounds has a LD₅₀ ranging from 15 mg/kg (dogs, i.v.) to 434 mg/kg (mouse, i.p.) [124].

Even though these compounds are present in several herbal remedies and coffee plant tissues, high temperatures may reduce their toxicity. Chen et al. [125] showed that Atr could be disrupted by decomposition after heating with water for a long period (decoction). Furthermore, acidification might also accelerate the degradation process. It has been observed that, in some herbal medicines exposed to high temperatures (> 98 °C) during decoction and pH 2.3 for 2 h, Atr could be significantly reduced (40% of Atr was degraded). Consequently, these compounds can also be degraded during coffee roasting since high temperatures are necessary to perform this process [123]. Other studies proved that oxidation or light irradiation could also remove some of these compounds [126].

Coffee Leaf Extract Activities

Few studies on coffee leaf extract efficacy have been carried out, and most of them focused only on ARA. Segheto et al. [127] evaluated the inflammatory and antioxidant activity of coffee leaf extract and 5-caffeoylquinic acid in a mouse model. It has been shown that ear edema induced by croton oil was reduced after treatment with ARA leaf methanol extract and 5-caffeoylquinic acid. Particularly, tissue thickness passed from 157 µm to 33 µm after 0.1 mg extract/ear administration. Moreover, the ear weight was also monitored. An important difference between the control and the treated groups was observed (3 mg for the control group vs. 1 mg for the treated group). The administration of 0.1 mg/ear of 5-caffeoylquinic acid reduced the thickness from 154 µm to 70 µm after 24 h, and the ear weight was 1.7 mg. The methanol extract's 5-caffeoylquinic acid and mangiferin content was around 20 mg/g and 15 mg/g, respectively. Sixty-eight g of leaves were extracted with 1 L of methanol. Some information about CAN leaf extract is also available. Galam et al. [128] revealed that the aqueous extract exerts an important decrease of rat paw-induced edema. The 120 mg/kg dose produced the most significant decrease: from 20% at 20 minutes to 50% at 120 minutes. The dose that should be administered to an adult of 70 kg might be

around 8 g to get a possible benefit. However, the same study was not carried out on humans; consequently, any conclusions are premature.

Chen et al. [20] performed *in vitro* studies to evaluate the antioxidant and the anti-inflammatory activities of coffee leaves processed by different methods to make different styles of tea. They observed that the Japanese-style green tea-process mature leaves (JGTP-M) resulted in the extract with the highest total polyphenols content. In contrast, black-tea-process mature coffee leaves (BTP-M) was the method providing the extracts with the lowest content of polyphenols. The highest polyphenolic content of the extracts made by JGTP-M was also correlated with their highest anti-inflammatory activity. For example, among the various performed tests, the nitric oxide inhibitory activity was tested. Results showed that the IC₅₀ for JGTP-M extracts after 0.5 min infusion was 1.7 against 3.0 mg/mL for BTP-M extracts made with the same infusion duration. Another *in vitro* study was performed by Chiang et al. [129] to investigate how ARA leaf extract can prevent photoaging by suppressing matrix metalloproteinase (MMPs) expression and MAP kinase pathway. Five, 10, and 25 µg/mL of coffee leaf extracts could inhibit MMP-1, -3, and -9 by exerting a dose-dependent inhibition compared to the control group's MMPs levels. In a UVB-induced expression of MAP kinases model performed on human fibroblasts, coffee leaf extract at 5, 10, and 25 µg/mL inhibited JNK phosphorylation in a dose-dependent manner. Moreover, a dose of 25 µg/mL suppressed the ERK activation. It was also shown that a dose of 5 and 10 µg/mL of coffee leaf extract but not 25 µg/mL inhibited the p38 phosphorylation.

Conclusion

Although coffee beans have been widely studied, this review shows that coffee leaves could also be an important source of the 4 main classes of secondary metabolites found in the *Coffea* genus. For this reason, coffee leaf extracts might be used as an important feedstock in the nutraceutical industry. Additionally, the levels of some metabolites such as xanthone derivatives were found to be higher in coffee leaves and lower or absent in green and roasted coffee beans, and the content of some purine alkaloids such as caffeine, to which stimulant effects are attributed, was found tendentially lower in coffee leaves compared to *Camellia sinensis* tea leaves or coffee beans. The *in vitro* and *in vivo* activities of metabolites found in coffee leaves were observed at higher concentrations than those present in leaf water extracts. However, less is known about chronic consumption of coffee leaf water extracts and their potential benefits. If beneficial effects are to be highlighted, future formulations of new dietary supplements in tablet or capsule form may be a promising future perspective. However, if concentrated coffee leaf extracts have to be used in daily life as a dietary supplement, special attention should be paid to the presence of some toxic metabolites, and additional studies on the degradation process of these compounds should be carried out. This suggests that it would be preferable, in the first instance, to perform more quality control studies on coffee leaves to ensure the safe consumption of products made with its extracts.

Therefore, due to the strong biochemical differences between coffee species, more studies would be necessary to employ different coffee species other than *ARA* and *CAN* and clarify the potential benefits of their leaf extract.

Contributors' Statement

Conceptualization: A. Montis. Critical revision of the manuscript: F. Souard, C. Delporte, P. Stoffelen, C. Stévigny, P. Van Antwerpen.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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