Acta Pharmaceutica Sinica B 2013;3(1):8–19

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Research progress in the phytochemistry and biology of *Ilex* pharmaceutical resources

Dacheng Hao[a,b,*], Xiaojie Gu[a], Peigen Xiao[c,*], Zhanguo Liang[a], Lijia Xu[c], Yong Peng[c]

[a]*Biotechnology Institute, School of Environmental and Chemical Engineering, Dalian Jiaotong University, Dalian 116028, China*
[b]*Department of Metabolic Biology, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK*
[c]*Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine of Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, China*

Received 16 September 2012; revised 2 November 2012; accepted 30 November 2012

KEY WORDS

Ilex;
Chemical components;
Pharmacology;
Chemotaxonomy;
Phylogeny;
Pharmaceutical and food resource

Abstract *Ilex* is a botanical source for various health-promoting and pharmaceutically active compounds that have been used in traditional Chinese medicine and food for thousands of years. Increasing interest in *Ilex* pharmaceutical and food resources has led to additional discoveries of terpenoids, saponins, polyphenols (especially flavonoids), glycosides, and many other compounds in various *Ilex* species, and to investigation of their chemotaxonomy, molecular phylogeny and pharmacology. In continuation of our studies on *Ilex* pharmacology and phylogeny, we review the phytochemistry, chemotaxonomy, molecular biology and phylogeny of *Ilex* species and their relevance to health-promotion and therapeutic efficacy. The similarity and dissimilarity between *Ilex paraguariensis*, the source plant of mate tea, and the source plants of large-leaved Kudingcha (e.g., *Ilex kudingcha* and *Ilex latifolia*) are discussed. It is essential to utilize emerging technologies in non-*Camellia* tea studies to promote the sustainable utilization of *Ilex* resources and the identification and development of novel compounds with potential health and clinical utility. Systems biology and "-omics" technologies will play an increasingly important role in pharmaceutical and food research on the bioactive compounds of *Ilex* species.

*Corresponding authors.
E-mail addresses: hao@djtu.edu.cn (Dacheng Hao); xiaopg@public.bta.net.cn (Peigen Xiao).
Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.apsb.2012.12.008

1. Introduction

Ilex, whose common name is holly, is the only living genus of almost 600 species in the family Aquifoliaceae[1]. The species are evergreen and deciduous trees, shrubs and climbers from tropics to temperate regions worldwide. Various *Ilex* species are utilized by worldwide ethnic groups to make non-*Camellia* tea drinks for daily consumption and health promotion[2]. Mate tea from *I. paraguariensis* originated from the southern part of South America (Brazil, Argentina, Uruguay and Paraguay) and is now a popular health-promoting drink in western countries[3]. Large-leaved Kudingcha (bitter spikeleaf tea) is made from *I. kudingcha* and *I. latifolia* and has been consumed as a functional food in southern China for about 2000 years[2] (Table 1). Various compounds have been isolated from *Ilex* plants since the 1980's, and their chemistry and pharmacology has been reported[2,4]. Polyphenol constituents of green tea, mate tea and large-leaved Kudingcha are listed in Table 2. Studies on the chemotaxonomy and molecular phylogeny have been carried out to facilitate further conservation and exploitation of *Ilex* pharmaceutical and food resources[1,5]. In this brief review, we summarize the recent progress in phytochemical and biological research of *Ilex* plants.

2. Molecular taxonomy, molecular phylogeny, and genomics

The evolutionary history and diversification of the genus *Ilex*, comprised of 108 species, is deduced by analyzing two nuclear (ITS and *nepGS*) and three plastid (*rbcL*, *trnL-F* and *atpB-rbcL*) sequences[1]. Nuclear and plastid trees are highly dissimilar and the nuclear tree is more attuned with current taxonomic classifications. Many Chinese species, including *Ilex cornuta*, *Ilex zhejiangensis*, *Ilex integra*, *Ilex hylonoma*, *I. latifolia*, *Ilex pernyi*

Table 1 Traditional use of green tea and four kinds of non-*Camellia* teas

Name	Original region	Use history	Main application region	Traditional use
Camellia sinensis (green tea)	South of the Yang Tze River, China	Around 3000 years	Worldwide, raw materials for drink, food, cosmetic and health care products	Sweet and bitter taste, cool in property, refreshing, reduce stress and thirst, reduce phlegm, help digestion, diuresis, detoxify, treat headache, blurred vision, somnolence, dyspepsia, dysentery
Ilex paraguariensis (Mate tea)	South America	300–400 Years	drink and health care products in America, Europe and Asia	Hepatic protection, help digestion, anti-rheumatism, arthritis and inflammation, anti-obesity, hypertension and hypercholesterolemia
I. latifolia, *I. kudingcha* (large-leaved Kudingcha)	South of the Yang Tze River, China	Around 2000 Years	Tea drink in China and southeast Asia	Sweet and bitter taste, cold, liver meridian, lung meridian and stomach meridian, heat clearing, relieve summer-heat, smooth the liver, dispel wind, improve eyesight, help produce saliva, treat sunstroke and high fever, headache, toothache, red eye, aphtha, polydipsia, diarrhea, dysentery, mastitis, relieve swelling and pain, astringency and hemostasis
Ligustrum robustum (*L. purpurascens*, small-leaved Kudingcha)	Southwest China	Since ancient times	Tea drink and health care products in China and southeast Asia	Slightly bitter taste, sweet, slightly cold, heat clearing, detoxifying, stress-reducing, relieve swelling and pain, anti rheumatism and scald, furuncle and arthralgia therapy
Litsea coreana (eagle tea)	Guizhou, Sichuan Of China	Since ancient times	Southwest China	Detoxify, detumescence, improve eyesight, help produce saliva, decrease thirst, prevent sunstroke

Table 2 Polyphenol constituents of green tea, mate tea and large-leaved Kudingcha

Name	Total polyphenol	Caffeine	Total flavonoid(%)	Total saponin
Green tea	30%	1–4%	>0.4	NR
Mate tea	More abundant than green tea 178.32 mg/g spray-dried extract	0.7–2%	>1	0.35 g/L
Large-leaved Kudingcha	2–6%	No or trace (0.228-0.436 μg/g)	9–12	>20%
Small-leaved Kudingcha	20–30%	NR	>5	–
Eagle tea	>30%	NR	>10	>1%

Modified from Ref. 2. NR: not reported.

and *Ilex ficoidea*, are in the Aquifolium clade. *I. latifolia*, *I. pernyi* and *I. ficoidea* are closer to *I. hylonoma* than to *I. cornuta*, an adulterant of large-leaved Kudingcha. *Ilex pubescens* of Rotunda alliance is closer to Yunnanensis alliance than to Aquifolium. The Repandae clade includes *I. paraguariensis* and *Ilex vomitoria*, which are basal to the above taxa. *I. latifolia* was found to be closer to *Ilex liukiuensis* than to *I. cornuta*[6]. However, these studies did not include *I. kudingcha* and *Ilex pentagona*. We sequenced 11 chloroplast noncoding regions for five *Ilex* species[7]. *I. latifolia*, *I. kudingcha*, *I. cornuta* and *I. pentagona* intermingled in a cluster and cannot discriminate these species in the chloroplast phylogenetic tree. *I. paraguariensis* is basal to these four Chinese species. The significant discrepancy between nuclear and chloroplast phylogenies might be due to intraspecific polymorphism, hybridization and introgressions[1]. The most recent common ancestor (MRCA) of extant species is dated from the Miocene, while the fossil records suggest that the *Ilex* stem lineage originated in the late Cretaceous, implying extensive lineage extinctions between the Cretaceous and Miocene and explaining the difficulties in clarifying the relationships between *Ilex* and its closest relatives[1]. The MRCA ancestral area is in North America and/or East Asia. Several bidirectional North America/East Asia and North America/South America dispersal events are proposed to explain observed geographic and phylogenetic patterns. Hybridization and introgression events between distantly related lineages are inferred, indicating weak reproductive barriers between *Ilex* species. This characteristic might be useful in selecting cultivars with desired pharmaceutical substances. ISSR (Inter-simple sequence repeat), RAPD (random amplification of polymorphic DNA) and AFLP (amplified fragment length polymorphism) markers have been used to guide interspecific hybridization and germplasm improvement[8–10]. Population studies on *I. paraguariensis*[11], *Ilex aquifolium*[12], *Ilex leucoclada*[13], *Ilex canariensis*[14] and *I. cornuta*[15] reveal very high population diversity and polymorphism. More studies are needed at population or species level to ensure a better characterization of *Ilex* species.

Representational difference analysis (RDA) was adopted to screen *I. paraguariensis* genomes[16]. The occurrence of sex-related genomic differences was investigated to develop an early gender detection molecular method that could reduce energy requirements during mate tea processing, as well as be helpful in breeding programs. Fragments isolated in RDA assays fall into three categories: the first category is specific to *I. paraguariensis;* the second category comprises sequences identified as organellar or ribosomal plant DNA; the third category consists of clones representing conserved domains of retrotransposons (RNaseH, integrases and/or chromodomains), including Ty3/Gypsy retrotransposons and Ty1/Copia retroelements, which are associated with the sex determination regions of the Solanaceae, Caricaceae and Salicaceae families. These sequences could be used in phylogenetic analysis and species authentication. RDA and other powerful methods, such as fosmid genomic library[17] and second generation high throughput sequencing[18–20] could be of great help in gaining insight into *Ilex* genome structure and function, as well as regulation of gene expression and pharmaco-phylogeny.

3. Chemical components of the genus *Ilex* and their biological activities

3.1. *Terpenoids and triterpenoid saponins*

Most triterpenoid compounds in medicinal plants are saponin glycosides with the attachment of various sugar molecules to the triterpene unit. These sugars can be easily released in the gut by bacteria, allowing the aglycone (triterpene) to be absorbed. Terpenoid molecules could insert into cell membranes and modify the composition, influence membrane fluidity, and potentially affect signaling by many ligands and cofactors[21]. Both large-leaved Kudingcha and mate tea contain abundant saponins[2,22,23] (Tables 2 and 3). The newly discovered saponins and terpenoids are summarized in Figs. 1 and 2 and Tables S1 and S2 (see supplementary component).

3.1.1. *Anti-inflammatory activity*

Triterpenoid saponins from *Ilex mamillata*, endemic in Yunnan and Guangxi, China, showed inhibitory activities in an anti-inflammatory assay[24]. The saponin fraction from the ethanolic extracts of the root of *I. pubescens* exhibited potent anti-inflammatory effects on carrageenan-induced paw edema in rats[25]. A purified saponin fraction derived from the root of *I. pubescens* showed anti-inflammatory and analgesic activities[26]. The molecular mechanisms might be associated with inhibition of the elevated expression of cyclooxygenase (COX)-

Table 3 Major components of non-*Camellia* tea and green tea

Component	Green tea	Mate tea	Large-leaved Kudingcha
Caffeine	+	+	
Theobromine	+	+	
Theophylline	+	+	
Chlorogenic acid		+	+
Caffeic acid	+	+	
Quinic acid	+	+	
Caffeoyl derivative		+	+
Coumaric acid	+		
Feruloylquinic acid		+	
Gallic acid	+		
Caffeoyl shikimic acid		+	+
Gallocatechin gallate	+		
Catechin (C)	+		+
Epigallocatechin (EGC)	+		
Epigallocatechin gallate (EGCG)	+		
Epicatechin (EC)	+		+
Catechin gallate (CG)	+		+
Epicatechin gallate (ECG)	+		+
Gallocatechin gallate (GCG)	+		
Gallocatechin (GC)	+		
Kaempferol	+	+	+
Myricetin	+		+
Quercetin	+	+	+
Rutin	+	+	+

Modified from Ref. 2.

2 protein and the overproduction of the proinflammatory cytokines, as well as augmentation of the anti-inflammatory cytokines IL-4 and IL-10 in the carrageenan-injected paw tissues of rats. Saponins in mate tea and quercetin synergistically inhibit iNOS and COX-2 in lipopolysaccharide-induced macrophages through NFκB pathways[27].

3.1.2. Modulation of lipid metabolism and anti-obesity activity

Triterpenoid saponins from the leaves of *I. kudingcha* inhibit aggregated LDL-induced lipid deposition in macrophages[28]. The *I. kudingcha* total saponins may have a significant therapeutic application in hypercholesterolemia and atherosclerosis, as they improve abnormal hemorheological para-

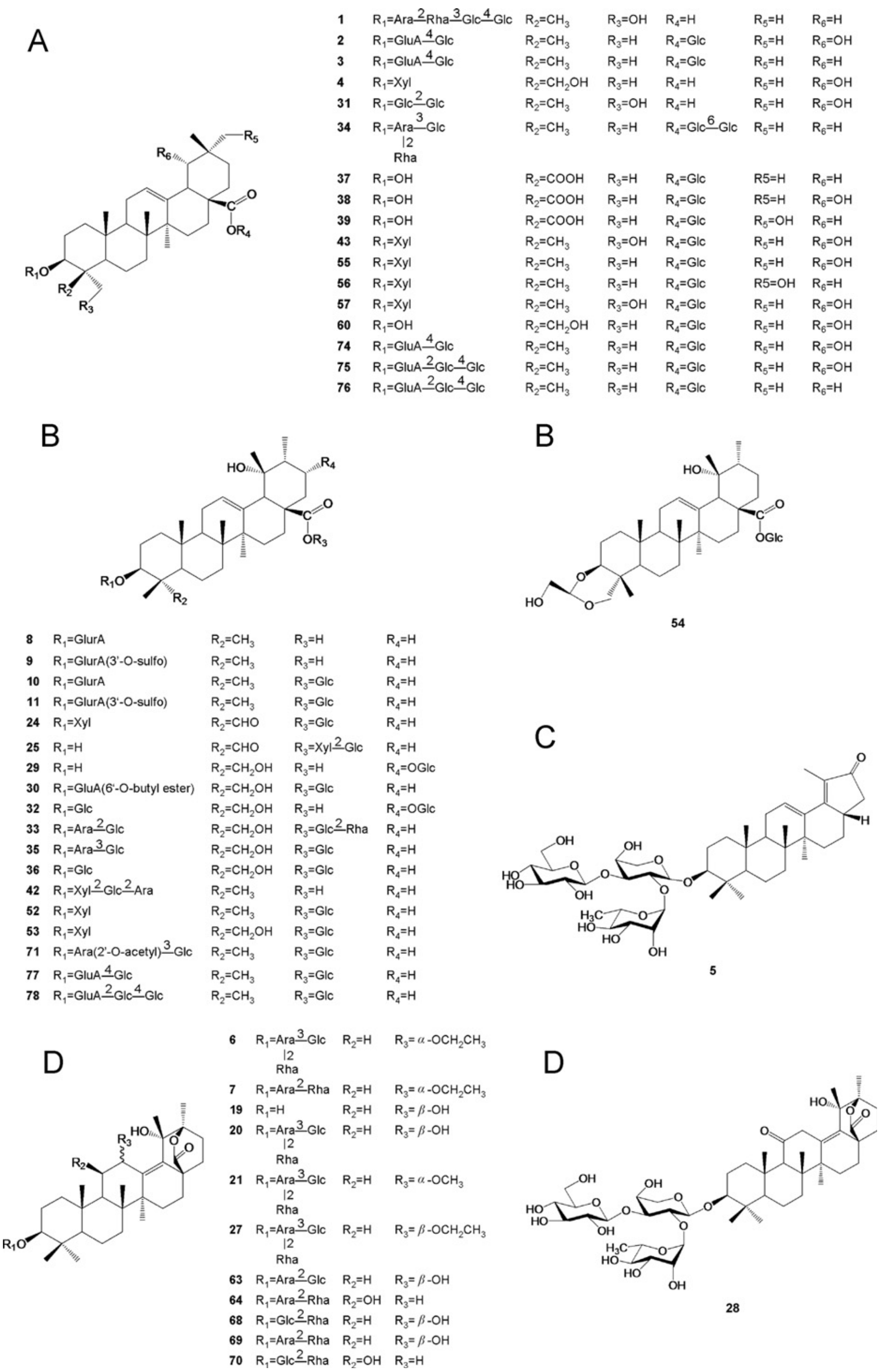

Figure 1 Triterpenoid saponins of *Ilex*. (A) oleanolic acid type; (B) ursolic acid type; (C) 3β-hydroxy-12(13),18(19)-diene-20-one-kudinone type; (D) kudinchalactone type; (E) kudinlactone type; (F) 3β-hydroxy-urs-12,18-diene-28-oic acid type; (G) 3β-hydroxy-urs-12,19-diene-28-oic acid type; (H) 3β-hydroxy-urs-12,19(29)-diene-28-oic acid type; (I) ilexgenin B type; (J) heterobetulinic acid type; (K) 3β,19α-dihydroxy-urs-12-en-28,20-lactone type; (L) ulmoidol type; (M) 3β,19α,23-trihydroxy-urs-12,20(30)-dien-28-oic acid type.

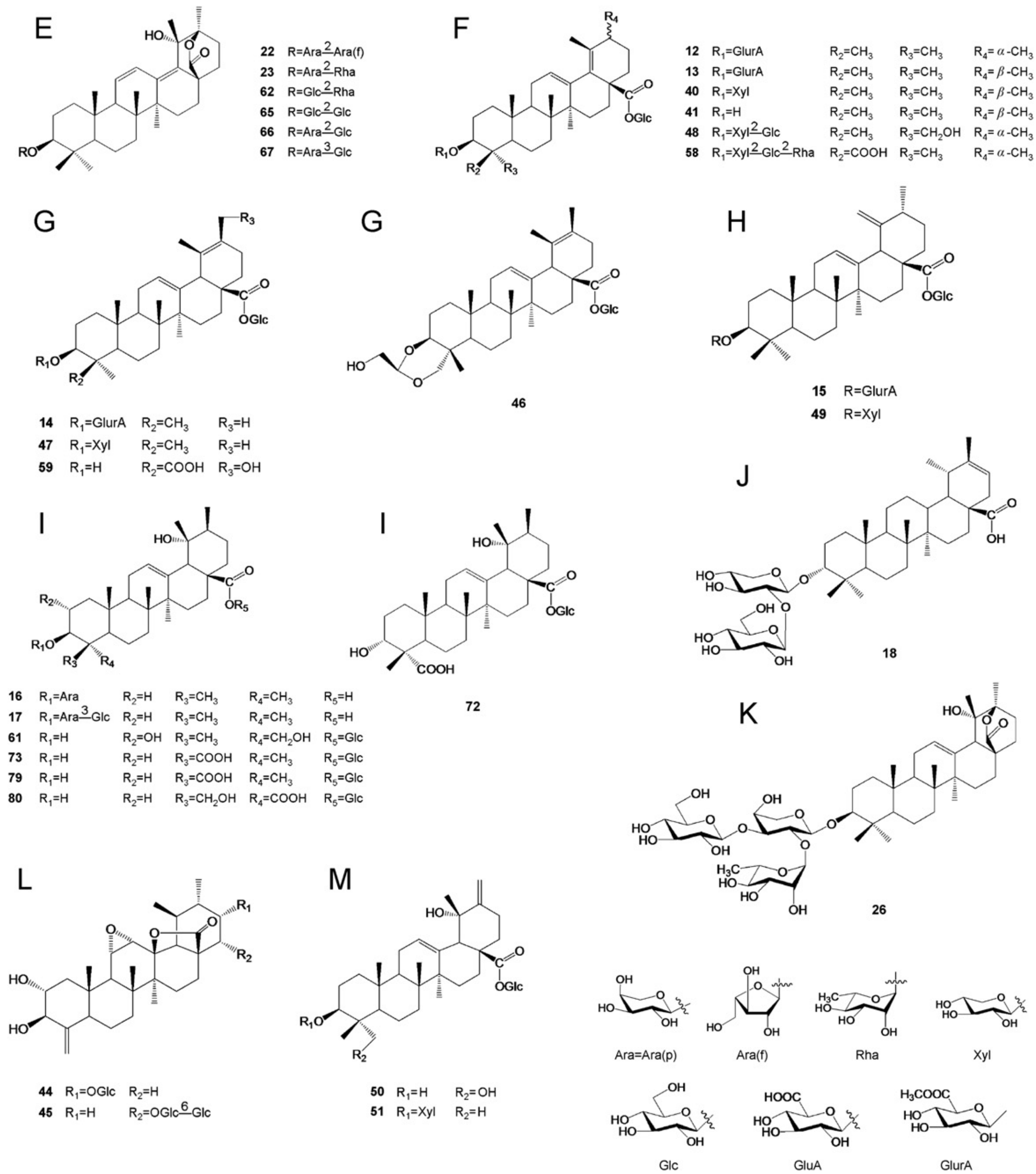

Figure 1 Continued.

meters in ApoE$^{-/-}$ mice[29]. Several triterpene saponins and monoterpene oligoglycosides were found to exhibit potent inhibitory activities and these compounds appear to be responsible for the antiobesity activity of mate tea[30]. Lupeol and betulin isolated from *Ilex macropoda* inhibit the activity of human Acyl-CoA: cholesterol acyltransferase-1 and -2 in a dose-dependent manner[31].

3.1.3. Anti-microbial activity

Monodesmosidic triterpenoid saponins, matesaponin-1 (a bidesmosidic saponin), caffeic and chlorogenic acids, and rutin, extracted from *I. paraguariensis*, synergistically inhibited herpes simplex virus types 1 and 2 replication[32]. Four triterpenoid saponins of the ursane type isolated from the leaves of *Ilex oblonga* showed appreciable inhibitory activity against tobacco mosaic virus replication[33,34]. Four triterpenoid saponins of *I. kudingcha* showed antibacterial activities against *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus*[35].

3.1.4. Anti-parasitic activity

Ursolic acid (a pentacyclic triterpene acid) derivatives inhibit hydrogen peroxide- and glutathione-mediated degradation of hemin, providing an additional mechanism of action for antimalarial activity[36]. Triterpenoid glycosides isolated from the leaves of *Ilex affinis* and *Ilex buxifolia*, two adulterant species of *I. paraguariensis*, showed antitrypanosomal activity *in vitro*[37].

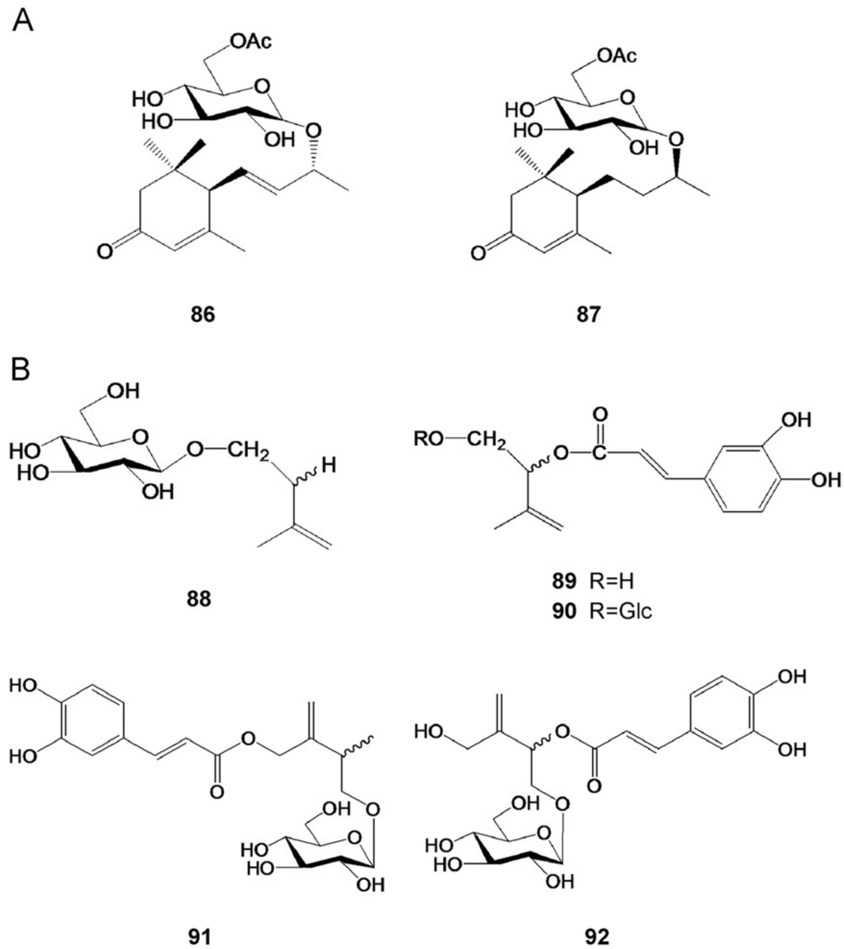

Figure 2 Terpenoids of *Ilex*. (A) megastigmane type; (B) hemiterpene type.

3.1.5. Other effects

Non-hemolytic saponins purified from *Ilex dumosa*, *Ilex argentina* and *I. paraguariensis* showed an alum-type adjuvant effect, *i.e.*, immunostimulating potential[38]. Hemiterpene glucosides from *I. pubescens* showed anti-platelet aggregation activities[39]. Two acetylated megastigmane glycosides of *I. paraguariensis* exhibited HNE inhibitory activity[40].

3.2. Polyphenols, flavonoids and phenolic glycosides

Phenolics are broadly distributed in plants and are the most abundant secondary metabolites. Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their significant effects in the prevention of multiple oxidative stress-associated diseases such as cancer. The common polyphenol components of *Ilex* are listed in Table 3. The newly discovered flavonoids and phenolic glycosides are summarized in Figs. 3 and 4 and Tables S3 and S4 (see supplementary component).

3.2.1. Modulation of lipid metabolism

The polyphenol extract from the dried leaves of *I. paraguariensis* was the most effective in inhibition of triglyceride accumulation in 3T3-L1 adipocytes, and rutin (100 μg/mL) likely accounted for a large portion of this activity[41]. Additionally, polyphenol extracts had a modulatory effect on the expression of genes related to adipogenesis such as PPARγ2, leptin, TNF-α and C/EBPα. Chlorogenic acid protects paraoxonase 1 activity in high density lipoprotein (HDL) from inactivation caused by physiological concentrations of hypochlorite in humans[42].

3.2.2. Anti-oxidant activity

High performance liquid chromatography (HPLC) analysis showed that 4,5-dicaffeoylquinic acid is the major component of the phenolic fraction of mate powder[43]. Among the extracts prepared with different solvents, the 80% methanol extract showed the highest total polyphenol content (11.51 g/100 g) and antioxidant activity. Organic extracts containing phenolic antioxidants might be used as natural antioxidants by the food industry, replacing the synthetic phenolic additives currently used[44]. Phenolic (*e.g.*, chlorogenic acid) and xanthine compounds (*e.g.*, caffeine) of *I. paraguariensis* are capable of preventing hydrogen peroxide-induced red blood cell lysis[45]. Caffeoylquinic acid-derived free radicals, identified by electron paramagnetic resonance (EPR) spectroscopy during antioxidant reactions of bitter tea (*I. latifolia* and *I. kudingcha*), have sufficient stability for biological activity, and contribute appreciably to the antioxidant chemistry of large-leaved Kudingcha[46]. Major phenolics in *I. kudingcha* and *I. cornuta* were mono- and dicaffeoylquinic acids, whereas those in *Ligustrum robustum*, i.e., the popular small-leaved Kudingcha

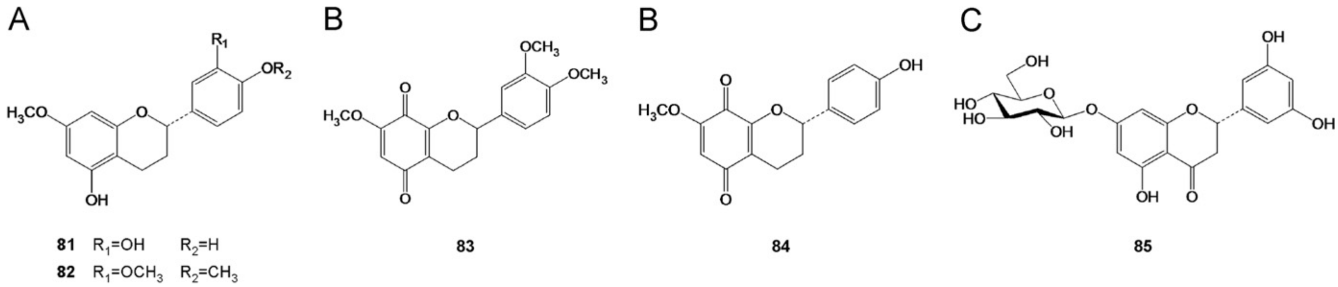

Figure 3 Flavonoids of *Ilex*. (A) flavanol type; (B) 5,8-quinoflavan type; (C) flavanone type.

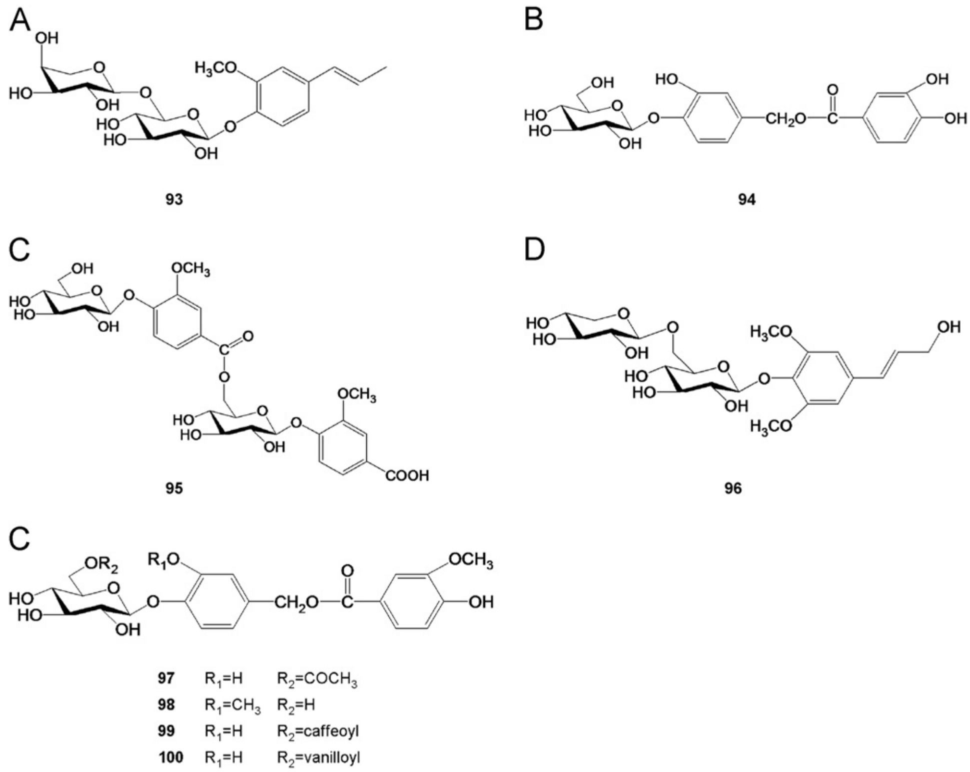

Figure 4 Phenolic glycosides of *Ilex*. (A) phenylpropanoid type; (B) 3,4-dihydroxy-benzoic acid type; (C) vanillic acid type; (D) syringinic acid type.

of southwest China, were phenylethanoid and monoterpenoid glycosides[47]. *Ilex* exhibited significantly stronger antioxidant capacities than *Ligustrum*. Huazhongilexone-7-*O*-*β*-*D*-glucopyranoside, a novel flavanone from *Ilex hainanensis*, showed antioxidant activity[48]. Two antioxidant phenylacetic acid derivatives, 2,4-dihydroxyphenylacetic acid and 2,4-dihydroxyphenylacetic acid methyl ester were isolated from the seeds of *I. aquifolium*[49]. In most cases, the DPPH spectrophotometric assay was used for detecting antioxidant activity.

3.2.3. Anti-diabetic activity

The leaves of *I. kudingcha* are used as an ethnomedicine in the treatment of diabetes mellitus and obesity in China. An "active components" group, consisting of three dicaffeoylquinic acids and three novel triterpenoid saponins, significantly reduced the elevated levels of serum glucose and lipids in type II diabetic mice[50]. Litseaefoloside C, a phenolic glycoside of *Ilex litseaefolia*, showed inhibitory activities *in vitro* for α-glucosidase and lipase[51]. Dicaffeoylquinic acids and matesaponins dramatically increased the satiety marker glucagon-like peptide 1 level and induced anorexic effects in mice[52]. Caffeic and chlorogenic acids in *I. paraguariensis* extracts are the main inhibitors of advanced glycation end product generation by methylglyoxal in the model proteins bovine serum albumin and histones[53].

3.2.4. Anti-inflammatory and anti-cancer activities

3,4,5-Tricaffeoylquinic acid inhibits tumor necrosis factor-α-stimulated production of inflammatory mediators in keratinocytes *via* suppression of Akt- and NF-κB-pathways[54]. Dicaffeoylquinic acids in mate tea inhibit NF-κB nucleus translocation in macrophages and induce apoptosis by activating caspases-8 and -3 in human colon cancer cells[55]. Flavonol-rich fractions of *I. vomitoria* leaves induce microRNA-146a and have anti-inflammatory and chemopreventive effects in intestinal myofibroblast CCD-18Co cells[56]. Epigallocatechin gallate showed greater cytotoxicity than quercetin and gallic acid against HepG2 cells[57].

3.2.5. Other effects

Rutin and caffeoylshikimic acid inhibit the extracellular glucosyltransferase activity from *Streptococcus mutans* and prevent the development of dental caries[58]. Biologically, elastase activity significantly increases with age, which results in a reduced skin elasticity and in the appearance of wrinkles or stretch marks. Dicaffeoylquinic acid derivatives and flavonoids of *I. paraguariensis* exhibited potent human neutrophil elastase (HNE) inhibitory activity[59].

3.3. Purine alkaloids

Stimulant properties of mate tea are attributed to methylxanthines, such as caffeine[43]. Despite its antioxidant capacity and well-known health benefits, mate tea has been shown to possess some genotoxic and mutagenic activities and to increase the incidence of some types of cancer[60]. Caffeine is one of the most abundant compounds found in the dry mass of mate (Table 2), and it was found that caffeine manifests more potent cyto- and genotoxic effects that may account, at least in part, for the detrimental effects observed for mate extract[60]. In contrast, large-leaved Kudingcha contains only trace amount of caffeine while polyphenol and flavonoids are abundant (Tables 2 and 3).

4. Pharmacology of *Ilex* tea and extracts

4.1. Effects on lipid metabolism and cardiovascular disease

The ingestion of mate tea, independent of dietary intervention, increased plasma and blood antioxidant protection in patients with dyslipidemia[61]. Consumption of mate improves serum lipid parameters in healthy dyslipidemic subjects and provides an additional LDL-cholesterol reduction in individuals on statin therapy[62], which may reduce the risk of cardiovascular diseases. Mate tea inhibits *in vitro* pancreatic lipase activity and has a hypolipidemic effect on high-fat diet-induced obese mice[63]. Unprocessed mate tea reduces fat more efficiently but produces a greater increase in blood glucose when compared to commercial mate tea[64]. Dietary supplementation with *I. latifolia* significantly decreased plasma total cholesterol levels and circulating immune complexes and increased HDL cholesterol in rats fed a high-cholesterol diet[65]. *I. latifolia*, similar to *C. sinensis* (green tea), could be used as a food supplement to protect against the development of hypercholesterolemia.

4.2. Anti-diabetic and anti-obesity effects

Mate extract has potent anti-obesity activity *in vivo*. Additionally, mate extract had a modulatory effect on the expression of several genes related to obesity[66]. *I. paraguariensis* extracts inhibit advanced glycation end-product formation more efficiently than green tea[67]. Mate extract has the ability to decrease the differentiation of pre-adipocytes and to reduce the accumulation of lipids in adipocytes, both of which contribute to a lower growth rate of adipose tissue, lower body weight gain, and obesity[68]. Mate tea consumption improved the glycemic control and lipid profile of type II diabetes mellitus (T2DM) subjects, and mate tea consumption combined with nutritional intervention was highly effective in decreasing serum lipid parameters of pre-diabetic individuals, which may reduce their risk of developing coronary disease[69]. Oral administration of mate (100 mg/kg) for seven weeks induced significant decreases in body weight, body mass index, and food intake in obese diabetic mice[70]. Mate extract ameliorates insulin resistance in mice with high fat diet-induced obesity[71]. Mate aqueous extract decreases intestinal SGLT1 (sodium–glucose linked transporter) gene expression but does not affect other biochemical parameters in alloxan-diabetic Wistar rats[72].

4.3. Antioxidant and anti-inflammatory effects

Ethanol extracts of *Ilex centrochinensis*, an endemic species in southern China, possess significant anti-inflammatory and free radical scavenging activities, whereas those of *Ilex ficoidea*, another Chinese species, exhibited a negligible anti-acute inflammatory and a moderate anti-chronic inflammatory activity[73]. Mate tea possesses potent antioxidant effects against hydroxyl and superoxide radicals in both chemical and cell culture systems, as well as DNA-protective properties[74]. The daily rectal application of enemas containing an aqueous extract of *I. paraguariensis* decreases oxidative tissue damage in the colon without fecal stream regardless of the time of irrigation[75]. After the supplementation period with mate tea, lipid peroxidation was acutely lowered, an effect that was maintained after prolonged administration. Total antioxidant status and the level of antioxidant enzyme gene expression were demonstrated after prolonged consumption[76]. Regular consumption of mate tea may increase the antioxidant defenses of the body by multiple mechanisms. Ingestion of mate tea increased LDL resistance towards *ex vivo* copper oxidation[77]. The ethanol extract of *I. latifolia* ameliorated ischemic injury induced by middle cerebral artery occlusion/reperfusion in rats, and this neuroprotective effect might be associated with its anti-apoptotic effect, resulting from anti-oxidative and anti-inflammatory actions[78]. An Argentinean *Ilex* specie, *Ilex brevicuspis*, has choleretic, intestinal propulsion and antioxidant activities, which may lead to the potential development of a new tea product and/or phytopharmaceutical products, without central nervous system stimulant activity[79].

4.4. Anti-microbial effects

Aqueous extracts of mate have antimicrobial activity against *Escherichia coli* O157:H7 and *S. aureus*[80,81]. The aqueous extract of *I. paraguariensis* (1,000 mg/mL) displays inhibitory activity against fungus *Malassezia furfur*[82].

4.5. Other effects

Mate consumption is associated with higher bone mineral density in postmenopausal women[83]. Acute administration of hydroalcoholic extract of *I. paraguariensis* differentially modulates short- and long-term learning and memory in rats probably through its antagonistic action on adenosine receptors, which partly substantiate the traditional use of mate tea for improvement of cognition[84]. *I. pubescens* extracts decreased significantly the number of escape failures relative to the control and showed antidepressant effects[85]. Mate tea, ardisia tea (*Ardisia compressa*) and green tea are cytotoxic to HepG2 cells, with mate tea demonstrating dominant cytotoxicity[37]. The cytotoxic activity and the inhibition of topoisomerase II may contribute to the overall chemopreventive activity of mate tea and ardisia tea extracts. Mate and ardisia teas may be used as chemopreventive agents.

5. Chemotaxonomy of *Ilex*

I. paraguariensis is one of the most commercialized plants of South America and grows naturally or in cultivation in Argentina, Uruguay, Brazil and Paraguay, where other *Ilex* species, such as *I. brevicuspis*, *I. buxifolia*, *I. affinis* and *Ilex theezans*, are also distributed. HPLC was used to distinguish the adulterant species from *I. paraguariensis*[79]. Novel triterpenoid glycosides detected in *I. buxifolia* and *I. affinis* confirm the structural specificity of the *I. paraguariensis* triterpenoids and reinforce the proposal to detect mate adulteration by triterpenoid analysis[37]. Arbutin-2′-sulphonyl could be a discriminating metabolite of *I. theezans*[86]. However, the metabolic similarities between species which can be used for chemotaxonomy of the species are not yet clear.

The metabolomic analysis of 11 *Ilex* species, *I. argentina*, *Ilex brasiliensis*, *I. brevicuspis, Ilex dumosa* var. *dumosa*, *I. dumosa* var. *guaranina*, *Ilex integerrima*, *Ilex microdonta*, *I. paraguariensis* var. *paraguariensis*, *Ilex pseudobuxus*, *Ilex taubertiana* and *I. theezans*, was carried out by nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis[87]. *Ilex* samples can be discriminated by the principal component analysis (PCA) and classification of the ^{1}H NMR spectra based on the phytochemicals present in the organic and aqueous fractions. Notably, in the classification of metabolites obtained from aqueous fractions, no species overlapped. The major metabolites that contribute to the discrimination are arbutin, caffeine, phenylpropanoids (e.g., caffeic acid, chlorogenic acid and dicaffeoylquinic acid) and theobromine. For the first time, arbutin is reported as an ingredient of *Ilex* species, and is found to be a biomarker for *I. argentina*, *I. brasiliensis*, *I. brevicuspis*, *I. integerrima*, *I. microdonta*, *I. pseudobuxus*, *I. taubertiana* and *I. theezans*. There is a clear discrimination between *I. paraguariensis* and other species. The method based on the determination of multiple metabolites illustrates the feasibility of chemotaxonomical analysis of *Ilex* species.

Numerous metabolites including saponins and phenylpropanoids have been reported from *Ilex* species. Based on the above study, 1D (dimensional)- and 2D-NMR-based metabolomics was used to classify 11 South American *Ilex* species[5]. ^{1}H NMR combined with PCA, partial least square-discriminant analysis (PLS-DA) and hierarchical cluster analysis (HCA) showed a clear separation between species and resulted in four groups based on metabolomic similarities. The signal congestion of ^{1}H NMR spectra was overcome by the implementation of 2D-*J*-resolved and heteronuclear single quantum coherence. Species included in group A (*I. paraguariensis*) were characterized by a higher amount of xanthines, and phenolics including phenylpropanoids and flavonoids; group B (*I. dumosa* var. *dumosa* and *I. dumosa* var. *guaranina*) with oleanane type saponins; group C (*I. brasiliensis*, *I. integerrima*, *I. pseudobuxus* and *I. theezans*) with arbutin and dicaffeoylquinic acids; and group D (*I. argentina*, *I. brevicuspis*, *I. microdonta* and *I. taubertiana*) with the highest level of ursane type saponins. The *Ilex* species used in this study were grown in the same conditions after collecting the seeds from diverse places in South America. Thus, it is expected that the metabolomic differences observed could not be due to environmental factors such as climate, soil condition or biotic stress but rather to the inherent character of each species, *i.e*., their genetic composition. The above findings agree with the molecular analysis of amplified fragment length polymorphism (AFLP)[6]. For example, AFLP results showed a very close relationship between *I. brasiliensis*, *I. integerrima* and *I. theezans*, which was congruent to the metabolomic analysis. However, the above studies did not include East Asia species, especially the source plants of Kudingcha.

The band differences of esterase isoenzymes of 25 Kudingcha germplasm materials in Aquifoliaceae, which involved five species, *I. kudingcha*, *I. latifolia*, *I. cornuta*, *I. pentagona* and *I. huoshanensis*, were analyzed by polyacrylamide gel electrophoresis[88]. The similarity coefficients of the test samples were calculated for a cluster dendrogoam. There were 18 bands of esterase isoenzymes in all samples. Based on the band differences, the 25 germplasm materials could be distinguished at the species level. The five Kudingcha species could be unmistakably distinguished. *I. latifolia* is closer to *I. pentagona* than to *I. kudingcha*, and *I. cornuta* is further to *I. latifolia* than to *I. kudingcha*. Different germplasm materials from the same species were clustered in line with their origins, which is congruent with the morphology. The analysis results of the esterase isoenzymes are useful references for the classification of Kudingcha species, and could be used to determine the origin of Kudingcha species.

The large-leaved Kudingcha from the genus *Ilex*, a traditional Chinese tea, contains several distinctive triterpenoid saponins that can be useful in classification and quality control[42]. The reverse-phase ultra-performance liquid chromatography coupled with evaporative light scattering detection (UPLC–ELSD) is used to simultaneously determine five triterpenoid saponins: kudinoside L (**1**), kudinoside C (**2**), kudinoside A (**3**), kudinoside F (**4**) and kudinoside D (**5**) in several species of the large-leaved Kudingcha and mate tea (*I. paraguariensis*). UPLC separation took only 13 min with low detection and quantification limits, and can be used for the quality control of large-leaved Kudingcha. The different *Ilex* species showed differences in the distribution of the five triterpenoids. *I. kudingcha*, the *bona fide* species of large-leaved Kudingcha, contains the maximum amount of triterpenoid saponins. The chemical profile of *Ilex kaushue* is almost identical to that of *I. kudingcha*, thus supporting the view that these two species are synonymous. The kudinosides (**1**–**5**) are abundant in *I. kudingcha* of Hainan and Guangxi, China. Compounds **2** and **3** are present in *I. latifolia*, while **1**, **4** and **5** are not. Notably, none of the kudinosides were detected in *I. pentagona*, *I. cornuta* and *I. paraguariensis*. Therefore, *I. pentagona* and *I. cornuta* should be considered as adulterants and removed from the large-leaved Kudingcha for tea quality control and utilization. Actually *I. cornuta* is the raw plant of Gougucha, another health-promoting tea consumed in southwest China. *I. kudingcha* and *I. paraguariensis* are chemically dissimilar although they both have a bitter taste and share similar pharmaceutical effects.

6. Conclusions

Mate tea and large-leaved Kudingcha are widely consumed nonalcoholic beverages in South America and East Asia respectively, and are gaining rapid popularity in the world market, either as tea itself or as elements in formulated foods or dietary supplements. Local people have used them for centuries as a social and medicinal beverage. Mate is hypocholesterolemic, hepatoprotective, a central nervous system stimulant, diuretic, and is beneficial to the cardiovascular system and obesity management. Mate has a high antioxidant capacity and is associated with both the prevention and the cause of some types of cancers. Kudingcha and related Chinese *Ilex* compounds regulate lipid metabolism, antagonize cardiovascular diseases and exert antioxidant and anti-inflammatory effects. Terpenoids/saponins and polyphenols/flavonoids extracted from *Ilex* plants have distinct pharmacological effects. Novel pharmacological effects of different species and various constituents are being revealed. Both mate and Kudingcha have gained public attention outside of their traditional use region, and research on non-*Camellia* tea has been expanding. This review presents the usage, chemistry, biological activities, health effects and some taxonomical considerations of Mate tea and Kudingcha. *I. paraguariensis*, *I. kudingcha* and other *Ilex* plants have great potential as the source of biological compounds for the nutraceutical and pharmaceutical industry. To date, the regulation of *Ilex* biological processes at the genomic level, epigenomic level, transcriptional and post-transcriptional levels, and translational and post-translational levels is unknown, although such knowledge is essential for the sustainable development and utilization of *Ilex* medicinal and food resources. The integration of systems biology and ''omics'' techniques could dramatically enhance *Ilex* research and development.

Acknowledgments

The study was funded by Dalian Jiaotong University and Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, China. This work is also supported by the National Natural Science Foundation of China (No. 81274188).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2012.12.008.

References

1. Manen JF, Barriera G, Loizeau PA, Naciri Y. The history of extant *Ilex* species (Aquifoliaceae): evidence of hybridization within a Miocene radiation. *Mol Phylogenet Evol* 2010;**57**:961–77.
2. Li L, Xu LJ, Peng Y, Shi RB, Xiao PG. Comparison of green tea and four other kind of teas. *China J Chin Mater Med* 2011;**36**:5–10.
3. Bracesco N, Sanchez AG, Contreras V, Menini T, Gugliucci A. Recent advances on *Ilex paraguariensis* research: minireview. *J Ethnopharmacol* 2011;**136**:378–84.
4. Liu J, Ding P. Research progress of *Ilex* medicinal plant resources, chemical constituents and pharmacology. *J Guangzhou Univ Trad Chin Med* 2008;**25**:277–80.
5. Kim HK, Saifullah S, Khan S, Wilson EG, Kricun SD, Meissner A, et al. Metabolic classification of South American *Ilex* species by NMR-based metabolomics. *Phytochemistry* 2010;**71**:773–84.
6. Gottlieb AM, Giberti GC, Poggio L. Molecular analyses of the genus *Ilex* (Aquifoliaceae) in southern South America, evidence from AFLP and ITS sequence data. *Am J Bot* 2005;**92**: 352–69.
7. Hao DC, Xiao PG, Peng Y, Dong JQ, Liu WX. Evaluation of the chloroplast barcoding markers by mean and smallest interspecific distances. *Pak J Bot* 2012;**44**:1271–4.
8. Qian YS, Wang HZ, Shi NN, Zhao Y, Li NL, Hu Z. Studies of genetic diversity among ten species of *Ilex* based on RAPD and AFLPs. *J Mol Cell Biol* 2008;**41**:35–43.
9. Zhou XJ, Zhang DM, Luo YL, Su JL, Li JX. Inter-simple sequence repeats (ISSR) marker analysis of *Ilex* plants species and its application. *J Henan Agric Univ* 2009;**43**:196–200.
10. Zhang JH, Gao YZ, Zhang B, Wang ZJ. Genetic diversity of *Ilex* L. tree species. *Acta Bot Boreal Occident Sin* 2011;**31**:504–10.
11. Gauer L, Cavalli-Molina S. Genetic variation in natural populations of mate (*Ilex paraguariensis* A. St.-Hil., Aquifoliaceae) using RAPD markers. *Heredity* 2000;**6**:647–56.
12. Rendell S, Ennos RA. Chloroplast DNA diversity of the dioecious European tree *Ilex aquifolium* L. (English holly). *Mol Ecol* 2003;**12**:2681–8.
13. Torimaru T, Tomaru N, Nishimura N, Yamamoto S. Clonal diversity and genetic differentiation in *Ilex leucoclada* M. patches in an old-growth beech forest. *Mol Ecol* 2003;**12**:809–18.
14. Manen JF. Are both sympatric species *Ilex perado* and *Ilex canariensis* secretly hybridizing? Indication from nuclear markers collected in Tenerife. *BMC Evol Biol* 2004;**4**:46–58.
15. Son SW, Kim JH, Kim YS, Park SJ. ITS sequences variations in populations of *Ilex cornuta* (Aquifoliaceae). *Korean J Plant Taxon* 2007;**37**:131–41.
16. Gottlieb AM, Poggio L. Genomic screening in dioecious yerba mate tree (*Ilex paraguariensis* A. St. Hill., Aquifoliaceae) through representational difference analysis. *Genetica* 2010;**138**:567–78.
17. Hao DC, Yang L, Xiao PG. The first insight into the *Taxus* genome *via* fosmid library construction and end sequencing. *Mol Genet Genomics* 2011;**285**:197–205.
18. Hao DC, Ge GB, Xiao PG, Zhang YY, Yang L. The first insight into the tissue specific taxus transcriptome *via* Illumina second generation sequencing. *PLoS One* 2011;**6**:e21220.
19. Hao DC, Chen SL, Xiao PG. Application of high-throughput sequencing in the medicinal plant transcriptome studies. *Drug Dev Res* 2012;73:487–98.
20. Hao DC, Ma P, Mu J, Chen SL, Xiao PG, Peng Y, et al. *De novo* characterization of the root transcriptome of a traditional Chinese medicinal plant *Polygonum cuspidatum*. *Sci China Life Sci* 2012;**55**:452–66.
21. Lindsey K, Pullen ML, Topping JF. Importance of plant sterols in pattern formation and hormone signalling. *Trends Plant Sci* 2003;**8**:521–5.
22. Li L, Peng Y, Ma G, He C, Feng Y, Lei Q, et al. Quantitative analysis of five Kudinosides in the large-leaved Kudingcha and related species from the genus *Ilex* by UPLC-ELSD. *Phytochem Anal* 2012;**23**:677–83.
23. de Souza LM, Dartora N, Scoparo CT, Cipriani TR, Gorin PA, Iacomini M, et al. Comprehensive analysis of mate (*Ilex paraguariensis*) compounds: development of chemical strategies for matesaponin analysis by mass spectrometry. *J Chromatogr A* 2011;**1218**:7307–15.
24. Che YY, Zhang L, Li N, Zeng KW, Tu PF. Triterpenoid saponins from *Ilex mamillata* C.Y. Wu ex C.J. Tseng. *Nat Prod Res* 2012;**26**:1991–5.
25. Wang JR, Zhou H, Jiang ZH, Liu L. Two new triterpene saponins from the anti-inflammatory saponin fraction of *Ilex pubescens* root. *Chem Biodivers* 2008;**5**:1369–76.
26. Wang JR, Zhou H, Jiang ZH, Wong YF, Liu L. *In vivo* anti-inflammatory and analgesic activities of a purified saponin fraction derived from the root of *Ilex pubescens*. *Biol Pharm Bull* 2008;**31**:643–50.

27. Puangpraphant S, de Mejia EG. Saponins in yerba mate tea (*Ilex paraguariensis* A. St.-Hil) and quercetin synergistically inhibit iNOS and COX-2 in lipopolysaccharide-induced macrophages through NFkappaB pathways. *J Agric Food Chem* 2009;**57**: 8873–83.
28. Zheng J, Tang L, Xian XD, Zhou SX, Shi HM, Jiang Y, et al. Inhibitory effect of triterpenoid saponins from the leaves of *Ilex kudingcha* on aggregated LDL-induced lipid deposition in macrophages. *Planta Med* 2009;**75**:1410–4.
29. Zheng J, Wang X, Li H, Gu Y, Tu P, Wen Z. Improving abnormal hemorheological parameters in ApoE$^{-/-}$ mice by *Ilex kudingcha* total saponins. *Clin Hemorheol Microcirc* 2009;**42**: 29–36.
30. Sugimoto S, Nakamura S, Yamamoto S, Yamashita C, Oda Y, Matsuda H, et al. Brazilian natural medicines. III. structures of triterpene oligoglycosides and lipase inhibitors from mate, leaves of *Ilex paraguariensis*. *Chem Pharm Bull* 2009;**57**:257–61.
31. Im KR, Jeong TS, Kwon BM, Baek NI, Kim SH, Kim DK. Acyl-CoA: cholesterol acyltransferase inhibitors from *Ilex macropoda*. *Arch Pharm Res* 2006;**29**:191–4.
32. Lückemeyer DD, Müller VD, Moritz MI, Stoco PH, Schenkel EP, Barardi CR, et al. Effects of *Ilex paraguariensis* A. St. Hil. (yerba mate) on herpes simplex virus types 1 and 2 replication. *Phytother Res* 2012;**26**:535–40.
33. Wu ZJ, Ouyang MA, Wang CZ, Zhang ZK. Six new triterpenoid saponins from the leaves of *Ilex oblonga* and their inhibitory activities against TMV replication. *Chem Pharm Bull* 2007;**55**: 422–7.
34. Wu ZJ, Ouyang MA, Wang CZ, Zhang ZK, Shen JG. Anti-tobacco mosaic virus (TMV) triterpenoid saponins from the leaves of *Ilex oblonga*. *J Agric Food Chem* 2007;**55**:1712–7.
35. Zuo WJ, Dai HF, Chen J, Chen HQ, Zhao YX, Mei WL, et al. Triterpenes and triterpenoid saponins from the leaves of *Ilex kudincha*. *Planta Med* 2011;**77**:1835–40.
36. Mullié C, Jonet A, Dassonville-Klimpt A, Gosmann G, Sonnet P. Inhibitory effect of ursolic acid derivatives on hydrogen peroxide- and glutathione-mediated degradation of hemin: a possible additional mechanism of action for antimalarial activity. *Exp Parasitol* 2010;**125**:202–7.
37. Taketa AT, Gnoatto SC, Gosmann G, Pires VS, Schenkel EP, Guillaume D. Triterpenoids from Brazilian *Ilex* species and their *in vitro* antitrypanosomal activity. *J Nat Prod* 2004;**67**:1697–700.
38. Silveira F, Rossi S, Fernández C, Gosmann G, Schenkel E, Ferreira F. Alum-type adjuvant effect of non-hemolytic saponins purified from *Ilex* and *Passiflora* spp. *Phytother Res* 2011;**25**: 1783–8.
39. Jiang ZH, Wang JR, Li M, Liu ZQ, Chau KY, Zhao C, et al. Hemiterpene glucosides with anti-platelet aggregation activities from *Ilex pubescens*. *J Nat Prod* 2005;**68**:397–9.
40. Xu GH, Kim YH, Choo SJ, Ryoo IJ, Yoo JK, Ahn JS, et al. Two acetylated megastigmane glycosides from the leaves of *Ilex paraguariensis*. *Arch Pharm Res* 2010;**33**:369–73.
41. Gosmann G, Barlette AG, Dhamer T, Arçari DP, Santos JC, de Camargo ER, et al. Phenolic compounds from Mate (*Ilex paraguariensis*) inhibit adipogenesis in 3T3-L1 preadipocytes. *Plant Foods Hum Nutr* 2012;**67**:156–61.
42. Gugliucci A, Bastos DH. Chlorogenic acid protects paraoxonase 1 activity in high density lipoprotein from inactivation caused by physiological concentrations of hypochlorite. *Fitoterapia* 2009;**80**:138–42.
43. Vieira MA, Maraschin M, Pagliosa CM, Podestá R, de Simas KN, Rockenbach II, et al. Phenolic acids and methylxanthines composition and antioxidant properties of mate (*Ilex paraguariensis*) residue. *J Food Sci* 2010;**75**:280–5.
44. Bastos DH, Saldanha LA, Catharino RR, Sawaya AC, Cunha IB, Carvalho PO, et al. Phenolic antioxidants identified by ESI-MS from Yerba mate (*Ilex paraguariensis*) and green tea (*Camelia sinensis*) extracts. *Molecules* 2007;**12**:423–32.
45. Peralta, I.N. Cogoi, L. Filip, R. Anesini, C. Prevention of hydrogen peroxide-induced red blood cells lysis by *Ilex paraguariensis* aqueous extract: participation of phenolic and xanthine compounds. *Phytother Res*. 2012; DOI:10.1002/ptr.4700.
46. Pirker KF, Goodman BA. Caffeoylquinic acid derived free radicals identified during antioxidant reactions of bitter tea (*Ilex latifolia* and *Ilex kudincha*). *Food Funct* 2010;**1**:262–8.
47. Zhu F, Cai YZ, Sun M, Ke J, Lu D, Corke H. Comparison of major phenolic constituents and *in vitro* antioxidant activity of diverse Kudingcha genotypes from *Ilex kudingcha*, *Ilex cornuta*, and *Ligustrum robustum*. *J Agric Food Chem* 2009;**57**:6082–9.
48. Chen XQ, Zan K, Yang J, Lai MX, Wang Q. A novel flavanone from *Ilex hainanensis* Merr. *Nat Prod Res* 2009;**23**:442–7.
49. Nahar L, Russell WR, Middleton M, Shoeb M, Sarker SD. Antioxidant phenylacetic acid derivatives from the seeds of *Ilex aquifolium*. *Acta Pharm* 2005;**55**:187–93.
50. Song C, Xie C, Zhou Z, Yu S, Fang N. Antidiabetic effect of an active components group from *Ilex kudingcha* and its chemical composition. *Evid Based Complement Alternat Med* 2012;**2012**:423690.
51. Zhang AL, Ye Q, Li BG, Qi HY, Zhang GL. Phenolic and triterpene glycosides from the stems of *Ilex litseaefolia*. *J Nat Prod* 2005;**68**:1531–5.
52. Hussein GM, Matsuda H, Nakamura S, Hamao M, Akiyama T, Tamura K, et al. Mate tea (*Ilex paraguariensis*) promotes satiety and body weight lowering in mice: involvement of glucagon-like peptide-1. *Biol Pharm Bull* 2011;**34**:1849–55.
53. Gugliucci A, Bastos DH, Schulze J, Souza MF. Caffeic and chlorogenic acids in *Ilex paraguariensis* extracts are the main inhibitors of AGE generation by methylglyoxal in model proteins. *Fitoterapia* 2009;**80**:339–44.
54. Lee CS, Lee SA, Kim YJ, Seo SJ, Lee MW. 3,4,5-Tricaffeoylquinic acid inhibits tumor necrosis factor-α-stimulated production of inflammatory mediators in keratinocytes *via* suppression of Akt- and NF-κB-pathways. *Int Immunopharmacol* 2011 1715–23.
55. Puangpraphant S, Berhow MA, Vermillion K, Potts G, de Mejia EG. Dicaffeoylquinic acids in Yerba mate (*Ilex paraguariensis* St. Hilaire) inhibit NF-κB nucleus translocation in macrophages and induce apoptosis by activating caspases-8 and -3 in human colon cancer cells. *Mol Nutr Food Res* 2011;**55**:1509–22.
56. Noratto GD, Kim Y, Talcott ST, Mertens-Talcott SU. Flavonol-rich fractions of yaupon holly leaves (*Ilex vomitoria*, Aquifoliaceae) induce microRNA-146a and have anti-inflammatory and chemopreventive effects in intestinal myofibroblast CCD-18Co cells. *Fitoterapia* 2011;**82**:557–69.
57. Ramirez-Mares MV, Chandra S, de Mejia EG. *In vitro* chemopreventive activity of *Camellia sinensis*, *Ilex paraguariensis* and *Ardisia compressa* tea extracts and selected polyphenols. *Mutat Res* 2004;**554**:53–65.
58. Battagim J, Souza VT, Miyasaka NR, Cunha IB, Sawaya AC, Fernandes AM, et al. Comparative study of the effect of green and roasted water extracts of mate (*Ilex paraguariensis*) on glucosyltransferase activity of *Streptococcus mutans*. *J Enzyme Inhib Med Chem* 2012;**27**:232–40.
59. Xu GH, Kim YH, Choo SJ, Ryoo IJ, Yoo JK, Ahn JS, et al. Chemical constituents from the leaves of *Ilex paraguariensis* inhibit human neutrophil elastase. *Arch Pharm Res* 2009;**32**:1215–20.
60. Wnuk M, Lewinska A, Oklejewicz B, Bugno M, Slota E, Bartosz G. Evaluation of the cyto- and genotoxic activity of yerba mate (*Ilex paraguariensis*) in human lymphocytes *in vitro*. *Mutat Res* 2009;**679**:18–23.
61. Boaventura BC, Di Pietro PF, Stefanuto A, Klein GA, de Morais EC, de Andrade F, et al. Association of mate tea (*Ilex paraguariensis*) intake and dietary intervention and effects on oxidative stress biomarkers of dyslipidemic subjects. *Nutrition* 2012;**28**: 657–64.
62. de Morais EC, Stefanuto A, Klein GA, Boaventura BC, de Andrade F, Wazlawik E, et al. Consumption of yerba mate

(*Ilex paraguariensis*) improves serum lipid parameters in healthy dyslipidemic subjects and provides an additional LDL-cholesterol reduction in individuals on statin therapy. *J Agric Food Chem* 2009;**57**:8316–24.
63. Martins F, Noso TM, Porto VB, Curiel A, Gambero A, Bastos DH, et al. Mate tea inhibits *in vitro* pancreatic lipase activity and has hypolipidemic effect on high-fat diet-induced obese mice. *Obesity* 2010;**18**:42–7.
64. Silva RD, Bueno AL, Gallon CW, Gomes LF, Kaiser S, Pavei C, et al. The effect of aqueous extract of gross and commercial yerba mate (*Ilex paraguariensis*) on intra-abdominal and epididymal fat and glucose levels in male Wistar rats. *Fitoterapia* 2011;**82**:818–26.
65. Luo XY, Li NN, Liang YR. Effects of *Ilex latifolia* and *Camellia sinensis* on cholesterol and circulating immune complexes in rats fed with a high-cholesterol diet. *Phytother Res* 2012; DOI:10.1002/ptr.4693.
66. Arcari DP, Bartchewsky W, dos Santos TW, Oliveira KA, Funck A, Pedrazzoli J, et al. Antiobesity effects of yerba mate extract (*Ilex paraguariensis*) in high-fat diet-induced obese mice. *Obesity* 2009;**17**:2127–33.
67. Lunceford N, Gugliucci A. *Ilex paraguariensis* extracts inhibit AGE formation more efficiently than green tea. *Fitoterapia* 2005;**76**:419–27.
68. Kang YR, Lee HY, Kim JH, Moon DI, Seo MY, Park SH, et al. Anti-obesity and anti-diabetic effects of Yerba Mate (*Ilex paraguariensis*) in C57BL/6J mice fed a high-fat diet. *Lab Anim Res* 2012;**28**:23–9.
69. Klein GA, Stefanuto A, Boaventura BC, de Morais EC, Cavalcante LS, de Andrade F, et al. Mate tea (*Ilex paraguariensis*) improves glycemic and lipid profiles of type 2 diabetes and prediabetes individuals: a pilot study. *J Am Coll Nutr* 2011;**30**:320–32.
70. Hussein GM, Matsuda H, Nakamura S, Akiyama T, Tamura K, Yoshikawa M. Protective and ameliorative effects of mate (*Ilex paraguariensis*) on metabolic syndrome in TSOD mice. *Phytomedicine* 2011;**19**:88–97.
71. Arcari DP, Bartchewsky Jr. W, dos Santos TW, Oliveira KA, DeOliveira CC, Gotardo ÉM, et al. Anti-inflammatory effects of yerba mate extract (*Ilex paraguariensis*) ameliorate insulin resistance in mice with high fat diet-induced obesity. *Mol Cell Endocrinol* 2011;**335**:110–5.
72. Oliveira DM, Freitas HS, Souza MF, Arcari DP, Ribeiro ML, Carvalho PO, et al. Yerba Mate (*Ilex paraguariensis*) aqueous extract decreases intestinal SGLT1 gene expression but does not affect other biochemical parameters in alloxan-diabetic Wistar rats. *J Agric Food Chem* 2008;**56**:10527–32.
73. Li L, Zhang Y, Zhang P, Pi H, Ruan H, Wu J. Appraisal of anti-inflammatory and free radical scavenging activities of ethanol extract of *Ilex ficoidea* Hemsl and *Ilex centrochinensis* S.Y. Hu. *Environ Toxicol Pharmacol* 2011;**32**:122–7.
74. Leonard SS, Hogans VJ, Coppes-Petricorena Z, Peer CJ, Vining TA, Fleming DW, et al. Analysis of free-radical scavenging of Yerba Mate (*Ilex paraguriensis*) using electron spin resonance and radical-induced DNA damage. *J Food Sci* 2010;**75**:14–20.
75. Cunha FL, Silva CM, Almeida MG, Lameiro TM, Marques LH, Margarido NF, et al. Reduction in oxidative stress levels in the colonic mucosa without fecal stream after the application of enemas containing aqueous *Ilex paraguariensis* extract. *Acta Cir Bras* 2011;**26**:289–96.
76. Matsumoto RL, Bastos DH, Mendonça S, Nunes VS, Bartchewsky W, Ribeiro ML, et al. Effects of mate tea (*Ilex paraguariensis*) ingestion on mRNA expression of antioxidant enzymes, lipid peroxidation, and total antioxidant status in healthy young women. *J Agric Food Chem* 2009;**57**:1775–80.
77. Matsumoto RL, Mendonça S, de Oliveira DM, Souza MF, Bastos DH. Effects of mate tea intake on *ex vivo* LDL peroxidation induced by three different pathways. *Nutrients* 2009;**1**:18–29.
78. Kim JY, Jeong HY, Lee HK, Yoo JK, Bae K, Seong YH. Protective effect of *Ilex latifolia*, a major component of kudingcha, against transient focal ischemia-induced neuronal damage in rats. *J Ethnopharmacol* 2011;**133**:558–64.
79. Filip R, Ferraro GE. Researching on new species of Mate: *Ilex brevicuspis*: phytochemical and pharmacology study. *Eur J Nutr* 2003;**42**:50–4.
80. Burris KP, Davidson PM, Stewart Jr. CN, Harte FM. Antimicrobial activity of Yerba Mate (*Ilex paraguariensis*) aqueous extracts against *Escherichia coli* O157:H7 and *Staphylococcus aureus*. *J Food Sci* 2011;**76**:456–62.
81. Burris KP, Davidson PM, Stewart Jr. CN, Zivanovic S, Harte FM. Aqueous extracts of yerba mate (*Ilex paraguariensis*) as a natural antimicrobial against *Escherichia coli* O157:H7 in a microbiological medium and pH 6.0 apple juice. *J Food Prot* 2012;**75**:753–7.
82. Filip R, Davicino R, Anesini C. Antifungal activity of the aqueous extract of *Ilex paraguariensis* against *Malassezia furfur*. *Phytother Res* 2010;**24**:715–9.
83. Conforti AS, Gallo ME, Saraví FD. Yerba Mate (*Ilex paraguariensis*) consumption is associated with higher bone mineral density in postmenopausal women. *Bone* 2012;**50**:9–13.
84. Prediger RD, Fernandes MS, Rial D, Wopereis S, Pereira VS, Bosse TS, et al. Effects of acute administration of the hydroalcoholic extract of mate tea leaves (*Ilex paraguariensis*) in animal models of learning and memory. *J Ethnopharmacol* 2008;**120**: 465–73.
85. Xu C, Luo L, Tan RX. Antidepressant effect of three traditional Chinese medicines in the learned helplessness model. *J Ethnopharmacol* 2004;**91**:345–9.
86. Andrade FD, Piacente S, Pizza C, Vilegas W. Arbutin-2′-sulphonyl from the infusion of *Ilex theezans* leaves. *Fitoterapia* 2004;**75**:782–4.
87. Choi YH, Sertic S, Kim HK, Wilson EG, Michopoulos F, Lefeber AW, et al. Classification of *Ilex* species based on metabolomic fingerprinting using nuclear magnetic resonance and multivariate data analysis. *J Agric Food Chem* 2005;**53**:1237–45.
88. Zhang GH, Zheng DJ, Liu GM, Li LY. Study on esterase isozymes of five Kudingcha species in Aquifoliaceae. *Chin Agric Sci Bull* 2008;**24**:50–6.