



Review

Mulberry: A review of bioactive compounds and advanced processing technology



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ABSTRACT

Background: Historically, mulberry has been effectively used as a traditional medicine in Asia for the treatment of various infectious and internal diseases. It is a rich source of bioactive compounds that can promote human health. However, to date, it has not been officially or scientifically recognized because of a lack of accepted and standardized methodology for its evaluation. Numerous studies have been conducted by applying modern biotechnological tools to explore the relationship between the active ingredients in mulberry and their biological activities to solve ambiguities in their mechanism of action, opening a new horizon in traditional Chinese medicine with the potential for modernization in the near future.

Scope and approach: This paper presents a concise overview on mulberry by examining its major bioactive components, including anthocyanins, polysaccharides, phenols, alkaloids and flavonoids. Recent technical advances are discussed for extraction (solid-liquid extraction, pressurized liquid extraction, supercritical-fluid extraction, microwave-assisted extraction, ultrasonic assisted extraction, enzymatic assisted extraction, solid-phase extraction) and separation (macroporous resins adsorption, silica gel chromatography, ion exchange chromatography, gel filtration chromatography, preparative liquid chromatography, countercurrent chromatography).

Key findings and conclusions: The mystery behind mulberry lies in various bioactivities that have been explored by modern technologies. Studies employing efficient, systematic, and practical protocols for sample preparation and isolation integrated with various analytical systems have been applied to discover, separate, and identify analytes in natural plants especially traditional Chinese medicines and to explore the scientific principles that will benefit the standardization and modernization of natural product research.

1. Introduction

In recent years, there has been a worldwide trend towards the use of plants as sources for various biologically active substances with anti-oxidant, anti-inflammatory, anticancer, anti-diabetic, and weight-loss activities (Afrin et al., 2018; Baranauskaitė et al., 2017; Cianciosi et al., 2018; Pan et al., 2018; Pan et al., 2017; Pereira, Franco, Vitorio, & Kummerle, 2018; Santhakumar, Battino, & Alvarez-Suarez, 2018; Sharma, Kashyap, Sak, Tuli, & Sharma, 2018). Numerous studies have been carried out on some plants such as mulberry, curcuma, garlic, sage, rosemary, oregano, and actinidia resulting in the development of

natural functional formulations as foods and for other applications (Choi et al., 2016; Hettihewa, Hemar, & Rupasinghe, 2018; Kukula-Koch et al., 2018; Marchese et al., 2016; Oswell, Thippareddi, & Pegg, 2018). However, the comprehensive utilization of various plants, rich in functional components, particularly those that are less widely used in culinary and medical applications, is still rare. Therefore, the processing of such plants remains an interesting and useful task, particularly in discovering new sources for functional foods and nutraceuticals.

Mulberry, belonging to the Moraceae family, is a fast-growing deciduous plant found in a wide range of climatic, topographical and soil conditions, and it is widely distributed, from temperate to subtropical

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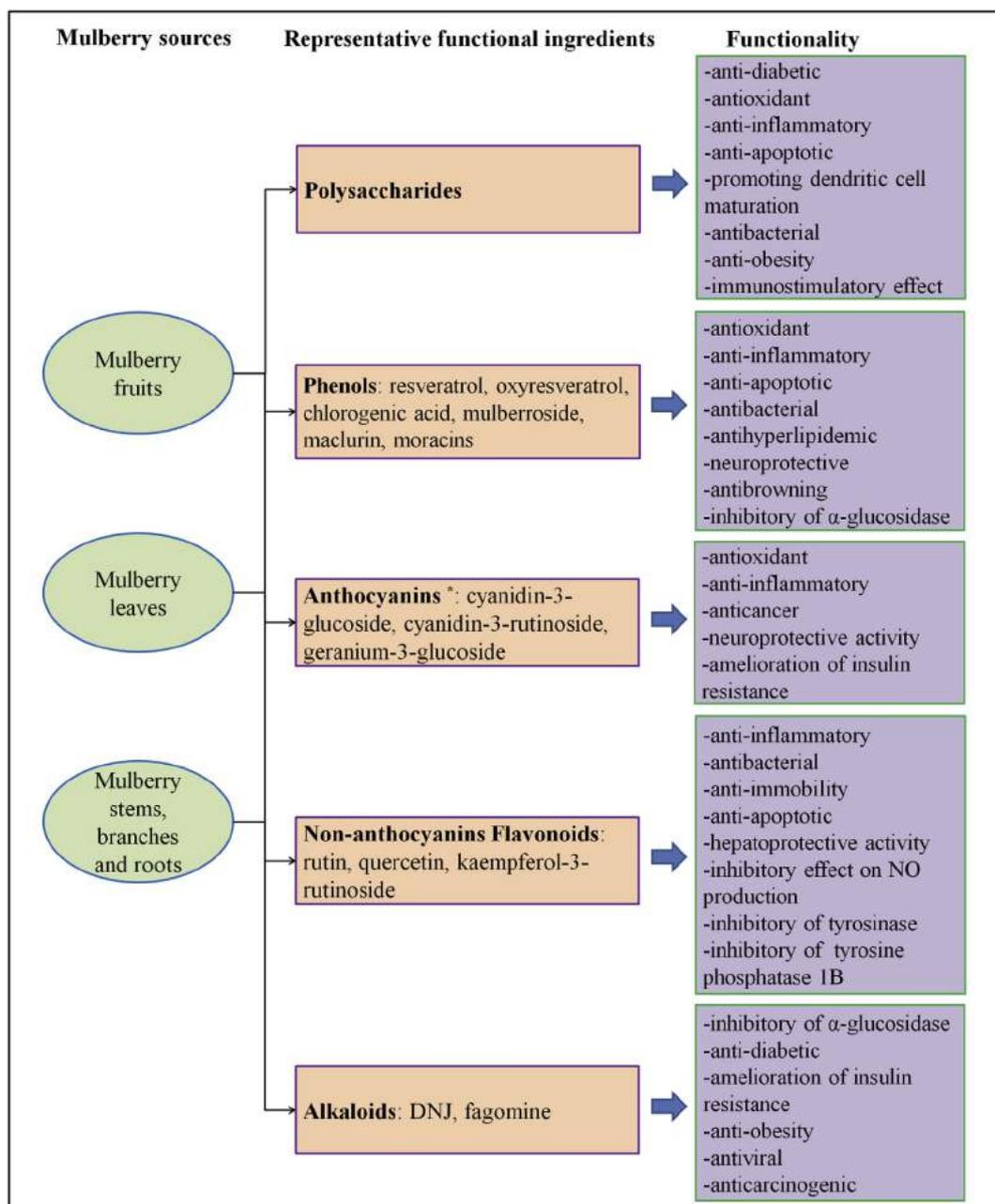


Fig. 1. Main mulberry functional ingredients and inherent functionality. ^aAnthocyanins are only found in mulberry fruit.

regions of the Northern hemisphere to the tropics of the Southern hemisphere. Since the ancient Chinese *Material Medica* describes many medicinal benefits of mulberry, the potential nutritional and medicinal values of mulberry have attracted increasing research interest. Recent studies have demonstrated the nutritional value and health benefits associated with mulberry consumption. The functional components of mulberry, mainly including anthocyanins, polysaccharides, phenols, alkaloid, and flavonoids, have been studied in detail. These bioactive compounds often exhibit a wide range of physiological activities. Therefore, mulberry represents a medicinal and edible plant source that poses no health risk to consumers.

According to the literature, mulberry fruit exhibits a variety of biological activities, including anti-thrombotic (Yamamoto et al., 2006), antioxidant (Kamiloglu, Serali, Unal, & Capanoglu, 2013; Lucia, Olga, Eva, Terentjeva, & Jan 2016), anti-obesity (Khalifa, Zhu, Li, & Li, 2018), anti-inflammation (He et al., 2018), anti-cancer (Cho et al., 2017), and neuroprotective (Kawvised, Wattanathorn, & Thukham-

mee, 2017; Yang, Liu, Zhang, Jin, & Li, 2016) effects.

The bark of mulberry stems, branches, and roots are herbs recorded in the Pharmacopoeia of the People's Republic of China (2000 edition) with applications in removing heat from the lung, relieving asthma, and inducing diuresis. The extracts of mulberry bark have been shown to exhibit anti-inflammatory (Soonthornsit, Pitaksutheepong, Hemstapat, Utaisincharoen, & Pitaksutheepong, 2017), anti-oxidative (Ku et al., 2015), anti-hyperlipidemia (Jo, Kim, & Lim, 2014), and anti-immobility activities (Lim et al., 2016).

Mulberry leaves have long been used in Chinese medicine to treat fevers, protect the liver, improve eyesight, strengthen joints, and modulate dendritic cell maturation (He et al., 2018; Xue et al., 2015), and have been applied in anti-obesity (Chang, Yang, Chen, & Wang, 2016; Kim, 2017), anti-diabetic (Li, Zhang, Chen, & Fu, 2017b; Ranjan et al., 2017), antibacterial (Gryn-Rynko, Bazylak, & Olszewska-Slonina, 2016; Thabti et al., 2014), and antioxidant (Ma et al., 2018; Zhang et al., 2018) treatments.

Despite these various applications of mulberry, the acceptance of mulberry, by the international community, as an herbal medicine for natural or alternative therapies, is still quite poor. The data reported on mulberry are far from sufficient to support its use worldwide. The reasons for the lack of these research data are not only national health care policies, but also a lack of adequate or accepted research methodology for evaluating mulberry. Hence, the standardization and quality control of mulberry and its components by employing modern science and technology are critically important. The first step for the utilization of mulberry's cellular bioactive compounds is extraction, which is a critical step because efficient extraction not only can increase the yield of bioactive components but also can facilitate their separation and characterization. Different extraction techniques, such as solid-liquid extraction (SLE), pressurized liquid extraction (PLE), supercritical-fluid extraction (SFE), microwave assisted extraction (MAE), ultrasonic assisted extraction (UAE), enzymatic-assisted extraction (EAE), solid-phase extraction (SPE) have been used to isolate bioactive components from mulberry. Furthermore, to obtain highly purified bioactive components and identify their mechanisms of action, the separation and analysis of mulberry components is critical to ensure the reliability and repeatability of biochemical and clinical research. Recently, an impressive range of new separation technologies, for the cleanup and enrichment of complex mixtures, has been developed. Modern separation technologies, such as macroporous resins adsorption (MRA), silica gel chromatography (SGC), ion exchange chromatography (IEC), gel filtration chromatography (GFC), preparative liquid chromatography (PLC), and countercurrent chromatography (CCC) have all been applied to mulberry research. When integrated with other analytical techniques, these technologies offer a powerful approach for the separation of mulberry extracts. Analytical techniques have been used for the identification and quantification of mulberry components in illuminating the relationship between these components and their bioactivities, as well as enhancing product quality control. Several analytical techniques, such as gas chromatography, high-performance liquid chromatography, and mass spectrometry, can be applied for mulberry bioactive components profiling and identification. The popularity of mulberry, as a nutritional, therapeutic, auxiliary material in functional foods and medicine, is enhanced as a result of recent improvements in extraction, separation, and analytical technologies. In this paper, we present an overview of recent studies on the bioactive components of mulberry. A summary of extraction and separation technologies that have been developed to improve the production and isolation of mulberry components is also provided.

2. Mulberry functional ingredients

Mulberry is a source of high value-added compounds, with nutraceutical application as functional ingredients, which include anthocyanins, phenols, flavonoids, alkaloids, and other bioactive compounds. Supplementation of foods with functional or bioactive ingredients has become an increasingly interesting way to develop new functional foods for health-conscious consumers. The main mulberry functional ingredients and their inherent functionality are presented in Fig. 1.

Enhancement of mulberry utilization has driven consumers to be more aware that a product can serve both nutrition and health promotion goals. Studies on mulberry functional ingredients have attracted many scientists throughout the world including ones from China, Japan, Korea, and some European countries (Hunyadi, Martins, Hsieh, Seres, & Zupko, 2012).

2.1. Polysaccharides

Polysaccharides, the major active components of mulberry resources and the compositions, listed in Table 1, have attracted increasing attention because of their multiple biological activities. Polysaccharides extracted from mulberry reportedly possess antioxidant

Table 1
The composition of polysaccharide isolated from mulberry.

Source	Variety	Components of polysaccharide	Molecular weight	Reference
Leaf	<i>Morus alba</i>	Mannose (44.93 mol%), rhamnose (5.35 mol%), glucose (33.61 mol%), xylose (10.96 mol%), and arabinose (5.15 mol%)	8 kDa	Zhang et al. (2014a)
	Unknown	Three components, one of them was composed of mannose (4.56 mol%), rhamnose (41.49 mol%), glucose (10.45 mol%), galactose (11.48 mol%), and arabinose (32.02 mol%)	–	Zhang et al. (2016)
Fruit	Unknown	Two components, the first was composed of sorbose (2.27 mol%), arabinose (1.52 mol%), xylose (12.88 mol%), and glucose (83.33 mol%); the second was composed of mannose (19.20 mol%), rhamnose (19.63 mol%), glucose (13.66 mol%), galactose (13.23 mol%), xylose (20.48 mol%), and arabinose (13.80 mol%)	24898 Da and 61131Da, respectively	Ying, Han, and Li (2011)
	<i>Morus alba</i>	Mannose (1.60 mol%), rhamnose (18.40 mol%), glucose (3.10 mol%), galactose (37.60 mol%), xylose (1.70 mol%), fucose (1.30 mol%) and arabinose (36.30 mol%)	1639 kDa	Lee et al. (2013)
	<i>Morus alba</i>	Rhamnose (25.98 mol%), glucose (13.06 mol%), galactose (23.10 mol%), galacturonic acid (16.35 mol%) and arabinose (21.51 mol%)	136.6 kDa	(Chen et al., 2016)
	<i>Morus nigra</i>	Mannose (18.88 mol%), rhamnose (5.94 mol%), glucose (27.62 mol%), galactose (25.00 mol%), xylose (1.75 mol%), galacturonic acid (7.87 mol%) and arabinose (12.94 mol%)	–	Ma et al. (2018)
Unknown	Unknown	Three components, the first was composed of mannose (8.51 mol%), glucose (28.20 mol%), galactose (18.51 mol%), xylose (6.55 mol%), galacturonic acid (3.97 mol%) and arabinose (34.15 mol%); the second was composed of mannose (2.61 mol%), glucose (38.33 mol%), galactose (29.27 mol%), xylose (4.33 mol%), galacturonic acid (3.18 mol%) and arabinose (22.45 mol%); the third was composed of mannose (5.17 mol%), glucose (12.14 mol%), galactose (30.33 mol%), xylose (11.25 mol%), galacturonic acid (3.19 mol%) and arabinose (37.45 mol%)	838 kDa, 49.5–163 kDa and 1.41–15.30 kDa, respectively	(Chen, Huang, You, & Fu, 2017d)
	<i>Morus alba</i>	Rhamnose (3.67 mol%), glucose (7.80 mol%), galactose (45.87 mol%), xylose (16.97 mol%) and arabinose (25.69 mol%)	137 kDa	Chen, et al. (2018)
Branch	<i>Morus alba</i>	Two components, the first was composed of mannose (11.73 mol%), rhamnose (11.38 mol%), glucose (44.85 mol%), galactose (14.77 mol%), xylose (6.26 mol%) and arabinose (11.01 mol%); the second was composed of mannose (7.05 mol%), rhamnose (43.05 mol%), galactose (5.11 mol%), galacturonic acid (7.86 mol%), glucose (9.78 mol%), xylose (22.12 mol%) and arabinose (5.03 mol%)	114,901 kDa and 124,785 kDa, respectively	Qiu, He, and Zhang (2016)

properties. Deng, Zhou, and Chen (2014) optimized the extraction process of *Morus alba* fruit polysaccharides by using response surface method and demonstrated that these polysaccharides possess optimal antioxidant activity as measured by validated pharmacological tests. According to studies by Liao, et al. (2017) and Yuan et al. (2015), polysaccharides from *Morus alba* leaf exhibit potent Fe²⁺ chelating capability and scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, superoxide, and 2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) (ABTS) radicals.

Numerous studies have demonstrated that mulberry polysaccharides have the effect of regulating glucose metabolism, which provides direct evidence of the hypoglycemic function of these polysaccharides. For example, polysaccharides extracted from *Morus alba* leaf effectively normalizes hepatic glucose metabolism and insulin signaling by inhibiting the expression of protein-tyrosine phosphatase 1B, activating the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) pathway, and mitigating oxidative stress in the liver of rat with type 2 diabetes induced by high fat and streptozotocin (Ren et al., 2015). Study of the anti-diabetic effects of polysaccharides isolated from the *Morus alba* branch found that these polysaccharides play a significant role in lowering blood glucose and normalizing metabolism, which might also improve pancreas function by inhibiting the inflammatory response and attenuating oxidative stress (Guo et al., 2013). The molecular mechanism of hypoglycemic activity of mulberry polysaccharides (isolated from *Morus alba* leaf, fruit) have been suggested to take place through the inhibition of pancreatic islet cell apoptosis and amelioration of insulin secretory capacity in diabetic rats (Chen, Huang, Li, & Fu, 2017c; Zhang et al., 2014b). Additionally, mulberry polysaccharides (isolated from *Morus alba* fruit) were shown to target glucose metabolism by modulating gut microbiota, enriching functional bacteria and reducing microbial diversity (Chen et al., 2018), repairing damaged pancreatic tissues and increasing the expression of the insulin receptor, insulin receptor substrate 2, AKT, and glucose transporter type 4 (Jiao et al., 2017).

Moreover, anti-inflammatory and anti-apoptotic activities of mulberry polysaccharides (isolated from *Morus alba* fruit) have been reported (Liu & Lin, 2012, 2014). Mechanistic studies indicate that mulberry fruit polysaccharides significantly ($P < 0.05$) decreased pro-inflammatory cytokines including interleukin (IL)-1b and IL-6, thus, markedly increasing the anti-inflammatory cytokine IL-10 and protecting lipopolysaccharide (LPS)-stimulated macrophages from apoptotic cell death through the regulation of anti- and pro-apoptotic protein levels.

Furthermore, mulberry polysaccharides (obtained from *Morus alba* fruit, leaf), as an adjuvant in dendritic cell-based cancer immunotherapy, can induce phenotypic maturation of dendritic cells by the increasing the expression of cluster of differentiation (CD) 40, CD80/86, major histocompatibility complex-I/II molecules, IL-12, IL-1 β , tumor necrosis factor- α (TNF- α), and interferon- β , decreasing antigen capture capacity, and enhancing allogenic T cell stimulation, thus, inducing functional maturation of dendritic cells (Shin et al., 2013; Xue et al., 2015).

A study has been carried out to evaluate the antibacterial activities of mulberry polysaccharides (extracted from *Morus alba* leaf). The results of this study showed mulberry polysaccharides exhibit distinctive antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* with minimum inhibitory concentrations against of 10, 2.5, and 5 mg/L, respectively (Wang, Li, & Jiang, 2010).

In vitro evidence suggests that mulberry polysaccharides might be useful as a functional ingredient in health-beneficial food supplements for the treatment or prevention of obesity disorders by inhibiting pre-adipocyte proliferation, reducing the number of fat cells and the mass of adipose tissue. Further work demonstrated that a water-soluble polysaccharide (JS-MP-1), isolated from *Morus alba* fruit, can dose-dependently reduce the viability of 3T3-L1 pre-adipocyte cells by increasing the expression of apoptosis-related proteins, such as caspases 9 and 3,

and poly (adenosine diphosphate-ribose) polymerase, decreasing the ratio of B-cell lymphoma/leukemia-2 (Bcl-2) to Bax, and aggravating mitochondrial dysfunction of pre-adipocyte cells (Choi et al., 2016).

Evidence demonstrates that mulberry polysaccharides also have immunomodulating activity. Mulberry polysaccharides (isolated from *Morus alba* fruit and leaf) regulate the maturation of dendritic cells, the most potent antigen-presenting cells, to initiate the majority of immune responses (Shin et al., 2013; Xue et al., 2015). Additionally, *Morus alba* fruit polysaccharide can act as a immunomodulator by stimulating murine RAW264.7 macrophage cells to release chemokines (RANTES and macrophage inflammatory protein-1 α) and proinflammatory cytokines (TNF- α and IL-6), and to induce the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, which are responsible for the production of nitric oxide (NO) and prostaglandin E2, respectively (Lee et al., 2013). These observations suggest the potential application of mulberry polysaccharides as an immunotherapeutic adjuvant and a beneficial health food for immunomodulation.

2.2. Phenols

Phenols have been receiving increasing interest from researchers and food manufacturers. The major reason for this interest is the recognition of the physiological functions of phenols. Phenols, abundant micronutrients present in the human diet, exhibit a probable role in the prevention of various diseases associated with oxidative stress. Researchers have isolated phenols from mulberry, which constitute the main active substances of these plants, including resveratrol, oxyresveratrol, chlorogenic acid, mulberroside, moracin, and maclurin (Fig. S1a). There is much evidence for the protective health effects of phenols isolated from mulberry (*Morus alba*, *Morus nigra*, *Morus multicaulis*, *Morus laevigata*, *Morus atropurpurea*, *Morus rubra*), such as antioxidant, anti-diabetic, anti-inflammatory, cholesterol-lowering, and antiproliferative effects (Gundogdu, Muradoglu, Sensoy, & Yilmaz, 2011; Turan et al., 2017; Yang et al., 2016; Zhang, Du, Zhang, Zhao, & Wang, 2017).

2.2.1. Resveratrol

Resveratrol, a phenolic compound and an excellent antioxidant, can be found in *Morus alba*, *Morus nigra*, and *Morus rubra* (Abbas, Bar, Baraka, Gohar, & Lahloub, 2014; Oh et al., 2009; Shrikanta, Kumar, & Govindaswamy, 2015). Furthermore, it is a prominent anti-inflammatory agent; and the effect of resveratrol (extracted from *Morus alba*) on LPS-induced IL-8, which plays a central role in the initiation and maintenance of inflammatory response production in the human monocytic cell line, THP-1, was investigated by Oh et al. (2009). The results indicate that the treatment of THP-1 cells with resveratrol results in a decrease of IL-8 production and COX-2 expression.

2.2.2. Oxyresveratrol

Oxyresveratrol, a naturally occurring compound found in mulberry, exhibits a wide spectrum of biological activities. Lorenz, Roychowdhury, Engelmann, Wolf, and Horn (2003) reported that oxyresveratrol (separated from mulberry *Morus alba* wood) is a potent antioxidant and effective scavenger through its protective effects against reactive oxygen and nitrogen species in microglial cells. In addition, oxyresveratrol (obtained from *Morus alba* wood) regulates fatty acid oxidation and hepatic lipogenesis to ameliorate nonalcoholic fatty liver (Lee et al., 2018).

Research has revealed that oxyresveratrol exhibits an anti-diabetic effect, as evidenced by the fact that: (i) oxyresveratrol significantly reduced fasting blood and plasma glucose level in a dose dependent manner and at the dose of 600 mg/kg bodyweight oxyresveratrol significantly lowered fasting plasma glucose levels; (ii) the treatment of oxyresveratrol (separated from *Morus alba* branch) stimulated hepatic glucose uptake and glycogen storage by increasing hepatic glucose transporter 2 transcription and glycogen content (Ahn, Lee, Jeon, Choi,

& Kim, 2017); and (iii) oxyresveratrol (separated from unknown mulberry variety) has been demonstrated to be a potent *in vitro* inhibitor of α -glucosidase (He & Lu, 2013).

The anti-inflammatory effect of oxyresveratrol (separated from *Morus alba* branch) on leukocyte migration, one key step in inflammation, has been studied by Chen et al. (2013). Their results showed that oxyresveratrol treatment involves the inhibition of the chemokine receptor type-4-mediated chemotaxis and the mitogen-activated extracellular signal-regulated kinase/extracellular signal-regulated kinase pathway in T-cells and other immune cells. In addition, evidence suggested oxyresveratrol (obtained from *Morus alba* cortex) can significantly down-regulate nuclear factor κ B(NF- κ B) binding activity and COX-2 activity, inhibiting the expression of iNOS in macrophage cells (Chung et al., 2003). Another study showed oxyresveratrol (obtained from *Morus alba* branch) can remarkably attenuate acute colitis by increasing mucin 2 production and trefoil factor 3 mRNA expression, suppressing inflammation (Hwang, Jo, Kim, & Lim, 2017).

Oxyresveratrol could be beneficial for a therapeutic strategy to limit brain injury. It was shown that oxyresveratrol (extracted from *Morus alba* wood) had a neuroprotective ability and significantly inhibited neuronal death produced by trauma and reactive gliosis to repair trauma at 100 μ M (Weber et al., 2012). A similar report about the neuroprotective effect of oxyresveratrol demonstrated that oxyresveratrol (separated from mulberry *Morus alba* wood) treatment can inhibit the apoptotic cell death in transient cerebral ischemia by diminishing cytochrome c release, decreasing caspase-3 activation, reducing brain infarct volume and the number of apoptotic nuclei in ischemic brain (Andrabi et al., 2004).

Studies showed that oxyresveratrol (extracted from *Morus alba* twigs) exhibited inhibitory activity against African swine fever virus (Galindo et al., 2011), *Staphylococcus aureus* (Joung et al., 2016), and *Trichopyton rubrum* (Lu et al., 2017). The underlying mechanisms of its antimicrobial activity were mainly through increasing membrane permeability and the inhibiting of adenosinetriphosphatase in bacteria, or restraining DNA replication, late protein synthesis and factory formation in the virus.

2.2.3. Chlorogenic acid

Chlorogenic acid is a natural chemical compound and an important intermediate in lignin biosynthesis (Boerjan, Ralph, & Baucher, 2003). Chlorogenic acid, another phenolic compound extracted from mulberry, exhibits many physiological functions, such as anti-oxidative, and anti-diabetic functions. Researchers found that chlorogenic acid (extracted from *Morus nigra* leaf, *Morus laevigata* fruit, *Morus alba* leaf) was the dominant phenolic constituent in mulberry and possessed excellent antioxidant activity (Radojkovic et al., 2018; Saracoglu, 2018; Zhang et al., 2018). For example, Lee, Hsu, Lin, Kao, and Wang (2017) reported chlorogenic acid and its derivatives (extracted from *Morus alba* leaf) attenuated alcoholic steatohepatitis by inhibition of oxidative stress.

Chlorogenic acid (extracted from *Morus alba*, *Morus nigra* leaf) has also been reported to exert anti-diabetic and anti-hyperlipidemia activity by decreasing non-fasting blood glucose levels, serum cholesterol, triglycerides and normalization lipoproteins in a dose-dependent manner (Hunyadi et al., 2012; Zeni et al., 2017). Furthermore, chlorogenic acid (extracted from *Morus alba* leaf) could prevent dyslipidemia-induced metabolic syndromes, such as non-alcoholic fatty liver disease, by regulating adipocytokines (leptin and adiponectin), inflammation (IL-6 and TNF- α) and oxidative stress (Peng, Lin, Chung, Huang, & Wang, 2018). Another study indicated chlorogenic acid and its derivatives (extracted from *Morus alba* leaf) inhibit hepatocellular carcinoma cell proliferation by depressing IL-6 and TNF- α , inducing the inflammatory response of adipocytes to activate the proliferation signaling pathway (Chang, Chang, Tseng, & Wang, 2018).

2.2.4. Mulberroside A

Mulberroside A is a natural glycosylated stilbene present at relatively high abundance in the roots and twigs of mulberry. The anti-hyperlipidemic effect of mulberroside A, isolated from *Morus alba* root, has been studied (Jo et al., 2014). The results showed pre-treatment with oral mulberroside A significantly ($P < 0.05$) reduced the levels of serum lipids, coronary artery risk index, and atherogenic index of hyperlipidemic rats, but increased hepatic fatty degeneration in a dose-dependent manner.

Another report investigated the ability of mulberroside A (isolated from *Morus alba*) to counteract the hypoxia-ischemia impairment of rat cortical neurons induced by oxygen-glucose deprivation and reperfusion. This study demonstrated that mulberroside A elicited neuroprotective effects by decreasing the expression of TNF- α , IL-1 β and IL-6, and by inhibiting the activation of neutrophilic alkaline phosphatase 3, caspase-1, NF- κ B, and the phosphorylation of ERK, the c-Jun N-terminal kinase and p38 (Wang, Liu, Zhang, & Zhang, 2014).

Evidence suggested mulberroside A (isolated from *Morus alba* branch) also attenuated the mRNA and protein expression of renal glucose transporter 9 and urate transporter 1, and upregulated the mRNA and protein levels the organic ion transporters to implement its nephroprotective and uricosuric effects (Shi et al., 2012).

Mulberroside A (isolated from *Morus alba* root) may be a potential substance to influence the role of P-glycoprotein in herb-drug interactions. As testified by the case that mulberroside A can suppress trans-activation mediated by prenanthene X receptor and gene expression of P-glycoprotein accompanied by the activation of PKC and NF- κ B (Li et al., 2017; Li et al., 2014).

Further studies on the underlying mechanisms of the inhibitory effect of mulberroside A (isolated from *Morus alba* root) on tyrosinase and melanin were associated with suppression the expression of melanogenic enzymes tyrosinase, tyrosinase-related protein-1 and 2, and microphthalmia transcription factor, demonstrate that mulberroside A may be a suitable cosmetic agent for skin whitening (Kim, Park, Lee, Kim, & Lim, 2012; Park, Kim, Hwang, Yoo, & Lim, 2011; Wang et al., 2014).

Besides, mulberroside A (isolated from *Morus alba*/unknown mulberry species twig) was effective in inhibiting tumors (Liu et al., 2017), inflammatory response (Zhang & Shi, 2010), and repairing alcoholic hepatic injury (Zhang, Jin, & Shi, 2008).

2.2.5. Maclurin

Maclurin is a lightly yellow-colored natural organic compound that can be extracted from mulberry. The antioxidant activity of maclurin from mulberry (*Morus alba*) twigs reportedly takes place through its radical scavenging and reducing activity, as well as its Fe²⁺ chelating activity (Chang et al., 2011). Maclurin (extracted from unknown mulberry variety) has also been demonstrated to be a promising candidate as an anti-metastatic therapeutic agent in the migration suppression and invasion of human non-small-cell lung cancer (NSCLC). The underlying mechanisms were clarified as follow: (i) maclurin exhibited anti-oxidative activity to significantly decrease the level of intracellular reactive oxygen species, thus leading to the inhibition of Src/focal adhesion kinase and extracellular signal-regulated kinase (ERK); and (ii) the suppression of Src/focal adhesion kinase and ERK-dependent signaling pathway activated glycogen synthase kinase-3 β , resulting in the nuclear accumulation of β -catenin and then the down-regulation of the transcriptional expression of MMP-2 and MMP-9 (Ku et al., 2015).

2.2.6. Moracins

Moracins are 2-arylbenzofuran derivatives isolated from mulberry. They (isolated from *Morus alba* leaf, root) are capable of free-radical scavenging and lipid peroxidation, and inhibit the activities of dual β -secretase 1 and cholinesterase, which play a crucial role in anti-Alzheimer's disease and anti-glycation (Sharma et al., 2001) (Seong, Ha, Min, Jung, & Choi, 2018).

Lee and coworkers carried out the study that evaluated the anti-inflammatory of morcains (isolated from *Morus alba* root) *in vitro* and *in vivo*, and found morcains interrupted the c-Jun N-terminal kinase (JNK)/c-Jun pathway to down-regulate IL-6 expression and suppressed iNOS to decrease the NO production (Lee et al., 2016).

The anti-tumor activities of moracins against A549 (lung cancer), BEL7402 (liver cancer), BGC823 (gastric cancer), HCT8 (colon cancer), and A2780 (oophoroma) (extracted from *Morus alba* leaf) were evaluated by Yang, Gong, Liu, and Chen (2010). These results demonstrate that moracins with more aromatic 2-arylbenzofurans exhibit stronger potent cytotoxic activity against A549, BEL7402, BGC823, HCT8, and A2780 cell lines, than those with long aliphatic chains.

2.3. Anthocyanins

Anthocyanins are a group of naturally occurring flavonoid polyphenols responsible for the color in mulberry fruit. The major anthocyanins identified in mulberry fruit are cyanidin-3-O-glucoside (C3G), cyanidin-3-O-rutinoside (C3R) and pelargonidin-3-glucoside (Fig. S1b) (Hassimotto, Genovese, & Lajolo, 2008). In addition to imparting color, anthocyanins possess known pharmacological properties and specific health-enhancing characteristics, such as antioxidant, anti-inflammatory, anti-cancer activities and improve chronic diseases.

Du, Zheng, and Xu (2008) investigated the direct correlation between antioxidant activity and anthocyanin content of *Morus alba* fruit using a free radical scavenging method relying on DPPH. The results showed that mulberry fruit anthocyanins are excellent antioxidants, as evidenced by their high scavenging ability towards DPPH. In another study, *Morus alba* anthocyanin extract eliminated excessive intracellular free radicals to ameliorate oxidative damage in HepG2 cells, regulated mitogen-activated protein kinases and nuclear factor-erythroid 2-related factor 2 signal pathways to prolong the lifespan of *Caenorhabditis elegans* (Yan, Chen, Azat, & Zheng, 2017). Another study showed anthocyanin (extracted from *Morus australis* fruit) prevented oxidative stress by increasing superoxide dismutase and glutathione peroxidase activities, suppressing inflammation through down-regulating the expression of TNF α , IL-6, iNOS and NF- κ B genes (Wu, Yin, Zhang, Long, & Zheng, 2016).

Additionally, the prophylactic or therapeutic efficiency in acute inflammation of anthocyanins (extracted from *Morus nigra* fruit) was investigated by Hassimotto et al. (2013). The study was carried out in two acute inflammation models, peritonitis and paw oedema, by oral administration of anthocyanins in two distinct protocols, 30 min before and 1 h after carrageenan stimulus. The treatment of anthocyanins suppressed the paw oedema in both administration times, which indicates that anthocyanins can not only prevent but also treat paw oedema induced by carrageenan. In the peritonitis model, anthocyanins reduced the polymorphonuclear leukocyte influx in the peritoneal exudates and suppressed mRNA and protein levels of COX-2 up-regulated by carrageenan in both protocols. It was found that anthocyanins were more efficient on reducing the polymorphonuclear leukocytes when administered 30 min before than 1 h after carrageenan induction, and that anthocyanins have an inhibitory effect on prostaglandin E2 production only when administration 30 min before carrageenan stimulus.

Anthocyanins show additional bioactivity on improving chronic diseases, such as diabetes, hyperlipidemia, and atherosclerosis. For example, *Morus alba* fruit anthocyanins can ameliorate insulin resistance in HepG2 cells, and diabetes-related metabolic changes in type 2 diabetic mice. Specifically, *in vitro*, anthocyanins inhibited the peroxisome proliferator-activated receptor (PPAR) γ coactivator 1 α and forkhead box protein O1, and increased the phosphorylation of AKT and glycogen synthase kinase-3 β , thus, decreasing the activities of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, and up-regulating the expression of glycogen synthase 2, then prompting glucose consumption, glucose uptake and glycogen production (Yan,

Zhang, Zhang, & Zheng, 2016). *In vivo*, anthocyanin extract supplementation markedly decreased fasting blood glucose, serum insulin, leptin, triglyceride, and cholesterol levels and increased adiponectin levels in db/db mice (Yan, Dai, & Zheng, 2016), up-regulated adenosine monophosphate activated protein kinase (AMPK) phosphorylation, and down-regulated acetyl-CoA carboxylase and the mechanistic target of rapamycin to alter the expression of p38-mitogen-activated protein kinase and peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC 1 α) in insulin sensitive tissues (Yan & Zheng, 2017). These results support the observation that anthocyanins have potential benefits on improving dysfunction in diabetic mice and mitigating insulin resistance in HepG2 cells. Moreover, anthocyanins (extracted from *Morus alba* fruit) were reported to ameliorate metabolic disease through activating brown adipose tissue thermogenesis with increasing the expression levels of fatty acid oxidation-related genes and brown adipose tissue specific gene-uncoupling protein 1, and the mitochondrial copy number (You et al., 2017).

In testing the effect of anthocyanins on tumors, Chen, et al. (2006) observed that C3R and C3G (extracted from *Morus alba* fruit) exerted a dose-dependent inhibitory effect on the migration and invasion of highly metastatic A549 human lung carcinoma cells without significant cytotoxicity. The result indicates that C3G and C3R treatments can decrease the expressions of matrix metalloproteinase (MMP)-2 and urokinase-plasminogen activator, thus, enhancing the expression of tissue inhibitor of MMP-2 and plasminogen activator inhibitor and inhibiting the activation of c-Jun and NF- κ B. Anthocyanins also could induce apoptosis and autophagy cell death of thyroid cancer cells (Long et al., 2018). The above evidence suggests that anthocyanins may be of great value as lead compounds in developing new cancer therapies.

C3G (extracted from *Morus alba* fruit), one monomer of anthocyanins, also has neuroprotective effects on the PC12 cells exposed to hydrogen peroxide *in vitro* and on cerebral ischemic damage *in vivo* (Kang, Hur, Kim, Ryu, & Kim, 2006).

In addition to aforementioned features, anthocyanins (extracted from *Morus alba*, *Morus nigra*, *Morus mongolica* fruit) are the main active ingredients in antinociceptive process, and show antibacterial activities toward *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Chen et al., 2018). Furthermore, anthocyanins (extracted from *Morus alba* fruit) inhibit the aryl hydrocarbon receptor signaling pathway, protecting skin from the damaging effect of benzo[a]pyrene (Woo, Lee, Park, & Jung, 2017).

2.4. Non-anthocyanin flavonoids

Flavonoids are secondary metabolites abundantly found throughout the plant kingdom. The major sources of flavonoids are fruits, vegetables, grains, barks, roots, stems, flowers, and leaves. Flavonoids can be divided into various classes by their molecular structures. In addition to anthocyanins, the other five major subclasses of flavonoids include the flavones (e.g., rutin, kuwanon, apigenin, luteolin), flavonols (e.g., quercetin, myricetin), flavanones (e.g., naringenin, hesperidin), catechins or flavanols (e.g., catechin, epicatechin, sanggenon, gallo catechin), and isoflavones (e.g., genistein, daidzein). The flavonoid compounds commonly found in mulberry are kuwanon, sanggenon, rutin, quercetin, and catechins (Fig. S1c).

2.4.1. Kuwanons

Kuwanons are natural isoprenylated flavonoids isolated from mulberry. Antioxidant assays of kuwanons (C and G, isolated from *Morus nigra* stem) show that they exhibit potential radical-scavenging capacity on ABTS and DPPH (Abbas et al., 2014; Mazimba, Majinda, & Motlhanka, 2011).

There is evidence suggesting that kuwanons are effective in treating chronic diseases, such as diabetes, hyperlipidemia, and atherosclerosis. For example, kuwanon G (isolated from *Morus alba* root) showed α -glucosidase and protein tyrosine phosphatase 1B inhibitory

activity, significantly enhancing glucose uptake and in a dose-dependent manner in insulin-resistant HepG2 cells (Chen et al., 2018; Paudel et al., 2018). Another study demonstrates that kuwanons (C and G, isolated from unknown mulberry variety stem) possess hypolipidemic effects, as evidenced by kuwanon inhibition of pancreatic lipase-mediated 4-methylumbelliferyl oleate hydrolysis with the K_i values less than $0.5 \mu\text{M}$ (Hou et al., 2018). Moreover, kuwanon G (isolated from *Morus alba* root) can attenuate atherosclerosis by up-regulation of Liver X receptor α -adenosine triphosphate binding cassette transporters A1/G1 and inhibiting NF- κ B activity in macrophages (Liu et al., 2018).

Kuwanon G (extracted from *Morus alba* leaf) may also have a beneficial effect on preserving the integrity of the gut epithelial barrier by increasing cell viability and tight junctions between cells, and decreasing pro-inflammatory cytokines (IL-1 β and TNF- α) and oxidative damage (Guo et al., 2016).

Kuwanons also showed good antimicrobial activity. Park, You, Lee, Baek, and Hwang (2003) reported kuwanon G (extracted from *Morus alba* root bark) shows activity against oral pathogens, completely inactivating *Streptococcus mutans* at a concentration of 20 mg/mL in 1 min and significantly inhibiting the growth of other cariogenic and periodontitis causing bacteria such as *Streptococcus sobrinus*, *Streptococcus sanguis*, and *Porphyromonas gingivalis*. Another study found kuwanon G (extracted from *Morus nigra* root bark) showed antifungal activity against *Botrytis cinerea*, *Aspergillus niger*, *Aspergillus carbonarius*, *Aspergillus terreus* and *Penicillium expansum* with minimal inhibitory concentrations of 32, 64, 32, 16, 32 and 16 g/mL, respectively (Simonetti et al., 2017). Furthermore, kuwanon G and H (extracted from *Morus nigra* root bark) exhibit suppressive activity on *Mycobacterium tuberculosis* by inhibiting its protein tyrosine phosphatases A and B (Mascarello et al., 2018). Kuwanon C (isolated from *Morus mongolica* root bark) shows antibacterial activity against *Candida albicans*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus epidermis* (Sohn, Son, Kwon, Kwon, & Kang, 2004). These results support the potential applications of kuwanons as antimicrobial agents.

Additionally, kuwanon (C, isolated from *Morus australis* root) is active in the arachidonic acid-activating and platelet-activating factor-induced platelet aggregation assays (Ko, Yu, Ko, Teng, & Lin, 1997).

Since NO produced by iNOS plays an important role in inflammatory disorders, inhibition of NO production by kuwanons may contribute to their anti-inflammatory and immunoregulation activities *in vivo*. Evidence shows that kuwanon C (isolated from *Morus alba* root) inhibits NO production from LPS-induced RAW 264.7 cells at $> 10 \mu\text{M}$. The underlying mechanism of the effect is that kuwanon C suppresses the induction of iNOS enzyme rather than directly inhibited the activity of iNOS enzyme (Cheon et al., 2000; Yang, Matsuzaki, Takamatsu, & Kitanaka, 2011).

Kuwanons were demonstrated to be potential hypopigmenting agents. Kuwanon G (extracted from *Morus alba* root bark) suppresses tyrosinase in a competitive manner with an IC_{50} of $67.6 \pm 2.11 \mu\text{M}$, thus restraining synthesis of melanin (Koirala et al., 2018). Similar effects were found for another kuwanon compound, kuwanon O (extracted from *Morus australis* root) (Hu, Zheng, Chen, & Wang, 2017). Kuwanon O (extracted from *Morus australis* root) also shows a photoprotective effect and significant suppression on ultraviolet A-induced or H_2O_2 -induced cellular reactive oxygen species and nitrotyrosine in human primary epidermal keratinocytes at low concentrations (0.25 and $0.5 \mu\text{M}$). The photoprotective effect of kuwanon O also involves the augmentation of p53 expression after ultraviolet A radiation to repair the 8-hydroxy-2'-deoxyguanosine and cyclobutane pyrimidine dimers (Hu, Chen, & Wang, 2015).

Further study showed kuwanons increase neurogenesis in rat neural stem cells (NSCs) and may represent a new drug candidate for the regeneration or protection neurons in neurodegenerative diseases. Specifically, kuwanon V (isolated from *Morus bombycis* root) enhanced neuronal differentiation and decreases NSC proliferation even in the presence of mitogens such as epidermal growth factor and fibroblast

growth factor 2, as evidenced by reduction the phosphorylation of ERK 1/2, increased mRNA expression levels of the cyclin-dependent kinase inhibitor p21, down-regulation of Notch/Hairy expression levels, and up-regulation of microRNA miR-9, miR-29a, and miR-181a in kuwanon V treated NSCs (Kong et al., 2015).

2.4.2. Sanggenons

Sanggenon, an active compound isolated from the root bark of mulberry, shows various biological activities. Lu et al. (2018) studied the antioxidant activity of sanggenon and found that sanggenon X (isolated unknown mulberry variety) shows significant antioxidant activity against Fe^{2+} -Cys-induced lipid peroxidation in rat liver microsomes. Moreover, sanggenon N (isolated from *Morus alba*) suppresses oxidative stress in HepG2 cells with an IC_{50} value of $23.45 \pm 4.72 \mu\text{M}$, thus, exhibiting protective effects on hepatic cells (Jung et al., 2015).

Sanggenon could be beneficial in diabetes by regulation of α -glucosidase activity. Research found that sanggenon V, as well as sanggenon C, (isolated from *Morus alba*) exhibits strong α -glucosidase inhibitory with IC_{50} s of 2.89 μM , 18 μM , respectively (Chen et al., 2018; Li, Wu, Wang, Shen, & Ren, 2018).

Under continued exploration of the anti-inflammatory activity of sanggenon, the inhibition of NO production in LPS-induced macrophages treated with sanggenons was evaluated. Sanggenons (D, E, F, G, H and N, extracted from *Morus alba* and *Morus nigra*) showed significantly NO production inhibitory effects (Cheon et al., 2000; In, 2017; Yang et al., 2011). The anti-inflammatory mechanism of sanggenons was associated with attenuation of TNF- α and IL-1 β secretion and the inhibition of NF- κ B nuclear translocation in LPS-stimulated macrophages (Zelova et al., 2014). In addition, sanggenon I (extracted from *Morus alba*) enhanced cell viability in a dose-dependent manner against NO-induced cell death to protect neuronal cells from neurodegenerative diseases (Lee et al., 2012).

Sanggenon also shows remarkable antimicrobial activity. For example, *in vitro* demonstrated the germination inhibitory activity of sanggenons (B, C, D, E, G and O, isolated from *Morus alba*), which showed antifungal activity against *Venturia inaequalis* with IC_{50} values between 10 and 123 μM (Rollinger et al., 2006). Sanggenons (B and D, isolated from *Morus alba* and *Morus mongolica*) were also effective in inhibiting gram-positive bacteria (*Staphylococcus epidermis*, *Staphylococcus aureus*) with 12.5–50 $\mu\text{g/mL}$ minimum inhibitory concentration (Sohn et al., 2004). Sanggenon G (isolated from *Morus alba*) shows a dual activity against influenza virus and *Streptococcus pneumococcal* by inhibiting the activity of neuraminidase, a functional enzyme that plays important role in colonization and growth of influenza virus and *Streptococcus pneumococcal*. In contrast to the approved neuraminidase inhibitor oseltamivir, sanggenon G also restrained planktonic growth and biofilm formation of *Streptococcus pneumococcal* (Grienke et al., 2016). Sanggenon O (isolated from unknown mulberry variety) can inhibit Dengue virus by blocking the Asn-130 glycosylation site of the virus (Qamar et al., 2014). These results support the use of sanggenons in Asian traditional medicine to treat microbial and viral infection and indicate the utility of sanggenons as anti-infective agents as well as anti-inflammatory agents.

Furthermore, sanggenon G (extracted from *Morus alba* root bark) has also been studied for its anti-immobility activity in forced swim test-induced depression. It was found that sanggenon G significantly decreased the immobility time and enhanced the antidepressant-like behavior because of the interaction between sanggenon G and the serotonergic system (Lim et al., 2016; Lim et al., 2015).

Sanggenon G, isolated from *Morus alba* root bark, is also a natural cell-permeable small-molecular inhibitor of the X-linked apoptosis inhibitory protein, an anti-apoptotic protein resulting in defects in the regulation of cancer cell development (Seiter et al., 2014). Another mechanism of sanggenon's anticancer activity is illustrated for sanggenon C (isolated from *Morus cathayana*), which induces apoptosis of colon cancer cells by increasing reactive oxygen species generation,

inhibiting of iNOS expression and activating the mitochondrial apoptosis pathway (Chen, Liu, Zhang, Yao, & Wang, 2017). Besides inducing apoptosis of tumor cells, sanggenon can protect normal cells and stimulate their proliferation and differentiation. Sanggenon C (isolated from *Morus alba*) promotes osteoblastic proliferation and differentiation to ameliorate osteoporosis in Zebrafish model (Wang et al., 2018). Additional research suggest that sanggenon C possesses a direct cytoprotective effect against hypoxia injury in cardiac cells by signaling mechanisms involving the activation AMPK and concomitant inhibition of mechanistic target of rapamycin and forkhead box O3a (Gu et al., 2017).

In addition to aforementioned properties of sanggenons, they have also been shown to modulate arachidonic acid metabolism including COX and lipoxygenase inhibition. For example, sanggenon D (obtained from unknown mulberry variety) inhibits the formation of thromboxane B₂ by affecting the activity of COX-2, 12-hydroxy-5,8,10-heptadecatrienoic acid and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (Kimura, Okuda, Nomura, Fukai, & Arichi, 1986). Sanggenon also inhibits tyrosinase and tyrosine phosphatase 1B. Specifically, sanggenons C, D, M, O and T (extracted from *Morus nigra* twig, *Morus mongolica* and *Morus australis* root) exhibit IC₅₀ values of 1.17, 7.30, 13.06, 1.15 and 1.2 μM on tyrosinase, respectively, and are more effective than kojic acid (IC₅₀ = 24.8 μM) (Hu et al., 2018; Lee et al., 2004; Zheng, Tan, & Wang, 2012). Sanggenons C and G (extracted from *Morus alba* and *Morus bombycis* root bark) inhibit tyrosine phosphatase 1B with IC₅₀ values ranging from 2.6 to 1.6 μM (Cui et al., 2006).

Due to the limited space, the bioactivity of rutin, quercetin, and catechin isolated from mulberry are summarized in supporting information.

2.5. Alkaloids

Alkaloids, a group of naturally occurring chemical compounds, are produced by a wide variety of organisms including bacteria, fungi, plants, and animals. Alkaloids can generally be purified from crude extracts of these organisms. Two common alkaloids, 1-deoxynojirimyacin (DNJ), and fagomine (Fig. S1d) have been obtained from mulberry.

2.5.1. DNJ

DNJ is a polyhydroxylated piperidine alkaloid containing a 5-amino-1,5-deoxy-D-glucopyranose moiety. As a promising competitive inhibitor of intestinal α-glucosidases, DNJ has been received special attention. DNJ competitively inhibits α-glucosidase to suppress glucose absorption, which leads to a decrease in the blood glucose levels and an increase in insulin sensitivity. Kwon, Chung, Kim, and Kwon (2011) demonstrated that DNJ (extracted from *Morus alba* leaf) inhibits α-glucosidase, significantly reducing cellular glucose uptake in Caco-2 cells. Mulberry (extracted from unknown mulberry variety) leaf DNJ shows the same hypoglycemic function on injection into the stomach of mice having diabetes mellitus induced by alloxan treatment (Liu, Hua, & Wang, 2012). Another study shows DNJ can significantly reduce the levels of serum parameters (triglyceride, total cholesterol and low density lipoprotein cholesterol), liver indexes (triglyceride, TNF-α, IL-1, IL-6), activities of serum alanine aminotransferase and aspartate transaminase, as well as glucose-6-phosphatase, glycogen phosphorylase, and phosphoenolpyruvate carboxykinase in liver, and significantly increases hepatic glycogen content, the activities of hexokinase, pyruvate kinase in liver tissue (Liu et al., 2016). Moreover, Liu, Li, Li, Zheng, and Peng (2015) discovered that DNJ (obtained from *Morus alba* leaf) treatment significantly improves insulin sensitivity by activating the insulin signaling PI3K/AKT pathway, increasing glucose transporter type 4 translocation and phosphorylation of Ser473-AKT, p85-PI3K, and Tyr1361-insulin receptor-β and Tyr612-insulin receptor substrate 1.

In addition to its role in modulating glucose metabolism and insulin

function, DNJ also exhibits lipid-regulating and anti-obesity effects. The administration of a *Morus alba*-derived DNJ for 12 weeks decreases both the visceral fat weight and adipocyte size, thus, suppressing lipid accumulation in liver and reducing plasma triacylglycerol (Tsuduki, Kikuchi, Kimura, Nakagawa, & Miyazawa, 2013). Another study indicates that DNJ (extracted from unknown mulberry variety) also inhibits adipogenesis by down-regulating PPARγ and the phosphorylation of extracellular regulated protein kinases 1/2 in intramuscular adipocytes (Wang et al., 2015).

DNJ (isolated from *Morus alba* leaf) can also act as an anti-infective agent with demonstrated anti-adherence activity to control the overgrowth of *Streptococcus mutans* (Islam et al., 2008).

A study was carried out to evaluate the neuroprotective effect of DNJ (obtained from *Morus alba* leaf) on cognitive impairment, β-amyloid deposition, and neuroinflammation in the senescence-accelerated-prone mice. The results indicate that DNJ treatment (160 mg/kg/day) can significantly inhibit β-secretase expression, attenuate β-amyloid deposition, remit neuroinflammation (IL-1β, IL-6 and TNF-α), and up-regulate brain-derived neurotrophic factor/tyrosine kinase receptors signal pathway in the brain. Therefore, DNJ potentially has a neuroprotective effect and might be a valid agent to improve pathological changes in brains of Alzheimer's patients (Chen et al., 2018).

In addition, DNJ (extracted from unknown mulberry variety) inhibit metastasis of B16F10 melanoma cells possibly by attenuating the activities and expression of MMP-2/9, enhancing of the MMP-2 mRNA expression, and alternating cell surface-binding motifs. These findings further confirm the antimetastatic potential of DNJ against melanoma cells (Wang et al., 2010). In another study, the effect of DNJ (isolated from *Morus alba* leaf) against colorectal cancer induced by azoxymethane dextran sodium sulfate was examined in ICR mice. The results show that the tumor incidence and number were significantly reduced in a dose dependent manner by DNJ by increasing the mRNA expression of pro-apoptotic Bax, and decreasing the mRNA expression of anti-apoptotic Bcl-2 (Shuang et al., 2017). DNJ (extracted from unknown mulberry variety) also alleviates the growth suppression of epithelial cell by up-regulating lymphoid enhancer factor-1, a key gene regulating mammary gland growth and development, mRNA expression (Ji, Liu, Zhang, & Zhang, 2018).

2.5.2. Fagomine

Fagomine, an N-containing pseudo-sugar and a naturally occurring iminosugar, is another polyhydroxylated piperidine alkaloid having remarkable biological properties. For example, fagomine (isolated from *Morus bombycis*) shows inhibitory activity against mammalian intestinal α-, β-glucosidase, and α-, β-galactosidase, exhibiting potent anti-hyperglycemic effect on streptozotocin-induced diabetic mice, and potentiating glucose-induced insulin secretion (Nojima et al., 1998). Moreover, Taniguchi, Asano, Tomino, and Miwa (1998) have reported the potentiation of glucose- and glyceraldehyde-induced insulin secretion by fagomine isolated from the *Morus bombycis* leaf, as evidence by: (i) glycolysis assessed by lactate production from glucose was significantly enhanced after fagomine (4 mM) treatment; (ii) fagomine treatment defended all intermediates (from glucose 6-phosphate to glyceraldehyde 3-phosphate) of the upper part of the glycolytic pathway from the influence by high glucose concentration (20 mM) in islets; and (iii) fagomine stimulated the formation of acetyl-CoA and glyceraldehyde 3-phosphate, thus, resulting a rise of the ATP/ADP ratio.

3. Extraction techniques for mulberry bioactive compounds

Extraction is the first step of the analysis and utilization of the cellular bioactive compounds contained in plants. The identification of suitable extraction methods is thus a critical step for increasing the extraction yield of such bioactive components from mulberry. Since various approaches have been already reviewed, this section focuses on

the sample-preparation techniques used for the extraction of analytes from mulberry.

3.1. Solid-liquid extraction (SLE)

SLE is a traditional extraction method that has been widely applied to isolate bioactive compounds from solid matrices in laboratory and industry by using organic solvents or water. Target active compounds can be extracted by SLE technique according to their differences in polarity by employing suitable solvents. Hence, the solvent is an important factor during SLE process. Some of the most widely used solvents in the extraction procedures include ethanol, methanol, acetone, and dichloromethane, which are commonly used in different ratios with water (as shown in Table S1).

There are numerous studies on the extraction of bioactive compounds from mulberry by using SLE during the past decade. For example, Li, Zhang, Chen, and Fu (2017a) evaluated the effect of extraction solvent on the *Morus alba* fruit polyphenols profile, antioxidant and antiproliferation capacities against human hepatoma HepG2 cells. There were significant differences among solvents, including acetic acid/water (0.1/99.9, v/v), methanol/acetic acid/water (75/0.1/24.9, v/v/v), ethanol/acetic acid/water (75/0.1/24.9, v/v/v) and acetone/acetic acid/water (75/0.1/24.9, v/v/v). These are supported by the results: (i) ethanol/acetic acid/water took advantage in obtaining mulberry polyphenols with the highest yield (49.81 mg gallic acid equivalent/g dry weight), and cellular antioxidant capacity (63.2 μmol quercetin/100 g), as well as low cell cytotoxicity (≥ 50.0 mg/mL); and (ii) acetone/acetic acid/water was the best choice for extracting mulberry polyphenols with the most various compositions (two phenolic acids, four anthocyanins and four flavonols), greatest potential in inhibiting proliferation of HepG2 cells ($\text{IC}_{50} = 28.2$ mg/mL) and highest intracellular antioxidant activity (38.0 μmol quercetin equivalent/100 g). Another systematic study about the effect of solvents including ethanol/water (50/50, v/v), acetone water/acetic acid (70/29.5/0.5, v/v/v) and acetone/water (50/50, v/v) on the extraction of anthocyanin compounds from *Morus nigra* fruit was conducted. Results indicate acetone/water/acetic acid (70/29.5/0.5, v/v/v) solvent mixture was the most efficient co-solvent for the extraction, which could be attributed to the destruction of cell membranes by this mixed solvent combination, simultaneous dissolution and stabilization of the anthocyanins (Boeing et al., 2014). The effects of extraction time, temperature, particle size, number of extraction, and the concentration of raw material on the yield of *Morus alba* leaf polysaccharides were investigated by Yuan et al. (2015). The results indicate that increasing extraction time, temperature, and extraction time, small powder size, and low concentration can favor the solubility and the yield of polysaccharides. The highest yield was achieved at an extraction temperature of 85 °C with extraction time 5 h, the concentration of raw material 55.56 mg/mL, and extracted for four times after response surface methodology optimization. Similar work was carried out to elevate the antioxidant capacities of *Morus alba* leaf extract against on ABTS, DPPH, cupric ion and Fe^{2+} by optimizing these parameters (Tchabo et al., 2018). Additional examples of application SLE for the optimum extraction of analytes from mulberry employing different extraction conditions are presented in Table S1.

SLE is advantageous compared with other methods due to low processing cost and convenience of operation. However, this method often uses toxic solvents, requires an evaporation/concentration step for recovery. Therefore, it usually calls for large amounts of solvents, which are hazardous to health and environment, difficult to be removed completely, and time-consuming. Moreover, the possibility of thermal degradation of bioactive ingredients cannot be neglected because of the high temperatures of the solvents during extended extraction time. For these reasons, new methods and procedures are rapidly developed to address the limitations inherent to conventional SLE techniques.

Over the past few years, new extraction techniques have been

continuously developed with the aims of shortening operating time, reducing organic solvent consumption, increasing extraction efficiency, and reducing costs and environmental pollution. Several new methodologies with significant potential benefits are reviewed in the following paragraphs. It is critical to focus on the unique characteristics of the various target compounds being isolated before adopting these new technologies.

3.2. Assisted extraction

The applications of enzymes, ultrasound wave, and microwave have been adopted to enhance various extraction methods. These assisted extraction techniques are often applied in a pretreatment step to enhance the recovery of bioactive compounds.

3.2.1. Microwave-assisted extraction (MAE)

MAE is a method for extracting soluble products into a fluid from a wide range of materials by using microwave energy. When microwaves pass through a medium, their energy can be absorbed and converted into thermal energy, which is the theoretical basis for the development of MAE. By heating and evaporating the moisture with microwave, high pressure on the cell wall and organelles is generated, which enhances the porosity of the biological matrix and accomplishes increased penetration of the extracting solvent, thus resulting in the improved recovery of the desired intracellular component.

In contrast to conventional SLE, which requires a relatively long extraction time, the use of microwave energy for solution heating significantly reduces the extraction time. In addition to having the advantage of a high extraction speed, MAE is also less labor intensive with increased extract yield and reduced solvent consumption. Recently, these characteristics have been demonstrated in the extraction of bioactive compounds from mulberry. Li, Li, and Tang (2009) developed an optimized process of MAE with a high extraction yield of 25 mg/g flavonoids from *Morus alba* leaves relying on the following parameters: 60% ethanol, 66.67 mg/mL raw material concentration, 560 W microwave powder and 5 min extraction time. Zuo et al. (2013) investigated the effects of microwave parameters on the MAE of mulberry (unknown variety). Their results indicate that factors affecting MAE of mulberry anthocyanin from greatest to least were the extraction time, the solvent composition, and the substrate concentration. The process was efficient with the yield of 2.098 mg/g anthocyanin at extraction time of 267s. Thirugnanasambandham, Sivakumar, and Maran (2015) used a response surface method to optimize the process variables (substrate concentration, microwave power, and extraction time) on the yield of polysaccharides from mulberry leaves. The most promising results were obtained by employing MAE (10 min) with an up to 9.41% polysaccharides yield, which is closed to the maximum polysaccharides yield of 10% by using SLE processing technology with 3.5 h of extraction time in a report by Yuan et al. (2015). An analogous study characterized the effect of the above microwave parameters of MAE on anthocyanin content from *Morus nigra* fruit and showed that MAE (10 min) was more effective than SLE (6 h), as the evidence by the fact that MAE resulted in better outcomes compared to SLE (12.63 mg/mL vs. 10.93 mg/mL anthocyanin and IC_{50} 1.60 mg/mL vs. 2.81 mg/mL for tyrosinase inhibitory activity) (Koyu, Kazan, Demir, Haznedaroglu, & Yesil-Celiktas, 2018). MAE applied to polyphenol extraction from *Morus nigra* leaf showed similar results with MAE affording higher total polyphenol content and better antioxidant activities against Fe^{2+} , DPPH, and oxygen radical than SLE and UAE (Radojković et al., 2017).

As a result, left off MAE is increasingly regarded as a viable approach for the extraction of bioactive compounds due to its distinct advantages over traditional SLE, which is indeed worthy of follow-up and further advanced research. However, one area where MAE is not suitable is for the extraction of heat-sensitive bioactive compounds because it is usually performed at high temperature. Another important limitation hindering its application in industry is that it is less

compatible with a flow system.

3.2.2. Ultrasonic assisted extraction (UAE)

Ultrasound, like microwaves, passes through a medium by creating compression and expansion. Ultrasound produces a phenomenon called cavitation, which results in the growth and collapse of bubbles. The large amount of energy, produced from the conversion of kinetic energy of motion, can be converted into heating within the bubble. Furthermore, bubbles can result in a rarefaction cycle or collapse leading shock waves, thus, affording extreme changes in pressure and temperature. The implosion of cavitation bubbles hits the solid matrix and disintegrates the cells, resulting in the release of the desired compounds. For example, UAE has been employed to disrupt cell of *Morus atropurpurea* leaf to isolate protein fractions and the fractions showing encouraging functional properties and antioxidant activities (Sun et al., 2018). Furthermore, UAE has been developed into an alternative to conventional SLE. The advantages of UAE include a reduction in extraction time, energy, and required solvent. Ultrasound energy for extraction also facilitates more effective mixing, faster energy transfer, reduced thermal gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, quick start-up, increased productivity, and reduced process steps.

Today, UAE is used extensively in the extraction of valuable molecules. For example, polysaccharides have been extracted from mulberry (unknown variety) fruit and leaf using UAE, in which the extraction process was optimized for substrate concentration, ultrasonic power, extraction temperature and time (Chen, You, Abbasi, Fu, & Liu, 2015; Zhang et al., 2016). Zou, Wang, Gan, and Ling (2011) maximized the extraction yield of anthocyanins (64.70 ± 0.45 mg/g) from *Morus alba* fruit, identifying the optimal conditions of UAE (63.8% methanol contains 1% (v/v) trifluoroacetic acid, 43.2 °C temperature, 23.8 (v/w) liquid-to-solid ratio, and 40 min time) using response surface method. Similarly, Espada-Bellido et al. (2017) investigated the effect of solvents and pH in addition to the above parameters of MAE on the extraction of anthocyanins and phenolic compounds from *Morus nigra* jam. Finally, extraction temperature and solvents were found to be the most influential parameters for UAE of anthocyanins (48 °C and 76% methanol) and phenolic compounds (64 °C and 61% methanol). Another study reported using acidic methanol and 60 min sonication in UAE resulted in significantly higher phenolic (isolated from *Morus alba* fruit) contents than extractions with other solvents (ethanol, methanol, and acidic ethanol) and with other sonication times (0, 5, 15 and 30 min) (Kim & Lee, 2017).

Additionally, UAE has been developed as an efficient method for the extraction of mulberry bioactive compounds. It was utilized to establish an estimation model to extract the flavonoid contents of 86 varieties of mulberry (*Morus alba*, *Morus multicaulis*, etc.) leaves (Zhao & Zhang, 2018). Moreover, Vichasilp et al. (2009) improved the extraction efficiency (98% vs. 55%) and productivity (20% vs. 11%) of DNJ from mulberry leaves over standard SLE at 50 °C for 2 h by using UAE under optimized conditions (50% methanol, 0.14 g/mL mulberry leaf powder, 180 W, and 260 s). A similar result using MAE showed more effective extraction yields than for SLE (22.66 mg/g vs. 15.29 mg/g) in isolating phenolic compounds from *Morus alba* leaf (Zhou et al., 2018).

Notably, UAE has been employed for the isolation bioactive compounds in processes ranging from laboratory-scale to large-scale industrial operations, including full-scale commercialized extraction applications. Particular challenges in UAE technologies include wave attenuation in dispersed phase systems because of the decrease in sound wave amplitude with distance and difficulties in the extraction of unstable compounds.

3.2.3. Enzyme-assisted extraction (EAE)

Some phytochemicals in the plant matrices are dispersed in the cell

cytoplasm, and some are retained in the polysaccharide-lignin network through hydrogen-bonding or hydrophobic interactions that are not accessible to solvent during routine extraction processes. Enzymatic pre-treatment is a novel and effective way to degrade cell wall structure by hydrolyzing plant cell wall polysaccharides, facilitating the release of desired compounds, and increasing overall extraction yield. Some commonly used enzymes include cellulase, β -glucosidase, xylanase, β -glucuronase, and pectinase. EAE represents a useful approach for optimizing the extraction of compounds trapped or linked by plant cell walls, and EAE is eco-friendly and nontoxic, and often can eliminate organic solvents from a process. Since enzymes are both catalytic and specific, EAE is not only efficient but also generally preserves the original characteristics of the compounds being isolated.

There are several reports using EAE on mulberry plant tissues for the extraction of bioactive compounds from mulberry. The enzymes Pectinex USP-L, Pectinex UF, Pectinex Ultra Colour, and Klerezyme 150 have been independently employed to improve the release of phytochemical compounds and volatiles from *Morus nigra* must, resulting in a maximum level of extraction of phenolics and volatiles when only Pectinex UF was used alone, and a maximum level of flavonoid and anthocyanin treated with Klerezyme 150 (Tchabo, Ma, Engmann, & Ye, 2015; Tchabo, Ma, Engmann, & Zhang, 2015). Polysaccharide, efficiently extracted from *Morus alba* leaves using pectinase and protease, represents a promising natural additive in liquid dairy products like yogurt (Yang, Li, Jia, Yao, & Liu, 2017).

EAE technology is a feasible alternative to traditional extraction methods for the recovery of mulberry bioactive compounds and can also be applied in large-scale operations. The versatility of enzymes to catalyze a variety of processes for the production of bioactive compounds represents an interesting issue needed to be further investigated regarding their activity, robustness, recoverability, and efficiency.

3.3. Pressurized liquid extraction (PLE)

PLE allows for the fast extraction of compounds with minimum solvent consumption. In the method, high pressure is used to maintain the solvents in their liquid state at high temperatures (frequently at temperatures above solvent boiling point). These high temperatures generally increase the capacity of solvents to solubilize solutes and solute diffusion rates, enhance the disruption of solute-matrix bonds, and reduce the viscosity of the solvent and solvent surface tension.

The use of PLE in the extraction of phytochemical constituents, however, raises certain concerns. In comparison to the traditional solvent extraction, since PLE dramatically decreases extraction time and solvent consumption, it has been successfully applied to extract bioactive compounds from mulberry. In PLE, the nature of the solvent or solvent mixture, the ratio of solvent volume to sample mass, the extraction pressure and temperature, the number of extraction cycles and the duration of each cycle can result in variable extraction efficiency. Yang, Ou, Zhang, Zhou, and Ma (2017) investigated the effect of different solvents on the recovery of the total phenolic compounds and their antioxidant activities when treated *Morus atropurpurea* fruit with PLE. The results showed that water-organic solvent mixtures (acidified methanol, and acidified acetone) increased the antioxidant activities of the extracts compared with these prepared with water or pure organic solvents. Another study examined the influence of temperature, extraction time, and extraction cycle on PLE of rutin and quercetin from mulberry (unknown variety). Compared to UAE and heat-reflux extraction, an optimized PLE method achieved the highest extraction efficiency in the shortest extraction time with the lowest solvent consumption (Wu, Chen, Fan, Elsebaei, & Zhu, 2012). PLE showed a higher extraction efficiency for total phenolic compounds (2186.09 μ g/g vs. 1916.37 μ g/g) from *Morus nigra*, and required less methanol (47.2% vs. 76.0%, v/v), shorter times (10 min vs. 60 min) and lower temperature (48 °C vs. 75.5 °C) than UAE (Espada-Bellido et al., 2018). PLE has also been applied to extract bioactive and low molecular weight

carbohydrates (iminosugars and inositols) from *Morus alba* leaves. Under optimized conditions, PLE provided a similar yield to the conventional SLE process used to recover these bioactives but cost much less time (5 min vs. 90 min) (Rodriguez-Sanchez, Ruiz-Aceituno, Sanz, & Soria, 2013).

3.4. Supercritical fluid extraction (SFE)

SFE, an emerging and environmentally friendly technology, is a process in which supercritical fluids (at the vapor-liquid critical point) are used to extract the components of interest from a solid or even liquid matrix, such as plants and food by-products. This technique often relies on nontoxic organic solvents to accomplish reduced pollution, high selectivity, fast extraction, and no degradation of active ingredients, which circumvent the prolonged exposure to elevated temperatures and atmospheric oxygen, and produce products without toxic residues. SFE is based on the properties of the fluids used, such as density, diffusivity, dielectric constant, and viscosity. In general, modified conditions such as pressure and temperature are employed to obtain a supercritical fluid (SF). Carbon dioxide is currently the fluid of choice in SFE. Under these conditions, the fluid is between gas and liquid because the density of an SF is similar to that of liquid while the viscosity is similar to that of a gas. Thus, the supercritical state of a fluid is the state in which liquid and gas are identical to each other. SF has better transport properties than liquid because the transport properties depend on the density of SF which, unlike liquid solvents, is adjustable by changing pressure and temperature.

SFE may become a standard extraction technique for studying herbal, food and agricultural samples, including mulberry, because of increasing public interest in safety and purity of natural products. Radojkovic et al. (2016) have applied supercritical carbon dioxide to extract biological components from *Morus alba* and *Morus nigra* leaves. It was found that the efficiency of SFE technique was 1.15-fold higher than that of Soxhlet technique in the isolation of non-polar compounds with non-polar solvents such as hexane. SFE was carried out to obtain α -amyrin acetate from the root bark of *Morus alba*. The extraction yield increased with the rise of temperature when the pressure was above 20 MPa and reached the maximum at the temperature of 40 °C and the pressure of below 15 MPa. Consequently, the highest yield of α -amyrin acetate obtained at 60 °C and 20 MPa was 3.68 ± 0.32 mg/g (Choi, Kim, Noh, Choi, & Yoo, 1997). Moreover, Boeszormentyi et al. (2009) established the best SFE conditions to extract β -sitosterol from *Morus alba* leaf and stem bark that were 30 MPa for 60 min and 40 MPa for 60 min, respectively. These studies indicated that the SFE had been developed primarily as an alternative method for the extraction of low volatile and thermal liable compounds that can be used as nutritional dietary supplements. However, SFE cannot be widely used due to its high costs.

3.5. Solid phase extraction (SPE)

SPE is a sample preparation technique by which compounds that are dissolved or suspended in a liquid mixture (the mobile phase) can be separated into groups of desired and undesired components by passing through a solid phase (the stationary phase).

Traditional and more recently developed extraction methods (i.e., SLE, MAE, and PLE, etc.) have poor selectivity and result in the co-extraction of relatively large amounts of undesirable components (i.e., lipids, sterols, chlorophylls, etc.), which severely stress subsequent separation, purification and analytical methods, such as GC or HPLC analysis. Thus, additional clean-up procedures are often required before gas or liquid chromatographic analysis of extracts. Since the integration of extraction with sample preparation in SPE enables the on-site sampling and analysis, this technology has recently been widely applied to recover bioactive constituents from mulberry. For example, polyamide SPE was applied to purify the crude methanolic extract from *Morus*

nigra fruit before analysis of the anthocyanin pigments by employing spectroscopies and HPLC (Hassimotto, Genovese, & Lajolo, 2007). Similarly, Chen et al. (2015) and Chen et al. (2015) have used SPE to recover free volatile chemicals exhibiting strong herbaceous notes and glycosidically linked volatiles that were responsible for the sweet and spicy qualities from *Morus atropurpurea* fruit efficiently. SPE was also used to isolate aroma compounds from *Morus nigra*, *Morus macroua* and *Morus alba* fruits. The results found that benzaldehyde, ethyl butanoate, (*E*)-2-nonenal, 1-hexanol, hexanal, methional, 3-mercaptohexyl acetate and 3-mercaptohexanol were present with much higher odor activity values than other 2-(methylthio)ethanol, methional, dimethyl sulfide, and 3-ethylthiophene compounds (Zhu, Wang, Xiao, & Niu, 2018). As an alternative to the sole use of SPE, SPE combined with other extraction methods has been demonstrated quite effective for the purification and isolation of active components from mulberry plants. For instance, Pothinuch and Tongchitpakdee (2011) demonstrated the utility of integrated ULE and SPE for the fractionation of melatonin from mulberry (unknown variety) leaves. A higher recovery rate (76%) was obtained compared with a process relying on homogenization in combination with liquid-liquid extraction procedure (12% recovery).

SPE has been developed as an efficient extraction tool because of its various advantages, such as straightforward, rapid, and solvent-free sample extraction and concentration method. The combination of SPE with gas chromatography, high performance liquid chromatography, capillary electrophoresis, mass spectrometry or nuclear magnetic resonance spectroscopy also represents an important advance in analytical technology. For example, SPE coupled with high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy has been applied to study the polypharmacological properties (α -glucosidase/ α -amylase/protein tyrosine phosphatase-1B inhibitory activities/radical scavenging activity) of crude root bark extract of *Morus alba* (Zhao, Kongstad, Jager, Nielsen, & Staerk, 2018).

SPE is currently only suitable for lab-scale extractions and has not been widely used in industrial applications.

3.6. Combined extraction techniques

Due to the drawbacks of each above extraction techniques, combination of different techniques gives the most satisfactory results. For instance, combined treatment on *Morus alba* wood with SLE (2% NaOH at 65 °C for 2 h) and cellulase (15 U/g) were 31 times better than cellulase-assisted treatment, 3.88 times better than NaOH treatment, respectively (Wang et al., 2018). Isolation phytochemical compounds from *Morus nigra* must by ultrasound-assisted enzymatic extraction yielded 2.98 mg/mL total phenolics, 3.79 mg/mL total flavonoids, and 0.55 mg/mL total anthocyanins, which is more efficient than SLE (2.31 mg/mL total phenolics, 3.48 mg/mL total flavonoids, and 0.52 mg/mL total anthocyanins), EAE (2.72 mg/mL total phenolics, 3.56 mg/mL total flavonoids, and 0.51 mg/mL total anthocyanins), and UAE (2.87 mg/mL total phenolics, 3.65 mg/mL total flavonoids, and 0.53 mg/mL total anthocyanins) (Tchabo, Ma, Engmann, & Zhang, 2015).

In addition to the aforementioned extraction processes, mechanical means are occasionally employed to enhance molecular interactions, including vortex mixing, centrifugation, mechanical stirring, and continuous rotary extraction.

Extraction is just the first step in the study of natural bioactive compounds. Further separation and purification are needed to explore mechanisms of actions of these substances.

4. Separation techniques for mulberry bioactive compounds

Mulberry contains hundreds of chemical constituents, including anthocyanin, polysaccharide, phenols, flavonoids, and alkaloids, which have widely different nutraceutical and pharmaceutical activities and

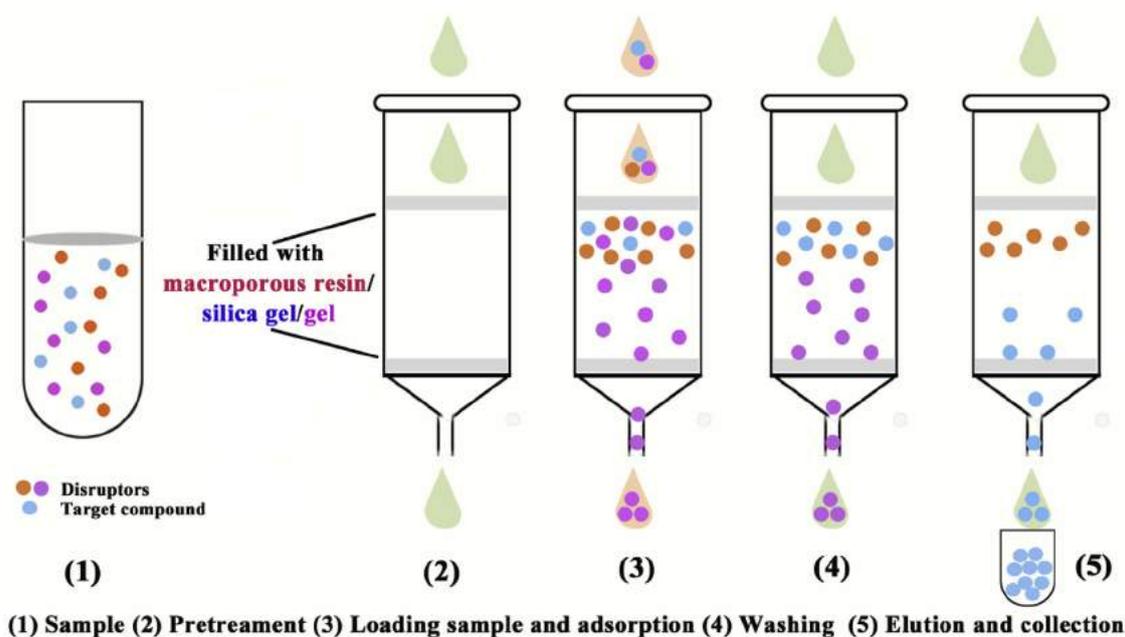


Fig. 2. Schematic diagram of MRA/SGC/GFC system.

sometimes toxicity. Therefore, both efficient and selective methods are required for the separation of these constituents from mulberry. Efficient and selective methods have become important separation techniques in natural products research, which are the topics in the subsequent sections of this review.

4.1. Column chromatography

Column chromatography is a chromatography technology used to separate bioactive compounds according to the distribution coefficients of the components in the stationary phase and the mobile phase. MAR, SGC, IEC and GFC are the common column chromatography technologies applied to isolate bioactive compounds from mulberry.

4.1.1. Macroporous resins adsorption (MRA)

Macroporous resin, a macroporous polymer resin with a terepiform structure, has a large specific surface area that can selectively and physically adsorb organic compounds from an aqueous solution. The schematic diagram of MRA adsorption is shown in Fig. 2. The interactions of organic compounds with MRA generally involve electrostatic interactions, hydrogen bonding, van der Waals forces, hydrophobic interactions, and size sieving action. Hence, the products of interest can be separated according to the intensity of interaction between MRA and an organic compound. The use of MRA has afforded an alternative method for the separation and enrichment of bioactive components from mulberry (see Table 2), showing that MRA is suitable for the separation of polysaccharides and anthocyanins, and marginally suitable for the isolation of polyphenols, flavonoids, alkaloids, and other compounds from mulberry. Because of MRA's convenience, low cost, high chemical stability, easy regeneration and adjustable selectivity by modification of surface chemistry and control of pore structure, it is often used to adsorb constituents selectively from aqueous solutions as well as non-aqueous solutions. MRA not only can enrich desired constituents that are found in mulberry extract but also can efficiently remove the impurities. For example, Chen et al., (2016) have systemically investigated five types of macroporous resins (XAD-7HP, AB-8, HP-20, D-101, and X-5) for the separation of *Morus alba* anthocyanins. Due to the similar polarity to anthocyanins, XAD-7HP was consistently more efficient than the other resins for adsorption/

desorption ratios of 86.45% and 80.81%, respectively, based on static tests. After the optimization of the dynamic adsorption curve of anthocyanins on XAD-7HP at different flow rates (1.0–2.5 mL/min), eluent ratios (20–100% ethanol), and pH values (1–7), the purity of anthocyanins reached 93.6% using 40% ethanol elution on XAD-7HP at the flow rate of 2.0 mL/min. Another study reported AB-8 with larger specific surface area and pore size than NKA-II resin was selected as the optimal discoloration reagent for the further purification of *Morus alba* leaf polysaccharide, in which three fractions were obtained with the purities of 63.56%, 68.16%, and 73.38%, respectively (Yang, Ou, et al., 2017). The 732 resin was the most appropriate resin for the separation of DNJ from *Morus alba* leaves extracts, with adsorption behavior described as Langmuir isotherms with a two-step adsorption kinetics model. The recovery and purity of DNJ in the final product were 90.51% and 15.3%, respectively (Wang et al., 2014). Chen and Li (2007) purified *Morus alba* leaf flavonoids extraction, a type of compounds with significant anti-hyperlipidemia effect using a NKA-9 macroporous resin and increased the content of flavonoids by approximately 19-fold–581.7 mg/g.

Although MRA is still a developing separation technology, it represents a promising technology for the purification bioactive substances because its selectivity and efficiency can be further improved. It is currently classified as one of the key methods for the purification of the traditional Chinese medicines because of its widespread application in the production of bioactive components of these medicines.

4.1.2. Silica-gel chromatography (SGC)

SGC is a technology that utilizes silica gel as stationary phase and separates target substances according to their absorption capacities on silica gel. Generally, compounds with relatively high polarity are easily adsorbed on silica gel, those with low polarity are on the contrary, thus selectively desorbing aim products from silica gel in solvents with appropriate polarity. The process of absorption and desorption of SGC is similar that observed for MRA, as shown in Fig. 2.

Thus, SGC is commonly used to separate polar substances, such as polyphenols and flavones, from mulberry. Specifically, Lim et al. (2015) loaded the ethyl acetate fraction of *Morus alba* root bark methanol (80%, v/v) extraction on silica gel column and eluted 41 fractions with *n*-hexane-ethyl acetate. The 37th fraction was subjected to a second

Table 2
Separation of bioactive compounds from mulberry by MRA.

Compounds	Source	Types of resins	Result	Reference
Polysaccharides	<i>Morus alba</i> leaf	AB-8	The retention rate and decolorization rate of mulberry leaf polysaccharide were 78.77 and 97.28%, respectively	Yang et al. (2017)
	<i>Morus alba</i> fruit	AB-8	The polysaccharide had antioxidant and hypo-glycemic properties and could provide a protective effect on diabetic mice	(Chen et al., 2017; Chen et al., 2016)
	Unknown mulberry variety leaf	ADS-17	The purity of the fraction reached 83.42%, and the fraction had excellent antioxidant activity against Fe ²⁺ and ABTS radical	Zhang et al. (2016)
Anthocyanins	<i>Morus alba</i> fruit	XAD-7	The fraction improved thyroid cancer progression mainly by inducing apoptosis and autophagy cell death	Long et al. (2018)
	<i>Morus alba</i> fruit	XAD-7HP	The purity of the fraction reached 93.6%	(Chen et al., 2016)
	<i>Morus atropurpurea</i> fruit	D3520, D4020, D101A, X-5, AB-8, NKA-9	X-5 was the best adsorbent capability for anthocyanins, the recovery and purity of anthocyanins were 99.33 and 76.33%, respectively	Liu, Xiao, Chen, Xu, and Wu (2004)
	<i>Morus nigra</i> fruit	AB-8	The composition of purified product: 237.5 mg/g anthocyanins, 98.1 mg/g C3G and 26.4 mg/g C3R	Jiang, Dai, Nie, Yang, and Zeng (2017)
Polyphenols	<i>Morus alba</i> fruit	AB-8	The fraction protected hepatocytes against oxidative stress	(Yan et al., 2017; Yan & Zheng, 2017)
	<i>Morus multicaulis</i> branch bark	D-101	The recovery rate of 30% ethanol elution fraction including mulberroside A was 21.84%	Wang et al. (2014)
	<i>Morus multicaulis</i> branches	D-101	The recovery rate of 100% ethanol elution fraction including morusin was 11.08%	Wan et al. (2014)
	<i>Morus alba</i> leaf	NKA-9	The flavonoids content was 581.7 mg/g in dried product post purification, increase 19 times compared to these in mulberry leaves	Chen and Li (2007)
	<i>Morus alba</i> leaf	H103	The recovery and purity of total flavonoids in the final product were 90.57 and 76.33%, respectively	Wang, Wu, Zhao, Liu, and Wu (2008)
Alkaloids	<i>Morus alba</i> leaf	732	The recovery and purity of DNJ in the final product were 90.51% and 15.3%, respectively	Wang et al. (2014)
γ-Aminobutyric acid	<i>Morus alba</i> leaf	D-101	Getting a preliminary fraction for further purification by IEC and polyamide resin column chromatographies	Wang et al. (2010)
	<i>Morus alba</i> leaf	S-8	The decolorization ratio and recovery rate of GABA were 91.17% and 95.79%, respectively	(Chen et al., 2016)

Table 3
Recent studies on separation of bioactive compounds from mulberry by SGC.

Compounds	Source	Result	Reference
Polyphenols			
Oxyresveratrol	<i>Morus alba</i> branch	The substance significantly reduced fasting plasma glucose level increased hepatic glucose transporter 2 transcription and glycogen content	Ahn et al. (2017)
Morusin and moracin C	<i>Morus alba</i> root bark	Morusin exhibited strong α -glucosidase inhibitory activities with IC_{50} less than $10 \mu M$, and showed moderate cytotoxic effects against lung cancer cells	Li et al. (2018)
Moracin M, O, P and S	<i>Morus alba</i> root bark	Moracin S possessed the highest β -site amyloid precursor protein cleaving enzyme 1 inhibitory activity in a dose-dependent fashion by interacting with key catalytic aspartyl residues of the enzyme.	Seong et al. (2018)
Flavonoids			
Saggenon C, M and O	<i>Morus nigra</i> twigs	The saggenons showed significant inhibitory effects on mushroom tyrosinase	Hu et al. (2018)
Saggenon A, F, G, L and N	<i>Morus alba</i> root bark	The saggenon F could inhibit lipopolysaccharide-induced NO production in RAW 264.7 macrophages	In (2017)
Saggenon G	<i>Morus alba</i> root bark	The product possessed antidepressant-like effect and maybe an alternative therapeutic approach for the treatment of depression	Lim et al. (2016)
Saggenon X	Unknown mulberry variety root bark	The fractions separated by SGC were further purified by GFC and PLC to obtain saggenon X	Lu et al. (2018)
Kuwanon G	<i>Morus alba</i> root bark	Kuwanon G displayed significant tyrosinase inhibitory activity with an IC_{50} value of $67.6 \mu M$, and potent activity toward L-DOPA with an IC_{50} value of $44.04 \mu M$	Koirala et al. (2018)

silica gel column and eluted with methanol (66.7%, v/v) to obtain sanggenon G, identified by several spectroscopic methods, including nuclear magnetic resonance, infrared spectroscopy, and mass spectrometry. Similarly, moracins were isolated from the ethyl acetate fraction of *Morus alba* root bark methanol extraction by SGC with a dichloromethane: methanol solvent system (1:0 to 0:1 gradient) and methanol-water solvent system (3:1, v/v) (Seong et al., 2018). In addition, kuwanon T, sanggenon F, N and G, along with moracin E were isolated from the root bark of *Morus alba* using SGC with the assistance of GFC (Jung et al., 2015). Some recently additional examples that have utilized SGC to separate mulberry bioactive compounds are summarized in Table 3.

4.1.3. Ion exchange chromatography (IEC)

IEC is a technological process that separates ions and polar molecules based on their affinities to an ion exchanger. Ingredients can be selectively separated based on their charges through the choice of different columns, by altering the pH of eluents, or by adding salt to the eluent. A schematic diagram for IEC separation is shown in Fig. 3.

There is a growing interest in the application of IEC in the preparation of functional mulberry compounds. IEC has been used to investigate the chemical nature and pharmacological activities of polysaccharides, DNJ and anthocyanins in mulberry. For example, Lee et al. (2013) applied DEAE-cellulose, a weak ion exchanger, to purify the crude polysaccharide extract from *Morus alba* leaf. One major carbohydrate-containing peak, eluted with 0.4 M NaCl, was comprised of neutral saccharide residues determined to be galactose (37.6%, in mole percent), arabinose (36.3%), rhamnose (18.4%), and minor amounts of glucose, xylose, mannose, and fructose. Bioactive carbohydrates (iminosugars and inositols) from PLE extracts of *Morus alba* leaves were fractionated by removing other carbohydrates that interfere with the bioactivity of iminosugars and inositols through cation-exchange chromatography. The results demonstrate that IEC can completely remove the major, soluble, and inactive carbohydrate components (fructose, glucose, galactose, and sucrose), without impacting the content and activities of iminosugars and inositols (Rodriguez-Sanchez et al., 2013). Liu et al. (2015) reported that DNJ was isolated by successive use of cation exchange resin, anion exchange resin and silica gel. The DNJ obtained was recovered in over 50% yield with a purity of 95%. This DNJ sample significantly improved insulin sensitivity by activating insulin signaling PI3K/AKT pathway in skeletal muscle of db/db mice. Moreover, a *Morus australis* fruit extract was loaded on a cation exchange resin to remove proteins, sugars, organic acids and other water-soluble compounds by distilled water and the binding anthocyanins were desorbed with 1% formic acid in methanol (Wu et al.,

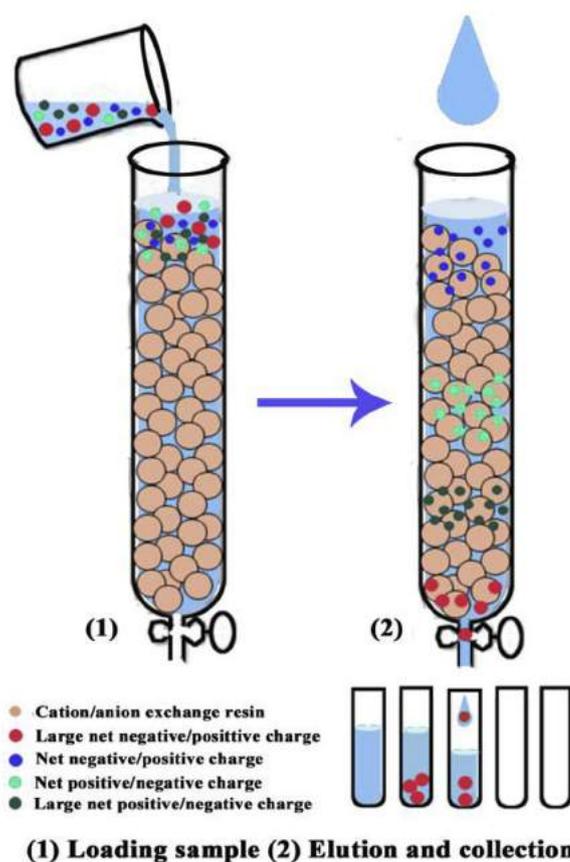


Fig. 3. Schematic diagram of IEC system.

2016). Other recent research on the application of IEC for separation active substances are presented in Table 4. These studies demonstrate that IEC is not only a controllable, highly selective and capable method affording high recovery, concentrate components, and also can be economically applied to the large-scale industrial purification of bioactives. Since IEC is a useful and popular method for the isolation of natural products in modern drug discovery, its application will continue to expand with the development of new IEC technologies.

4.1.4. Gel filtration chromatography (GFC)

GFC, also known as molecular-exclusion chromatography, is a

Table 4
Recent studies on separation of bioactive compounds from mulberry by IEC.

Compounds	Source	Types of resins	Result	Reference
Polysaccharides	<i>Morus multicaulis</i> fruit	anion	Three combined polysaccharide fractions were obtained through eluting with distilled water, 0.2 M NaCl, 0.3 M NaCl, respectively	Liao et al. (2018)
	<i>Morus alba</i> fruit	anion	Five polysaccharide fractions was eluted with 0.1–0.5 M NaCl	Wang et al. (2018)
	<i>Morus alba</i> leaf	anion	The molecular weights of the polysaccharide fractions were 7.812×10^3 (hot buffer extraction), 3.279×10^3 (chelating agent extraction), 6.912×10^3 (dilute alkali extraction), and 1.408×10^3 kDa (concentrated alkali extraction)	Liao et al. (2017)
DNJ	Unknown mulberry variety leaf	cation	After purification of IEC, the fractions was further separated by SGC; and the purity of final product was over 95%	Chen, Liang, et al. (2018)
	<i>Morus alba</i> leaf	cation	Getting a preliminary fraction for further purification by SGC	Liu et al. (2016)
	<i>Morus alba</i> leaf	unknown	The purity of DNJ was showed to be over 98%	Shuang et al. (2017)
Anthocyanins	<i>Morus alba</i> fruit	cation	Impurities including polysaccharides, organic acids and other water-soluble compounds were removed by 0.1% HCl solution; and the anthocyanins was subsequently eluted with ethanol/deionized water/HCl (80:20:0.1, v/v)	(Chen et al., 2017)
	<i>Morus alba</i> fruit	cation	The high purity of anthocyanins was obtained, mainly consisted of C3G and C3R	(Chen et al., 2016)

column chromatographic technology in which molecules in solution are separated based on their molecular weight. A schematic diagram of GFC, analogous to MAR and SGC, is shown in Fig. 2. The chromatography column is packed with porous reticular beads which are composed of dextran polymers (Sephadex), agarose (Sephacrose), or polyacrylamide (Sephacryl). In general, GFC is considered a low resolution chromatographic technique since it does not separate similar species very well, and is often used as a means for further separation after using other methods such as MAR, SGC and IEC. GFC is widely used as a final step in isolating polysaccharides, polyphenols, anthocyanins, and flavonoids from mulberry. For example, *Morus alba* polysaccharides were purified sequentially by IEC and GFC (Sephacryl S-300). The fraction eluted from DEAE-Sephacryl anion-exchange chromatography with 0.1 M NaCl is then further purified using Sephacryl S-300 gel-permeation chromatography affording final target polysaccharides with a purity of 95.1% that could exert a stimulatory effect on the SGC-7901 cells apoptosis (Chen, Jiang, Xie, Li, & Shi, 2018). Similarly, SGC combined GFC (Sephadex LH-20) were applied to purify sanggenon V, morusin and moracin C from *Morus alba* root bark methanol extracts (Li et al., 2018). Furthermore, C3G and C3R, both with purities above 98%, could be well separated from *Morus alba* pomace extract on Sephadex LH-20 by eluting with 10% ethanol containing 1% of acetic acid after purification with AB-8 macroporous resin (Zhang, He, Pan, Han, & Duan, 2011).

4.2. Preparative liquid chromatography (PLC)

Liquid chromatography (LC) is a well-established, demonstrated, and reliable method for mixture separation that is widely used for quality analysis and quality control, as well as for laboratory sample preparation. PLC not only affords the same ultra-high resolution as LC but also greatly enhances the sample loading amount. Columns can be heavily loaded to achieve high productivities, which is often performed by injecting large sample concentrations, testing solubility limits, or injecting large sample volumes. Consequently, large amounts of highly purified target compounds can be obtained from natural sources. Fig. 4 depicts the schematic diagram of PLC system.

Generally, PLC has been reserved for the final step of separation and purification small molecular entities, such as polyphenols and flavonoids, from mulberry. For example, high-purity morusin has been prepared using non-polar macroporous resin D101 to remove polar impurities from the ethanolic extract of *Morus multicaulis* branches. Sequentially, Sephadex LH-20 resin was used to enrich the main component of morusin followed by the purification of PLC. The resulting extract exhibited a remarkable inhibitory effect on liver cancer cells through a mechanism that maybe related to the increases in the expression of p53, survivin, cyclinB1, and caspase-3 and a decrease in NF- κ B gene expression (Wan, Ma, & Zhang, 2014). Lu et al. (2018) used a

similar process to separate sanggenon X from mulberry (unknown variety) root bark extract, and discovered sanggenon X showed significant antioxidant activity against Fe²⁺-Cys-induced lipid peroxidation in rat liver microsomes. Kuwanon G was also isolated and prepared by PLC after SGC and GFC (Kuk et al., 2017). In another study, PLC was used on a purified mulberroside A fraction isolated with a macroporous resin column, and a single major peak, corresponding to pure mulberroside A, was recovered. These results demonstrate the excellent separation efficiency of PLC. Finally, a high purity (98.3%) mulberroside A was separated by PLC that strongly inhibited monophenolase activity with an IC₅₀ of 1.29 μ M (Wang et al., 2014).

PLC has become a popular method for the final purification step of bioactive compounds since it can significantly improve sample loading amount and ensure an ultra-high resolution. PLC has numerous advantages including high separation efficiency, optimal selectivity, and high-level automation. Unfortunately, when used as a process scale, this technique is expensive and requires the optimization of preparative separation conditions. Therefore, it is very challenging to efficiently and cost-effectively scale-up PLC.

4.3. Countercurrent chromatography (CCC)

Conventional methods such as low-pressure chromatography (with MRA, SGC, IEC, GFC) and PLC are used to fractionate or isolate pure products from plants, but these are tedious, time and solvent consuming, and require multiple chromatographic steps. Under certain circumstances, CCC can be an ideal alternative to these conventional purification methods.

CCC does not use a solid stationary phase as it is a liquid-liquid partition chromatography process, in which both the mobile and the stationary phases are liquids. Compared with the traditional liquid-solid separation methods, CCC benefits from some advantages, such as high recovery of the injected sample, minimal tailing of peaks, low risk of sample denaturation, compatible with the particulate sample, and convenience of changing the solvent systems for each new sample. Currently, various studies are ongoing to improve this conventional technique CCC and develop some new CCC methods for natural product fractionation. CCC can be classified, based on their different principles of fluid mechanics, into hydrostatic equilibrium systems, such as droplet counter-current chromatography, or centrifugal partition chromatography, and hydrodynamic systems, such as high-speed counter-current chromatography (HSCCC). For a detailed explanation of these systems and their main characteristics, a comprehensive review has been published by Pauli, Pro, and Friesen (2008).

CCC, especially HSCCC (Fig. 5), is an emerging separation chromatographic technique that has been used for the large-scale separation of target compounds including polyphenols and anthocyanins from plant tissues derived from mulberry. Riviere et al. (2014) successfully

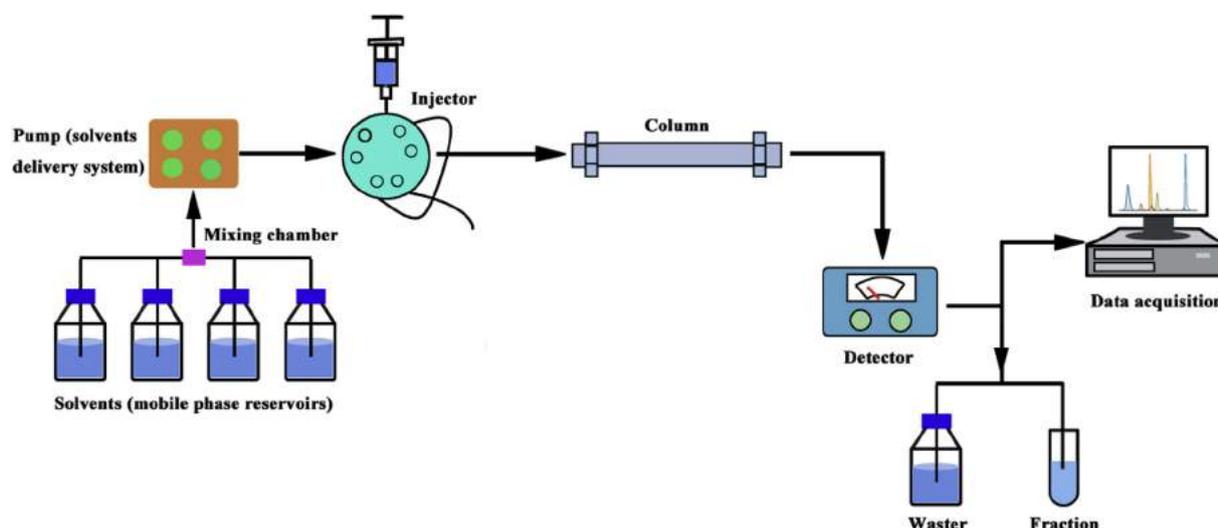


Fig. 4. Schematic diagram of PLC system.

applied HSCCC to the separation of bioactive polyphenols from stems of *Morus alba*. The crude polyphenol extractions by preparative HSCCC produced 20 sub-fractions, including 14 sub-fractions from the ascending mode and six sub-fractions from the descending mode. Then, with the help of ESI-mass spectrometry and nuclear magnetic resonance, these isolated fractions were identified as a new coumarin glycoside, isoscopoletin 6-(6-O-β-apiofuranosyl-β-glucopyranoside), together with seven known polyphenols (mulberroside A, dihydromorin

7-O-β-glucopyranoside, resveratrol, moracin M, steppogenin, dihydromorin and oxyresveratrol), of which resveratrol and moracin M were the most effective compounds at inhibiting NO production induced by LPS. Chen et al. (2017, a, b) reported a similar study. In this research, a strategy combining column chromatography with HSCCC was developed for the separation of high-purity anthocyanin monomers from *Morus alba* fruits. After purification using Amberlite XAD-7HP column with 80% ethanol (0.1% HCl), a fraction of anthocyanins

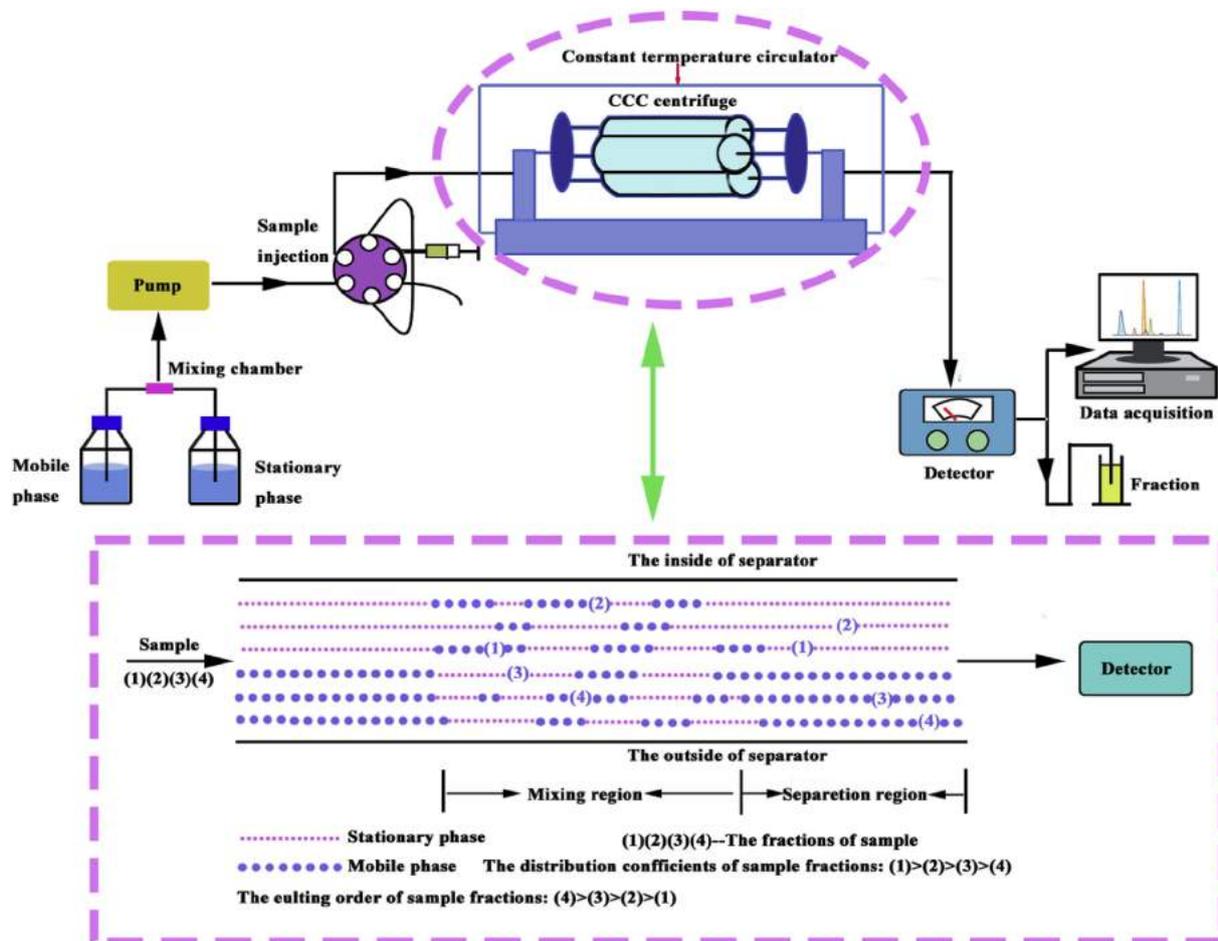


Fig. 5. Schematic diagram of HSCCC system.

mixture with a purity of 68.6% was obtained. Subsequently, three anthocyanin monomers of delphinidin-3-*O*-rutinoside, C3R, and C3G were separated by HSCCC with a biphasic solvent system of *n*-butanol/methyl tert-butylether/acetonitrile/water/trifluoroacetic acid (30:10:10:50:0.05, v/v). The yields of these three monomers were 2.27%, 18.07% and 43.19%, respective, which demonstrated HSCCC to be practical strategy for large-scale separation of pure anthocyanin monomers. Choi et al. (2015) optimized HSCCC parameters using a biphasic solvent systems composed of ether/*n*-butanol/acetonitrile/0.01% trifluoroacetic acid, flow rate, sample amount, and rotational speed to improve the efficiency of the HSCCC process. High purities anthocyanins monomers (C3R and C3G) from crude mulberry (unknown variety) fruit extract were rapidly separated within 60 min under optimum conditions (*tert*-butyl methyl ether/*n*-butanol/acetonitrile/0.01% trifluoroacetic acid, 1:3:1:5, v/v; flow rate, 4.0 mL/min; sample amount, 200–1000 mg; rotational speed, 1600 rpm). Furthermore, the semi-preparative-scale parameters of HSCCC were transferred successfully to preparative scale to achieve efficient and satisfactory separation results for C3G and C3R, as evidenced by the HSCCC recovery and purity of C3G/C3R of 96.2%/87.5%, 97.8%/94.4%, respectively. A similar study performed by Sheng et al. (2014), using the HSCCC with ethyl *tert*-butyl ether, 1-butanol/acetonitrile/water/trifluoroacetic acid (10:30:10:50:0.05, v/v) system, which produced cyanidin 3-*O*-(6''-*O*- α -rhamnopyranosyl)- β -glucopyranoside, keracyanin and petunidin 3-*O*- β -glucopyranoside (yields of 25.7%, 41.3%, 5.1%, respectively, and purity over 95% for all fractions) in 35–60 min from *Morus alba* fruit crude extract at 1.5 mL/min flow rate and 850 rpm revolution speed. In summary, HSCCC is a convenient and rapid method that avoids chemical degradation and irreversible absorption onto solid adsorbents such as resins because it does not employ solid adsorbents.

5. Conclusions

Mulberry is widely used in Asia as natural food and traditional Chinese medicine. Among mulberry's many bio-functional constituents, DNJ has been used in clinical therapy to reduce the blood sugar level. Fruit, leaf, stem, and root bark of mulberry have been commercialized as food or herb to ameliorate constipation, hyperglycemia, and inflammation. Although the list of compounds in this review is by no means exhaustive, most important classes of active substances in mulberry and much of the breadth of biological activities exhibited by natural mulberry products are discussed. The significant potential of mulberry as a functional food ingredient is increasingly being recognized.

There is a great need to develop new and improved novel extraction and separation technologies to better exploit the potential of mulberry-derived natural products. Besides the standard extraction method, SLE is time-consuming and eco-unfriendly. This paper outlines novel extraction technologies, including PLE, SFE, MAE, UAE, EAE, and SPE, which show promising applications in future extraction methods. The new technologies are of great interest owing to their efficiency for promoting the potential of mulberry bioactives as functional foods, drugs, and tools for use in biochemical, pharmaceutical and medical research. However, some challenges need to be addressed before these new technologies can become a reliable option. These difficulties include the extraction of polar compounds by SFE, high susceptibility of matrix effects, thermal degradation of the analytes when using of PLE and MAE, high cost and lack of available substrate-specific enzymes required for EAE, and challenges in the industrial scale application of SPE.

Moreover, the development of modern separation techniques, such as MAR, SGC, IEC, GFC, PLC, and CCC, facilitates the purification and identification of the many different bioactive compounds identified in mulberry, leading to improved studies on the functions of mulberry bioactive compounds. Although MAR, SGC, IEC and GFC are only used

as primary screening tools of extracts when the purity of target compounds is relatively low, PLC and CCC involve very expensive processes and their applications in industrial scale extraction are challenging. Future research priorities in this area should be focused on overcoming the challenges of these extraction and separation technologies, incorporation and development of hybrid or hyphenated methods so that the significant benefits can be achieved by using improved extraction and isolation techniques exploited by industry.

In conclusion, high recovery and environmentally friendly sample preparation and isolation methods are essential in the discovery and enrichment of analytes in plants, particularly in traditional Chinese medicines. Additionally, the development of flexible and online integratable sample extraction and separation methods are the focus of today's traditional Chinese medicines research, commercialization and modernization.

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Abbreviations

SLE	solid-liquid extraction
PLE	pressurized liquid extraction
SFE	supercritical-fluid extraction
MAE	microwave assisted extraction
ULE	ultrasonic assisted extraction
EAE	enzymatic-assisted extraction
SPE	solid-phase extraction
MRA	macroporous resins adsorption
SGC	silica gel chromatography
IEC	ion exchange chromatography
GFC	gel filtration chromatography
PLC	preparative liquid chromatography
CCC	countercurrent chromatography
HSCCC	high-speed counter-current chromatography
DPPH	1,1-diphenyl-2-picrylhydrazyl
COX	cyclooxygenase
C3R	cyaniding-3- <i>O</i> -rutinoside
C3G	cyaniding-3- <i>O</i> -glucoside
NF- κ B	nuclear factor κ B
AKT	protein kinase B
AMPK	adenosine monophosphate activated protein kinase
PI3K	phosphatidylinositol 3-kinase
PPAR	peroxisome proliferator-activated receptor
MMP	matrix metalloproteinase; IL, interleukin; CD, cluster of differentiation
TNF- α	tumor necrosis factor α
Bcl-2	B-cell lymphoma/leukemia-2
iNOS	inducible NO synthase
NO	nitric oxide
LPS	lipopolysaccharide
NSCLC	non-small-cell lung cancer
ERK	extracellular signal-regulated kinases
DNJ	1-deoxynojirimycin
BAT	brown adipose tissue
NSC	rat neural stem cell
SF	supercritical fluid
LC	liquid chromatography

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tifs.2018.11.017>.

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