Could the Gut Microbiota Reconcile the Oral Bioavailability Conundrum of Traditional Herbs?

Feng Chen\textsuperscript{a,*}, Qi Wen\textsuperscript{a}, Jun Jiang\textsuperscript{a}, Hai-Long Li\textsuperscript{a}, Yin-Feng Tan\textsuperscript{a}, Yong-Hui Li\textsuperscript{a}, Nian-Kai Zeng\textsuperscript{a}

\textsuperscript{a}Hainan Provincial Key Laboratory of R&D of Tropical Herbs, School of Pharmacy, Hainan Medical College, Haikou 571199, China.

\*Corresponding author at: Hainan Medical College, 3 Xueyuan Road, Haikou 571199, China, Tel./Fax: + 86-898-66895337, E-mail address: cy.chen508@gmail.com (F. Chen).
Abstract

Ethnopharmacological relevance: A wealth of information is emerging about the impact of gut microbiota on human health and diseases such as cardiovascular diseases, obesity and diabetes. As we learn more, we find out the gut microbiota has the potential as new territory for drug targeting. Some novel therapeutic approaches could be developed through reshaping the commensal microbial structure using combinations of different agents. The gut microbiota also affects drug metabolism, directly and indirectly, particularly towards the orally administered drugs. Herbal products have become the basis of traditional medicines such as traditional Chinese medicine and also been being considered valuable materials in modern drug discovery. Of note, low oral bioavailability but high bioactivity is a conundrum not yet solved for some herbs. Since most of herbal products are orally administered, the herbs’ constituents are inevitably exposed to the intestinal microbiota and the interplays between herbal constituents and gut microbiota are expected. Emerging explorations of herb-microbiota interactions have an opportunity to revolutionize the way we view herbal therapeutics. The present review aims to provide information regarding the health promotion and/or disease prevention by the interplay between traditional herbs with low bioavailability and gut microbiota through gut microbiota via two different types of mechanisms: (1) influencing the composition of gut microbiota by herbs and (2) metabolic reactions of herbal constituents by gut microbiota.

Materials and Methods: The major data bases (PubMed and Web of Science) were searched using “gut microbiota”, “intestinal microbiota”, “gut flora”, “intestinal flora”, “gut microflora”, “intestinal microflora”, “herb”, “Chinese medicine”, “traditional medicine”, or “herbal medicine” as keywords to find out studies regarding herb-microbiota interactions. The Chinese Pharmacopoeia (2010 edition, Volume I) was also used to collect the data of commonly used medicinal herbs and their quality control approaches.

Results: Among the 474 monographs of herbs usually used in the Chinese Pharmacopoeia, the quality control approach of 284 monographs is recommended to
use high-performance liquid chromatography approach. Notably, the major marker compounds (> 60%) for quality control are polyphenols, polysaccharides and saponins, with significant oral bioavailability conundrum. Results from preclinical and clinical studies on herb-microbiota interactions showed that traditional herbs could exert heath promotion and disease prevention roles via influencing the gut microbiota structure. On the other hand, herb constituents such as ginsenoside C-K, hesperidin, baicalin, daidzin and glycyrrhizin could exert their therapeutic effects through gut microbiota-mediated bioconversion.

**Conclusions:** Herb-microbiota interaction studies provide novel mechanistic understanding of the traditional herbs that exhibit poor oral bioavailability. “Microbiota availability” could be taken consideration into describing biological measurements in the therapeutic assessment of herbal medicine. Our review should be of value in stimulating discussions among the scientific community on this relevant theme and prompting more efforts to complement herb-microbiota interactions studies.

**Keywords:** gut microbiota; traditional herbs; oral bioavailability; herb-microbiota interaction; microbiota availability

**Chemical compounds studied in this article:** Baicalin (PubChem CID: 64982); Berberine (PubChem CID: 2353); Daidzein (PubChem CID: 5281708); Daidzin (PubChem CID: 107971); (-)-Epigallocatechin gallate (PubChem CID: 65064); Equol (PubChem CID: 91469); Ginsenoside C-K (PubChem CID: 9852086); Ginsenoside Rb1 (PubChem CID: 9898279); Glycyrrhizin (PubChem CID: 14982); trans- Resveratrol (PubChem CID: 445154)
Introduction

The practice of traditional Chinese medicine (TCM)—mainly herbal medicine—plays an important role in health maintenance not only for the peoples of Asia, and is becoming more frequently used in the West as a form of complementary and alternative medicine (CAM). According to a 2014 market report on the China pharmaceutical industry released by the SFDA's Southern Medicine Economic Research Institute, the China's production of the Chinese Patent Drug sector was valued at ¥ 524 billion in 2013 (http://www.docin.com/p-855174018.html). Meanwhile, after the 10th consecutive year increase, the sales of herbal dietary supplements in the United States were about $ 6 billion in 2013, according to data from previous HerbalGram herb market reports (Lindstrom et al., 2013). Further, two herb-based new drug applications (i.e., sinecatechins and crofelemer) have been approved by FDA in 2006 and 2012, respectively (Lee et al., 2015).

However, TCM differs in substance, methodology and philosophy to western medicine (Cheung, 2011). It stresses the maintenance of balance between the systems of the individual internal physiological systems and external environmental networks. The current challenge is to convince skeptic occidental medical doctors of the application of such medicines, serving better both the practitioners and the patients. However, research in TCM recently has been dominated by the search for its molecular, cellular and pharmacological bases, identifying active substances and investigating mechanisms of action (Tang, 2006). In spite of scientific advances of these works, limitations of this approach still exist. Alternatively, evidence-based approach based on modern scientific techniques (e.g., systems biology–based 'omics technologies) and comparative effectiveness research approach are regarded as valid strategies for exploring TCM and CAM (Verpoorte, 2012; Witt et al., 2015).

Systems biology endeavors to quantify all of the molecular elements of a biological system (Hood et al., 2004). As a proposed approach to biomedical research, systems biology consciously combines reduction and integration of information across multiple spatial scales to identify and characterize parts and explore the ways in which their interaction with one another and with the environment resulting in the
maintenance of the entire system (Kohl et al., 2010). Generally, humans contain proteins, polysaccharides, lipids and nucleic acids with which we can interfere using small-molecule therapeutic agents. Particularly, most of successful drugs achieve their activities by binding to and modifying the activity of a protein, with multiple consequences on various functions (Hopkins and Groom, 2002). Scientists in the pharmaceutical industry attempt to develop new chemical entities (NCEs) with desired actions against some particular families of ‘druggable’ proteins (Yıldırım et al., 2007). An assessment of ‘the druggable genome’, the subset of the ~25,000 genes in the human genome that express proteins able to bind drug-like molecules, is crucial to the development of post-genomic research strategies (Hopkins and Groom, 2002). Whole-genome sequencing approach presents enormous potential in personalized therapeutics (Cordero and Ashley, 2012).

However, human beings contain two interacting genomes, i.e., the onstructurally fixed and genetically inherited human genome and the plastic and environmentally acquired human microbiome, most of which reside in the gut (Zhao et al., 2012). The mutualism and symbiosis of humans with the commensal gut microbe extends the human genome with a collection of microbial genomes approximately 100-times larger than the host genome (Han et al., 2010). The two genomes exchange their respective metabolically active molecules and exert influences on each other. In a given environment, the humans’ health maintenance depends on harmonious integration work of them as a hologenome (Zhao and Shen, 2010). More importantly, the gut microbiota interacts with the host immune system, providing signals to promote the maturation of immune cells and the normal development of immune functions (Clemente et al., 2012). The intestinal microbe can also prompt immune cells to produce cytokines that can influence neurophysiology (Smith, 2015). The imbalance or dysbiosis of the gut microbiota has been implied in many human diseases, such as obesity, diabetes, inflammatory bowel disease, gastrointestinal cancers and infectious diseases (Han et al., 2010). Recent evidence suggests that diet and herbal medicines interact strongly with the gut microbiota which in turn would influence human health (Wang et al., 2011; Zhang et
Not the same as target-based approach, integrated network-based strategy, for TCM takes a systems approach to understanding the individual’s body as a whole and offers a comprehensive medical system that integrates fundamental theories, diagnostic methods and therapeutics based on a holistic and dynamic network-based approaches (Barabási et al., 2011, Leung et al., 2014). These strategies would be beneficial for bridging the gap between TCM theory and modern clinical utilization. But the major roles of gut microbiota have little been studied on TCM actions. As most Chinese herbal medicines are orally administered and are suitable for chronic treatments (Qiu, 2007), they are inevitably exposed to the microbiota in the whole gastrointestinal tract resulting in enough spatio-temporal opportunity for their “intimate” contact. TCM might work both by modulating gut microbiota to regain ecological balance and by regulating genes within the host to regain metabolic/immune homeostasis (Zhao et al., 2012). Metagenomics metatranscriptomics, metaproteomics and metabolomics, these network-based approaches could provide powerful tools for better understanding novel mechanisms of TCM with detailed analyses of gut microbial communities (Maccaferri et al., 2011; Martín et al., 2014; Rose et al., 2015; Wang et al., 2015). On the other hand, the availability of TCM by gut microbiota should be taken into consideration to assess the therapeutic contribution of TCM.

Here, the PubMed database and Web of Science database were searched from January 1995 to October 2015 using “gut microbiota”, “intestinal microbiota”, “gut flora”, “intestinal flora”, “gut microflora”, “intestinal microflora”, “herb”, “Chinese medicine”, “traditional medicine”, or “herbal medicine” as keywords to find articles that related to herb-microbe interactions. The literature findings were summarized from two aspects as follows: (1) the effects of traditional herbs on gut microbiota composition/balance; (2) the effects of absorbable metabolites of natural constituents undergoing microbiota enzymes transformations. This review focused on the non-clinical and clinical explorations of gut microbiota-targeted herbal interventions against some diseases including chronic inflammation, obesity and
cancer. In addition, oral bioavailability conundrum of herbal medicine was also underlined. Notably, recent evidence revealed that herbal medicine could exert its health promotion and disease prevention via influencing the gut microbiota structure and/or take effects after being processed by the commensal bacteria. “Microbiota availability” was proposed to describe biological measurements in the therapeutic assessment of herbal medicine.

The bioavailability conundrum of traditional herbs

Volume I of the Chinese Pharmacopoeia (Pharmacopoeia Commission of the People’s Republic of China, 2010) covers 474 monographs of commonly used herbs totally included 566 species, which are distributed into 340 genera, 110 families, 46 orders, 12 superorders belonging to Magnoliidae Novák ex Takht of Equisetopsida C. Agardh (Bremer et al., 2009). The detailed list of the herbs is summarized in Supplemental data. Among them, the qualities of the 284 monographs are encouraged to be controlled using high-performance liquid chromatography (HPLC) techniques. Particularly, more than 60% marker compounds monitored by HPLC for quality control are polyphenols and saponins. Meanwhile, various basic and preclinical studies, as well as some clinical investigations, focused on efficacy and safety of the two types of natural products. Obviously, current studies’ results indicate that these phytochemicals have potent biological activities. However, measurements of systemic exposure to these parent forms suggest that their bioavailabilities are very low after oral administration of pure compounds or herbal mixtures.

Resveratrol (3, 5, 4’-trihydroxy-trans-stilbene) is a polyphenol molecule found in many traditional herbs (e.g., Polygoni cuspidati rhizoma et radix from Polygonum cuspidatum Siebold & Zucc.) and processed products (e.g., red wine). Resveratrol has been reported to exert a plethora of health benefits through many different mechanisms of action (Fernández and Fraga, 2011; Vang et al., 2011; Tomé-Carneiro et al., 2013; Carter et al., 2014). However, low bioavailability but high bioactivity of resveratrol is a conundrum not yet solved. From a pharmacokinetic (PK) point of view, resveratrol has poor PK property. The oral bioavailability of resveratrol is almost zero.
due to rapid and extensive metabolism and consequent formation of various metabolites as resveratrol glucuronides and resveratrol sulfates (Wenzel and Somoza, 2005). In humans, systemic levels of unmodified resveratrol are only ~2% of the peak serum concentration of total free resveratrol and conjugates after a single oral administration (Goldberg et al. 2003). Moreover, resveratrol bioavailability shows a high inter-individual variability (Nunes et al., 2009). The circadian rhythm also influences resveratrol bioavailability (Almeida et al., 2009).

Epidemiological studies have revealed that more green tea intake led to relative lower risk for cancers. The major constituents in green tea are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin, (−)-epicatechin-3-gallate and (−)-epicatechin, of which EGCG may be the most abundant and effective chemo-preventive green tea catechin (Yang et al., 2009). The bioavailability of EGCG in rodents and humans was well studied and was rather poor and erratic (Mereles and Hunstein, 2011; Kim et al., 2014). The circulating levels of EGCG in humans were in the low μM range after tea consumption (Kim et al., 2014).

Proanthocyanidins, the oligomeric forms of flavan-3-ols possessing important bioactive properties, are contained in herbs such as the cortex of Cinnamomum tamala (Buch.-Ham.) T. Nees & Nees (Chen et al., 2014). Procyanidins are the commonest type of proanthocyanidin with (+)-catechin and (−)-epicatechin as their main constituent units. As condensed tannins, the oral bioavailability in animals and humans is relatively lower. The PK study by Serra and colleagues (Serra et al., 2010) revealed limited absorption of procyanidin dimers and trimers. However, the procyanidin B2, metabolite of procyanidin dimers mediated by gut microbiota, showed an improved oral bioavailability (8–11%) for [14C]procyanidin in male rats (Stoupi et al., 2010b).

Ellagitannins are a group of hydrolyzable tannins, occurring in some traditional herbs such as Polygonum capitatum Buch.-Ham. ex D. Don (Ma et al., 2014), Potentilla recta L. (Bazylko et al., 2013), Epilobium angustifolium L. (Ramstead et al., 2012) and Pelargonium reniforme (Andrews) Curtis (Latté et al., 2008). Various epidemiological studies have shown that regular consumption of
Ellagitannins-containing fruits and vegetables help reduce the risk of major chronic and degenerative diseases, such as cardiovascular diseases and certain types of cancer (Garcia-Muñoz and Vaillant, 2014). Ellagitannins’ bioavailability is very poor due to their largest molecular size and relatively high polarity. The systemic exposure to ellagitannins is significantly low or can’t be detected in human and animal studies (Garcia-Muñoz and Vaillant, 2014). However, antioxidant, anti-inflammatory and chemoprevention of cancer activities have been addressed (Bazylko et al., 2013; Garcia-Muñoz and Vaillant, 2014). The metabolites of ellagitannins from gut microbiota metabolism play a critical role for the addressed findings of pharmacological studies (see below in detail).

Ginsenosides are a class of active constituents present in the dried radix and rhizome of Panax ginseng C.A. Mey., P. quinquefolius L. and P. notoginseng (Burkill) F.H. Chen ex C.H. Chow. Ginsenosides have various pharmacological actions such as vasorelaxation, antioxidation, immune system modulatory actions and etc. (Yu et al., 2012). However, the oral bioavailability of ginsenosides is generally low. After oral dosing of the Sanqi extract to rats, the bioavailability values of ginsenosides Ra3, Rb1 and Rd ranged from 0.1 to 0.2%, whereas those of ginsenosides Re, Rg1 and notoginsenoside R1 were 0.2–0.6% (Liu et al., 2009). Similarly, the bioavailability values of ginsenosides Rb1, Rd, Re, Rg1 and notoginsenoside R1 in rats were 1.2%, 2.4%, 7.1%, 6.1% and 9.3%, respectively (Li et al., 2007). Most reported bioavailability values of ginsenoside Rb1 were <1% in rats, but those of ginsenoside Rg1 were quite inconsistent, ranging from <1% to 20% (Yu et al., 2012). In addition, the oral bioavailability values of purified ginsenoside Rd in dogs, ginsenoside Rh1 in rats, and ginsenoside Re in mice were reported to be 0.3%, 1.0% and 0.2–0.3%, respectively (Yu et al., 2012).

Bioavailability: a nice guy, but not the Mr. Right for traditional herbs

Bioavailability is defined in FDA’s Guidance for Industry as: the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be
absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action (http://www.fda.gov/downloads/Drugs/Guidances/ucm070124.pdf). Similar definition is also documented in the European Agency for the Evaluation of Medicinal Products (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003519.pdf). As the site of action may not be well defined, it is also stated that ‘bioavailability is understood to be the extent and the rate at which a substance or its active moiety is delivered from a pharmaceutical form, and becomes available in the general circulation. Therefore, bioavailability (denoted as \( F \) and generally expressed as a percentage, \( F\% \)) can be generally treated by a systemic exposure profile obtained by measuring drug and/or metabolite concentration in the systemic circulation over time.

For reasons of convenience for the patient and compliance to the therapy, most drugs are administered orally. To keep the dose at possible lowest level, high oral absorption and high \( F \) are prime properties to optimize in a new drug development. So, studies to measure \( F \) of a product are important elements in support of investigational new drug applications, new drug applications, abbreviated new drug applications and their supplements. Parameter \( F \) is specially tailored for evaluation of deliberately synthetic NCEs. For the body, drug is a xenobiotic substance and the body must do something to it (also referred as PK behaviors). Body may limit drug’s absorption in gastrointestinal tract; aggravate its metabolism in the gut and liver; accelerate its elimination through urine and/or bile; shorten its mean residence time in the body. From a therapeutic perspective, these initial PK properties of a compound may be inappropriate. But scientists in the pharmaceutical industry are wise enough to change things. For example, the medicinal chemist Christopher Lipinski and his colleagues analyzed the physicochemical properties of more than 2,000 drugs and candidate drugs in clinical trials, and concluded that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches
the following criteria: there are less than five hydrogen-bond donors; the molecular mass is less than 500 Da; the lipophilicity is not high (expressed as cLogP < 5); and the sum of nitrogen and oxygen atoms is less than 10 (Lipinski et al., 1997). Therefore, scientists can enhance the compound’s bioavailability through artificial structure modification based on the “rule-of-five” analysis by Lipinski. Thomas and his colleagues (Thomas et al., 2006) provided a “road map” to guide the selection of profiling assays that should be considered when optimizing oral bioavailability from an industrial perspective. Obviously, these strategies will drive the drug discovery process to achieve a common goal—an acceptable oral bioavailability, although they are always against the body’s will in nature.

However, nature has already carried out the combinatorial chemistry for traditional herbs and produced multi-components with low content, high chemical diversity, biochemical specificity and other molecular properties. The oral bioavailability of the natural chemicals has been predefined by nature when they are biosynthesized. We cannot change this situation when we investigate traditional herbs as a whole. It is inappropriate to evaluate herbal medicines using the parameter of bioavailability. For example, resveratrol is reported to extend lifespan and provide cardio-neuro-protective, anti-diabetic, and anti-cancer effects by initiating a stress response that induces survival genes (Sajish and Schimmel, 2015). But its oral bioavailability is very low due to extensive metabolism in the intestine and liver (Walle T, 2011). It seems not easy to explain the beneficial effects of resveratrol determined from in vitro and in vivo studies with its bioavailability data. Fortunately, scientists have found that a fundamental new mechanism for the known beneficial effects of resveratrol: it powerfully activates an evolutionarily ancient stress response in human cells by much lower doses of resveratrol (as much as 1,000 times lower) than were used in some of the more celebrated prior studies (Sajish and Schimmel, 2015). The benefits of resveratrol might due to mammals have evolved a symbiotic relationship with resveratrol-producing plants. Microbes-based mechanisms may also involve the efficacy of resveratrol.
Gut microbiota: partner in human health and disease

In humans, more than 100 trillion microbes colonize the oral-gastrointestinal tract, and most of these populations reside in the distal large intestine. The human gut microbiota is dominated by the Bacteroidetes and the Firmicutes stemming from over 50 reported bacteria phyla, whereas Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria and Cyanobacteria are present in minor proportions (Sekirov et al., 2010). Gut microbiota coevolves with the host and is critical for many host physiological processes including enhancement of the intestinal epithelial barrier through maintenance of cell-to-cell junctions and promotion of epithelial repair following injury, development of the immune system and acquisition of nutrients. The host immune system, of particular the mucosal immune system, has developed an intricate connection with the commensal microbiota. Both the innate and adaptive immune systems have evolved to require microbial interactions during their development (Round and Mazmanian, 2009; Clemente et al., 2012). Actually, the nutrient metabolism by gut microbiota is not carried out strictly for the host’s benefit; part of the energy extracted from luminal nutrients is designated for the microbiota itself, to maintain its numbers and fitness (Sekirov et al., 2010). The commensal microbiota also protects against overgrowth of indigenous pathobionts that can disrupt the healthy microbial community and colonization by pathogens via competitive exclusion, consumption of nutrient sources and production of antimicrobial substances, as well as stimulating the host to produce various antimicrobial compounds (Kamada et al., 2013a).

Because the gut microbiota seems to involve almost aspect of the host’s growth and development, various diseases and dysfunctions of host from gastrointestinal tract to remote organ systems have been linked with an imbalance in composition, numbers and/or habitat of the gut microbiota (Sekirov et al., 2010; Cho and Blaser, 2012). Some review articles have focused on the roles of gut microbiota in many diseases such as inflammatory diseases (Kamada et al., 2013b; Theodoratou et al., 2014), obesity (Walker and Parkhill, 2013; Zhao, 2013) and cancer (Louis et al., 2014).
For example, Turnbaugh and colleagues found that obese \( ob/ob \) mice (leptin-deficient mice) had a reduced number of Bacteroidetes and a proportional increase in Firmicutes, when compared to lean mice (\( ob/+ \) and \(+/+\)) from the same litter (Turnbaugh et al., 2006). Another animal study reported by Ley and colleagues observed a relative increase of 50% of Firmicutes and a decrease of 50% of Bacteroidetes in \( ob/ob \) mice. They also found that obese individuals presented an intestinal flora with a lower proportion of Bacteroidetes than lean rodents (Ley et al., 2006). Human studies showed that the gut microbiota of obese humans had a significantly greater ratio of Firmicutes (\( F \))/Bacteroidetes (\( B \)) (\( F/B \)) than their lean counterparts (Zhao, 2013). Therefore, “one microbe-one disease” viewpoint has been challenged and we have realized that some diseases might result from dysbiosis of the gut microbes.

**The effect of traditional herbs on gut microbiota composition/balance**

**Non-clinical studies**

Herbal medicines can affect health via gut microbiota in different ways. For some herbs, certain constituents influence the composition of the gut microbiota. *Ganoderma lucidum* (Curtis) P. Karst. (ganoderma) has been used for hundreds of years to promote health and contains triterpenes and polysaccharides as the main active ingredients showing anti-diabetic activity (Thyagarajan-Sahu et al., 2011; Pan et al., 2013). It was found that water extract of ganoderma and high molecular weight polysaccharides (> 300 kDa) could prevent high-fat diet (HFD)-induced obesity by modulating the composition of the gut microbiota (Chang et al., 2015). This water extract enhanced bacterial levels of *Clostridium* clusters IV, XVIII and XIVa (*Roseburia* spp.), and *Eubacterium* spp. in HFD-fed obese mice. Meanwhile, several bacterial species associated with inflammation and obesity was decreased after dosing, including *E. fergusonii* (linked with HFD-induced inflammation), *Oscillibacter* spp. (related to the expression of intestinal tight junction proteins) and *Mucispirillum* spp. belonging to *Deferribacteres* (colonized the mucus layer). In addition, these polysaccharides may contribute to the growth of specific bacterial species in the gut.
microbiota. The water extract of ganoderma and its high molecular weight polysaccharides has the potential as prebiotics to reduce obesity-related disorders in obese individuals.

Increasing studies show that Lentinula edodes (Berk.) sing (shiitake)-derived polysaccharides possess immune-modulating and anti-tumor characteristics, which might benefit from the gut microbiota to produce gut-absorbable metabolites and to reshape the bacteria structure (Flint et al., 2012). Xu and colleagues isolated a new heteropolysaccharide L2 with a molecular weight of 26 KDa from the fruit body of shiitake (Xu et al., 2012). Recently, Xu and Zhang found that L2 could alter the spatial structure of gut microbiota in mice (Xu and Zhang, 2015). After a 28-consecutive-day treatment of L2 (40 mg/kg), the diversity and evenness of gut microbiota along the mice cecum and colon were reduced. Compared with normal treatment, the overall composition shifts from Firmicutes-dominant to Bacteroidetes-dominant structure in L2-treated mice, especially with the ratio F/B changing from 0.77 to 0.2 in the colon. The increase of Bacteroidetes in the colon could be linked to degradation of L2 producing short chain fat acids (SCFCs) in mouse intestine. Proteobacteria is also significantly increased (~ 10.3 fold) in all samples, which might contribute to the immuno-stimulating activity of polysaccharide L2.

As a water-soluble-β-D-fructan polysaccharide, MDG-1 is commonly extracted and purified from the roots of Ophiopogon japonicas (L. f.) Ker Gawl (Ophiopogonis radix), as a commonly used herb for some centuries in China. MDG-1 could exert anti-obesity role in ob/ob mice and diet-induce obese mice model, but it was absorbed into bloodstream very hard due to its relatively high molecular weight (~ 3400 Da) (Lin et al., 2010). A recent animal study revealed that MDG-1 treatment for HFD-induced C57BL/6 mice at a dose of 300 mg/kg for 12 weeks had about a 28% increase of Bacteroidetes and a 15% decrease of Firmicutes gradually shifting the F/B ratio to the normal state (Shi et al., 2015a). The authors also found that the accumulation of pathogenic bacteria including Escherichia coli and Streptococcus was inhibited by MDG-1 chronic treatment in diabetic mice models (Wang et al., 2011). MDG-1 could dose-dependently improve the diversity of the intestinal probiotics and
promote their proliferation, especially *Lactobacillus taiwanensis* and *L. murinus* (Shi et al., 2015b). Moreover, in an *in vitro* study, Shi and colleagues revealed that MDG-1 treatment altered the microbial metabolism profiles. Most importantly, the levels of D-galactosamine displayed a 55% decrease in *L. taiwanensis* group and a 22% decrease in *L. murinus* group. This biomarker molecule is increased under liver necrosis and obesity conditions, indicating MDG-1 could protect liver from necrosis and ameliorate the metabolic syndrome associated with obesity or diabetes during treatment (Shi et al., 2015a). On the other hand, just as we know, microbe-related end-products such as acetate, propionate and butyrate also involve the anti-obesity activity. Butyrate can serve as an energy source for colonocytes and this compound is also effective in alleviating inflammation, mitigating oxidative stress and improving gut barrier function (Zhao, 2013). Acetate and propionate could stimulate adipogenesis in an adipocyte cell line and increase leptin release from adipose tissue in mice (Xiong et al., 2004; Hong et al., 2005). Shi and colleagues found that MDG-1 treatment increased the butyrate and its related products, such as vinyl acetic acid and acrylic acid in the *L. taiwanensis* group (Shi et al., 2015a).

Berberine, the major pharmacological component of the Chinese herb *Coptidis* rhizoma from *Coptis chinensis* Franch., was clinically effective in alleviating type 2 diabetes via various proposed mechanisms (Zhang et al., 2008; Zhang et al., 2010). However, a paradox remains regarding the mode of action of berberine due to its poor oral bioavailability and extremely low plasma maximum concentrations (Hua et al., 2007; Liu et al., 2009). Therefore, modulation of the gut microbiota has been hypothesized as one of the mechanisms of its anti-diabetic effect (Han et al., 2011). Both ethanol extract of *Coptidis* rhizoma and berberine significantly reduced the proportions of fecal Firmicutes and Bacteroidetes to total bacteria in HFD mice (Xie et al., 2011). Zhang and colleagues performed a MiWAS based on bar-coded 454 pyrosequencing of the V3 region of 16S rRNA genes analysis and observed significant reductions in bacterial diversity and total bacterial population in berberine-treated rats, which resulted in a decrease of free antigen load in the host, as confirmed by the decreased serum lipopolysaccharide (LPS)-binding protein levels in
berberine-treated HFD-fed rats (Zhang et al., 2012). Meanwhile, berberine feeding could enrich the abundance of SCFA producers such as *Bacteroides* spp. to promote colonic fermentation and SCFA production in the intestines of HFD-fed rats, leading to the gut barrier-protecting effects. We need to know whether these molecules contribute to the beneficial effects of *Coptidis* rhizoma and berberine.

Zhang and colleagues compared the modulation of gut microbiota by berberine and metformin using HFD-induced rat model (Zhang et al., 2015). Both berberine and metformin shifted the gut microbiota structure and significantly reduced the microbial diversity in the gut of obese rats and therefore reverting effects on the HFD-induced structural variations, but did not influence the F/B ratio. In particular, the putative SCFA-producing bacteria including *Blautia*, *Bacteroides*, *Butyricoccus* and *Phascolarctobacterium* were markedly enriched by both berberine and metformin treatments, indicating a critical role the SCFA-producing bacteria in the efficacies of both drugs. A traditional Chinese herbal formula (GeGeng QinLian Decoction using berberine as marker compound) has also been reported to recover the abundance of SCFA-producing bacteria (Zhang et al., 2013). In addition, *Allobaculum* and *Lactobacillus* were increased after both treatments. On the other hand, berberine and metformin both inhibited a wide range of intestinal microbes. But the cause and consequence of inhibited pathogens for obesity remains unknown.

*Loniceræ japonicæ flos*, coming from *Lonicera japonica* Thunb., contains various constituents such as organic acids, flavonoids, iridoid glycosides and saponins. This herb has been widely used as a traditional treatment for many diseases particularly based on its anti-inflammatory activity (Yoo et al., 2008). Wang et al. found that treatment of HFD- and HFD+LPS-fed rats with unfermented or fermented *Loniceræ japonicæ flos* resulted in a notable decrease in body and adipose tissue weights, ameliorated total cholesterol, high-density lipoprotein (HDL), triglyceride, aspartate transaminase and endotoxin levels in serum, reduced the urinary lactulose/mannitol ratio, and markedly alleviated lipid accumulation in liver. However, significant alterations of the distribution of gut microbiota in these
animals were observed, especially increasing the population of Akkermansia spp. and ratio of B/F, which could play a vital role in ameliorating HFD- or HFD+LPS- induced obesity and endotoximia (Wang et al., 2014). However, many efforts should be put into exploring which constituents contribute to the observed effects.

The rhizoma of Atractylodes macrocephala Koidz. (RAM) has long been widely used in eastern Asia. The anti-obesity effect of the water RAM extract via Akt/PI3K pathway-modulated inhibition of lipid accumulation has been observed (Kim et al., 2011). Recently, Wang and colleagues found that both unfermented RAM (URAM) and fermented RAM (FRAM) could combat HFD or HFD+LPS-induced inflammation probably via reduction of the serum endotoxin content and attenuation of the production or release of pro-inflammatory cytokines. These beneficial effects were influenced by the gut microbiota composition. Compared with control group, the relative abundance of Bifidobacterium spp. and Akkermansia spp. as well as the B/F ratio in the HFD+LPS group was significantly enhanced upon co-treatment with URAM or FRAM, whereas the relative abundance of Bacteriodes and Lactobacillus spp. in the HFD+LPS group was also significantly augmented by FRAM, but not by URAM (Wang et al., 2015).

As well known, it is estimated that about 90–95% of the unabsorbed polyphenol intake (e.g., quercetin and trans-resveratrol) reaches the colonic region (Cardona et al., 2013; Chen et al., 2013), which provides potential for the direct contact and the dual interaction between these natural bioactive compounds and the gut microbiota resulting in beneficial roles for both bacteria and the host. Etxeberria et al. found that administration of both quercetin and trans-resveratrol together prevented body weight gain and reduced serum insulin levels. Moreover, individual feeding of trans-resveratrol and quercetin effectively reduced serum insulin levels and insulin resistance. Quercetin treatment generated a great impact on gut microbiota composition at different taxonomic levels, attenuating F/B ratio and inhibiting the growth of bacterial species previously associated to diet-induced obesity (Erysipelotrichaceae, Bacillus, Eubacterium cylindroides) and increasing some that have been inversely related to obesity (Bacteroides vulgatus, Akkermansia
muciniphila). Overall, the administration of quercetin was found to be effective in lessening high-fat sucrose diet-induced gut microbiota dysbiosis which was associated with significant metabolic improvements. In contrast, trans-resveratrol supplementation alone or in combination with quercetin scarcely modified the profile of gut bacteria but acted at the intestinal level, altering the mRNA expression of tight-junction proteins and inflammation-associated genes (Etxeberria et al., 2015). The mechanisms of trans-resveratrol to exert beneficial effects due to its potential influence on modulating gut microbiota and its impact on host intestinal gene expression should be deciphered in the future.

Green tea has also been found to exert anti-obesity effect (Cunha et al., 2013) and to induce compositional changes in gut microbiota (Jin et al., 2012). Seo et al. observed that the F/B ratio was increased in HFD mice but dramatically reduced after subchronic administration of fermented green tea for 8 weeks, which was similar in the normal-chow-diet groups. Bacteroides abundance was increased by a Western diet (e.g., high protein and high fat), whereas Prevotella consumed carbohydrates and fibers. Feeding HFD resulted in increased Bacteroides and reduced Prevotella, i.e., increased the Bacteroides/Prevotella ratio, which is proposed to be closely linked with the development of obesity and insulin resistance. Fermented green tea treatment restored the changes in gut microbiota composition (Seo et al., 2015). Axling et al. also found green tea powder in combination with Lactobacillus plantarum was able to promote growth of Lactobacillus in the C57BL/6J mice intestine and to attenuate HFD-induced inflammation (Axling et al., 2012).

Guo et al. found that the water extract of red ginseng or Coicis Semen from Coix lacryma-jobi var. ma-yuen (Rom. Caill.) Stapf ex Hook. f. obviously increased the probiotics Bifidobacterium animalis, Bifidobacterium longum, and Lactobacillus rhamnosum in in vitro experiments. Red ginseng also inhibited the growth of pathogens Escherichia coli and Salmonella spp. However, Coicis Semen dose-dependently increased these pathogens. In vivo study revealed that Coicis Semen significantly increased the relative abundance of Lactobacillus, but also increased the relative abundance of E. coli at the same time. On the other hand, Red
ginseng increased *Lactobacillus* but inhibited *E. coli* in rats. Guo and colleagues also observed both red ginseng and *Coicis* Semen can relieve the symptoms of ulcerative colitis, of particular red ginseng, in which improvement of gut microbiota structure and anti-inflammatory effects might involve in these beneficial effects (Guo *et al.*, 2015).

*Gynostemma pentaphyllum* (Thunb.) Makino (GP), containing triterpenoid saponins as its main active constituents, has been consumed as an herbal tea and used as a folk medicine for the treatment of various diseases including tumor (Razmovski-Naumovski *et al.*, 2005). Chen *et al.* found that the xenografted tumors caused alteration of gut microbiota composition as compared to the nonxenograft mice. After GP saponins treatment, the *Clostridium cocleatum* and *Bacteroides acidifaciens* increased in number in both xenograft and nonxenograft mice. Meanwhile, the B/F ratio showed an increasing trend after 10 d of saponin treatment in xenograft mice. Of particular, the substantial increase of *C. cocleatum* contributed mainly to fecal bacteria community structures in saponin-treated tumor-bearing mice (Chen *et al.*, 2015).

**Clinical trials**

Investigation of the effects of herbs on the human gut microbiota has been reported in recent years, although inadequate. In a 12-week, double-blind, placebo controlled study revealed that the *Schisandra chinensis* (Turcz.) Baill. Fruits’ water extract could increase the relative abundance of *Akkermansia, Roseburia, Bacteroides, Prevotella* and *Bifidobacterium*, but decrease the number of *Ruminococcus*. Moreover, *Bacteroides* and *Bacteroidetes* negatively correlated with fat mass, aspartate aminotransferase, and/or alanine aminotransferase, respectively; whereas *Ruminococcus* negatively linked to HDL cholesterol and fasting blood glucose (Song *et al.*, 2015).

In another 10 obese middle-aged Korean women participating clinical study, Song *et al.* found that significant differences of gut microbiota between the effective weight loss group (EWG) and ineffective weight loss group (IWG) prior to ginseng
intake (Song et al., 2014). The three predominant genera in EWG were *Blautia*, *Anaerostipes* and *Oscillibacter*, whereas those in IWG were *Bifidobacterium*, *Blautia*, and *Clostridium_g4*. After 8-week chronic administration, each group exhibited changes in microbial composition; the three main dominant genera of EWG were changed to *Blautia*, *Faecalibacterium* and *Anaerostipes*, and those of IWG were changed to *Bifidobacterium*, *Blautia* and *Clostridium* at the genus level. Ginseng exerted a weight loss effect and slight effects on gut microbiota in all participants. Notably, the composition of gut microbiota prior to ginseng intake influenced its anti-obesity effects. Song and colleagues also found that the body weights (BW) and body mass index (BMI) of seven obese Korean women significantly decreased after chronic dosing of the water extract of *Ephedra sinica* Stapf. Furthermore, abundance of *Subdoligranulum*, *Oscillibacter* and *Akkermansia* showed a link with BW and BMI, whereas number of *Latobacillus* demonstrated correlation with body fat percentage, which could be looked as key bacteria for ephedra’s effects. But the influence of ephedra’s intake on gut microbiota exhibited obvious individual differences (Kim et al., 2014).

*Trametes versicolor* (L.) Lloyd, known as “Yun Zhi” in China, is a medicinal fungus world-wide used as a therapeutic for some diseases including cancer. Yu et al. have showed that the water extract of *T. versicolor* containing polysaccharide peptide (i.e., polysaccharopeptide, PSP) modified human fecal microbiota composition *in vitro* by increasing populations of *Bifidobacterium spp.* and *Lactobacillus spp.* while reducing *Clostridium spp.* population and decreasing culture pH, indicating PSP may be used as prebiotics (Yu et al., 2013). Recently, Pallav and colleagues reported the results of an open label, randomized clinical trial to compare and contrast the effects of PSP and amoxicillin on the gut microbiota of healthy adults (Pallav et al., 2014). They found that *Bacteroides* and *Sutterella* were significantly higher in the age 30 group, whereas *Coprococcus* and *Prevotella* were higher in the 40 through 50 year age groups. *Escherichia/Shigella* was significantly increased in the amoxicillin group during treatment returning to elevated levels after treatment. *Fusobacterium* was increased in the PSP group during treatment and returned to non-significantly different levels.
post-study. PSP ingestion is able to elicit distinctive changes in the human microbe consistent with its activity as prebiotics via host responses in turn reshaping the microbes.

In a double-blind, randomized, placebo-controlled clinical trial, Ko and colleagues found that combination therapy with HuoXiang ZhengQi powder and Duolac7S (a probiotics agent) synergistically increased significantly the number of *Bifidobacterium brevis, Bifidobacterium lactis, Streptococcus thermophilus, Lactobacillus rhamnosus, Lactobacillus plantarum* and *Lactobacillus acidophilus*, suggesting that HuoXiang ZhengQi powder could serve as prebiotics for the proliferation of beneficial bacteria in the human intestine (Ko et al., 2011; Ko et al., 2013). Meanwhile, HuoXiang ZhengQi powder group decreased the F/B ratio (p=0.211) among the 4 investigated groups indicating that this herbal formula could regulate harmful bacteria in the human intestine. Therefore, combination therapy with herbal medicine and probiotics appears to relieve overall irritable bowel syndromes by synergistically increasing abundance of beneficial gut microbiota.

**The effect of absorbable metabolites of natural constituents undergoing microbiota enzymes transformations**

For some herbal medicines, gut microbiota-mediated bioconversion is a critical step to exert their therapeutic effects or toxicities in vivo. As we know, berberine exhibits poor solubility partly contributing to its poor bioavailability. In fact, the limited absorption of berberine also associated with gut microbiota. Feng and colleagues recently revealed its absorption mechanism (Feng et al., 2015). Gut microbiota converted berberine into dihydroberberine (Figure 1 (a)) in the intestine via a reduction reaction mediated through bacterial nitroreductase. Dihydroberberine was easily absorbed than that of its parent forms by 5 folds. Dihydroberberine was then reverted to original form immediately after penetrating into the intestinal wall tissues. Antibiotics could inhibit the transformation resulting from the suppressed berberine absorption data. Therefore, gut microbiota play critical role in regulating the conversion-absorption-reversion process of berberine in the gut tract.
Ginsenoside compound K, the major metabolite of protopanaxadiol-type ginsenosides, is a main saponin that reaches the systemic circulation in the body after oral administration of ginseng extract in animals and human volunteers (Wang et al., 2011; Yu et al., 2012). Some studies have shown that this bacterial metabolite exhibit a variety of pharmacologic actions including anti-inflammation, anticancer and anti-diabetes (Kang et al., 2013; Jiang et al., 2014; Zheng et al., 2014; Chen et al., 2015; Igami et al., 2015), which could well be used to explain the effects of ginseng extract. Ginsenoside Rb1, from the radix and rhizome of Panax ginseng C.A. Mey., was converted into compound K via ginsenosides Rd by human intestinal strain Eubacterium sp. A-44 and the ginsenoside Rb1-hydrolysing enzyme isolated from the strain was β-D-glucosidase (Figure 1 (b)) (Akao et al., 1998). Recently, Kim and colleagues found that the numbers of Clostridiales uc_g, Oscillibacter, Ruminococcus, Holdemania and Sutterella in samples with fecal activity potently metabolizing Rb1 to compound K (FPG) were significantly higher than in samples with fecal activity non-metabolizing Rb1 to compound K (FNG), but that of Leuconostoc in FPG was lower than in FNG. The abundance of Bacteroides and Bifidobacterium, which potently converts Rb1 into compound K were dramatically increased in FPG (Kim et al., 2013).

Mostly occurring as glycosylated derivatives in herbs, polyphenols always undergo diverse intestinal transformations via gut microbiota prior to become bioactive in humans (Marín et al., 2015). For instance, hesperidin, a rutinose-conjugated polyphenol, was hydrolyzed to its active form hesperetin by Bifidobacterium catenulatum and Bifidobacterium pseudocatenulatum (Figure 1 (c)) (Almeida et al., 2015). For another example, baicalin (baicalein-7-glucuronide) is separated from the radix of Scutellaria baicalensis Georgi, which has long been used for treatment of some diseases such as hypertension, cardiovascular diseases and viral hepatitis. Liu and Jiang found that baicalein was more suitable to be administered orally than baicalin (Liu and Jiang, 2006). Actually, baicalin itself is poorly absorbed from the rat gut, but is hydrolyzed to baicalein by gut microbiota (Figure 1 (d)) and then restored to its original form from the absorbed baicalein in
the body. A comparative study has revealed that a very low concentration of baicalin was found in the circulation of germ-free rats compared with control groups after oral administration (Akao et al., 2000). The PK parameters of oral baicalin were significantly affected by antibiotics (cefadroxil, oxytetracycline and erythromycin) compared to those in normal rats, indicating that intestinal microbiota (via β-D-glucuronidases) might play a key role in the oral pharmacokinetics of baicalin (Yim et al., 2004; Kang et al., 2014). Take calycosin-7-O-β-D-glucoside (C7G) as the third example. This compound is the most abundant flavonoid in Astragali radix from Astragalus membranaceus Moench and is used as chemical marker for this herb. Calycosin-3′-O-glucuronide (G2) was the major circulating component together with a minor calycosin but not C7G in rat after receiving a single oral administration of C7G mainly due to the glycoside could be transformed to glucuronides in the enterocytes and in the liver. Rat gut microbiota converted C7G in vitro rapidly and produced its aglycone calycosin (Figure 1 (e)), which further underwent extensive glucuronidation to yield 3′-glucuronide as the dominant metabolite exhibiting similar or more potent proangiogenic effects than calycosin (Ruan et al., 2015).

Additionally, the formation of urolithins transformed from ellagitannins (urolithin A, B and C) was established by ex vivo incubation of human fecal samples with aqueous extracts from selected ellagitannin-rich plant materials such as Geranium pratense L., Lythrum salicaria L. and Potentilla anserina L.. Significant inhibition of TNF-α production was determined for all urolithins, while the urolithin A was found to be the most potent inhibitor of TNF-α production with nanomolar concentrations (0.625 μM). Moreover, urolithin C (0.625 μM) was the only compound inhibiting IL-6 production (Piwowarski et al., 2014). Similarly, ellagitannins from the water extract of Epilobium hirsutum L. were proven to be transformed by human gut microbiota into urolithins including urolithin C, which showed the strongest activity in the inhibition of prostate cancer cell proliferation, prostate specific antigen secretion and arginase activity (Stolarczyk et al., 2013). A recent study showed that colonic metabolite urolithin D selectively inhibited EphA2 phosphorylation in prostate cancer cells (Giorgio et al., 2015).
Ou and colleagues identified 9 metabolites from in vitro samples of procyanidin B2 incubated with human microbiota, including benzoic acid, 2-phenylacetic acid, 3-phenylpropionic acid, 2-(3’-hydroxyphenyl)acetic acid, 2-(4’-hydroxyphenyl)acetic acid, 3-(3’-hydroxyphenyl)propionic acid, hydroxyphenylvaleric acid, 5-(3’,4’-Dihydroxyphenyl)-γ-valerolactones and 5-(3’-hydroxyphenyl)-γ-valerolactones (Ou et al., 2014). Appeldoorn and colleagues utilized in vitro fermentation to study the role of human gut microbiota on the metabolism of purified procyanidin dimers. Two major metabolites were identified as 2-(3,4-dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)-γ-valerolactone (Appeldoorn et al., 2009). Another similar study reported by Stoupi and colleagues showed that 5-(3’-hydroxy phenyl) valeric acid, 3-(3’-hydroxyphenyl) propionic acid and phenyl acetic acid were the main catabolites of procyanidin B2 (Stoupi et al., 2010a). The diversity of the gut microbiota from different human fecal samples may contribute to the metabolic differences of procyanidin. The resulting SCFAs should be responsible for the health effects of this type compounds.

Isoflavones such as daidzin, genistin and glycitin in some herbs mostly (>80%) appear as glycoside conjugates (i.e., 7-β-D-glucosides and 6”-O-malonyl-7-β-D-glucosides), the bioavailability and bioactivity of which are relatively low. The various activities depend on their aglycones (i.e., daidzein, genistein and glycitein) liberated from these conjugated forms and then translated to more actively metabolites (e.g., equol and 5-hydroxy equol) mediated by intestinal microbiota (Figure 1 (f)), which also converted aglycones into inactive forms such as O-desmethylangolensin (Clavel et al., 2013). Some gut microbiota catalyzing the bioactivation of daidzein via dihydrodaidzein to equol have been identified as Adlercreutzia equolifaciens, Slackia isoflavoniconvertens, Slackia equolifaciens and Lactococcus garvieae (Matthies et al., 2011). However, only 30-50% humans are equol producers due to the inter-individual diversity in microbial composition (Tamura et al., 2015). Therefore, gut microbiota for producing equol plays a critical role in the efficacy of isoflavones. A comprehensive review has summarized the beneficial effects on human health in equol producers of gut
microbiota (Sánchez-Calvo et al., 2013). Here, some more recently published results are reviewed.

Tamura and colleagues observed a negative correlation between BMI and the dihydroadidzein production in healthy men, as well as BMI and the equol production in healthy women. But a positive correlation between intake of soluble dietary fiber and the dihydroadidzein production only was found in men (Tamura et al., 2015). Guadamuro and colleagues found the fecal microbes of 16 menopausal women significantly changed dependently on the equol-producing phenotype after 6 months treatment with isoflavones (Guadamuro et al., 2015). Compared to baseline, at 1 and 3 months the counts for all microbial populations in the feces of equol-producing women had increased strongly. In contrast, among the non-producers, the counts for all microbial populations at 1 month were similar to those at baseline, and decreased significantly by 3 and 6 months. Real-time quantitative PCR showed that the *Clostridium leptum* and *C. coccoides* populations increased in equol producers, while those of *bifidobacteria* and *enterobacteria* decreased, and vice versa in the non-producers. However, a significant increase in the relative proportion of *Bifidobacterium* was observed after soy consumption in postmenopausal American women, especially in S-(-) equol producers (Nakatsu et al., 2015). Meanwhile, *Bifidobacterium* and *Eubacterium* were significantly greater in equol vs non-S-(-) equol producers. A negative correlation with dihydroadidzein suggested that the *Bifidobacterium* in some of these subjects was involved in the conversion of dihydroadidzein to S-(-) equol. Another human pilot study demonstrated that fructooligosaccharide intervention (5 g/day for 2 weeks) did not significantly influence the capacity of gut microbiota producing equol in postmenopausal Japanese women, regardless of equol-producing phenotypes (Tousen et al., 2013).

Glycyrrhizin, presenting in the dried rhizoma and radix of *Glycyrrhiza* L. species, need to be stripped off its glucuronic acids by glucuronidase of gut microbiota prior to become active form. In the gut tract, glycyrrhizin is deglycosylated to glycyrrhetic acid as a dominant metabolite by *Eubacterium sp.* and *Bacteroides J-37* and to 18β-glycyrrhetic acid 3-O-mono(glucuronide as a minor product by *Bacteroides J-37*.
and *Streptococcus* LJ-22 (Figure 1(g)). In addition, conversion of 18β-glycyrrhetic acid 3-O-monomethylglucuronide to glycyrrhetic acid could also be mediated by *Eubacterium sp.* (Kim *et al*., 2008; Yu *et al*., 2012).

Taken together, the metabolism of traditional herbs with low oral bioavailability conundrum by gut microflora is a critical step towards the emergence of their bioactivities *in vivo*.

**Interplay between traditional herbs and gut microbiota: the availability of traditional herbs by gut bacteria**

The events following herbal medicines administration can be divided into two phases, a body disposition phase, in which the body regulates the absorption, distribution, metabolism and excretion of herbal constituents; and a microbe disposition phase, in which the bacteria utilize herbal constituents for themselves and the host through host-microbe interactions. In herbal medicine, many constituents have very poor oral bioavailability leading to limited concentration levels in the bloodstream. From apparently look, these ingredients just pass through the gut. Actually, when the herbal medicines reside in the intestinal tract, its constituents are intimate with the bacterial communities. The commensal cells inspect the xenobiotics in order to maintain the health and well-being of the host. During this process, the bacteria may recover valuable energy and absorbable substrates for the host, and also provide energy and nutrients for their growth and proliferation (O’Hara and Shanahan, 2006). Mass, energy and information exchange among herbal medicines, microbe and host occurs along mucosal surfaces in the intestine of the host. When exposure to herbal medicine, the gut microbiota will reshape its structure and the host intestinal epithelium cells could sense these changes and give signals to the enteric nervous system and then input the signal to brain areas. Meanwhile, the enteric nervous system receives efferent information from the brain in turn modulate microbe functions (Chen *et al*., 2013; Smith, 2015). In addition, microbes also produce
metabolites that could affect brain activity. So, just as “spearheads”, gut microbiota actively touch the herbal constituents, process these chemicals, change their own structure, gather information and “upload” the information to the host. Therefore, herbal constituents are bioavailable by the microbiota when herb passes through the gut.

In this context, the term “microbiota availability” is proposed to describe biological measurements in the therapeutic assessment of herbal medicines. When herbal medicines are orally administered, gut microbiota process the herbal ingredients for the host and themselves leading to the degradation of herbal ingredients and structural changes of their own composition (Figure 2). These changes might be used to define the microbiota availability.

(Insert Figure 2 here)

Conclusions

Taken together, the oral bioavailability conundrum of herb-derived constituents counts against deciphering the mechanisms of traditional herbs. This PK parameter designed for synthetic drugs seems not to be suitable for all the herbal medicines. The un-bioavailable constituents from some orally administered herbal medicine contact with gut microbiota intimately in the intestinal tract. These lines of evidence from non-clinical studies and clinical trials suggest that gut microbiota could be ultimately prove to be one of the main ways in which herbal medicines act on human health through reshaping the microbial structure and/or processing the herbal ingredients to form active metabolites. According to numerous studies, we propose that “microbiota availability” might be used to describe biological measurements in the therapeutic assessment of herbal medicines. Our review should be of value in stimulating discussions among the scientific community on this relevant theme and prompting more efforts to complement herb-microbiota interactions studies.
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Figure legends

**Figure 1** Selected examples of biotransformation of traditional herbal constituents by gut microbiota

**Figure 2** Schematic representation of the pattern of bidirectional herbal medicine-gut microbiota interactions. Three potential mechanisms by which gut microbiota-herb interactions could be referred for investigators. (1) Herb reshapes the structures of gut microbes; (2) the gut microbes could prompt immune cells to produce cytokines due to the influence of herb; (3) gut microbes metabolize herbs to produce active metabolites.
Dihydroberberin

Hesperidin
Hesperetin

Glycyrrhetic acid

Ginsenoside Rb1
Ginsenoside C-K

Berberin

Daidzin
daidzein
Equol

Hesperidin
Baicalein

Baicalin

Calycosin-7-O-β-D-glucoside 
Calycosin

18β-glycyrrhetic acid 3-O-monoglucuronide

Figure 1
Graphical Abstract