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Review

Quality control of herbal medicines in hyperlipidaemia: Metabolomics approach

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Abstract

Hyperlipidaemia is one of the essential public health risk factors that can cause other metabolic diseases such as cardiovascular diseases and diabetes. Dieting and healthy lifestyle have been among the primary approaches. However, medication is required to regulate the lipid profile in some instances. Therefore, there has been an increase in interest in using or integrating herbal medicine with modern medicine in treating hyperlipidaemia. Nonetheless, preparing standardised herbal extract or products has been one of the major challenges in the herbal industry. Standardising herbal extract or product (single plant-based or mixture of multiple herbs) is needed to ensure the quality, safety, and efficacy of the herbal maintained from batch to batch before it is released to the market. The present review thus evaluates several herbal plants with anti-hyperlipidaemic activities, quality control using chemical markers, and metabolomics application in herbal plants.

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Introduction

Non-communicable diseases (NCDs) comprise a group of non-infectious diseases which is non-transmissible; these include cancers, chronic respiratory diseases, stroke, heart diseases, and diabetes. According to the WHO fact sheet, NCDs alone are responsible for 71% of deaths globally (an estimated 41 million people), where 80% of these are premature deaths or before 70 years of age (WHO, 2021). Among the NCDs, cardiovascular diseases (CVD) had the highest mortality rate with 17.9 million deaths, followed by cancers at 9.3 million, respiratory diseases at 4.1 million, and diabetes at 1.5 million (WHO, 2021). In addition, the rise of NCDs globally has been associated with heavy usage of tobacco (*i.e*., smoking), sedentary lifestyle, alcohol abuse, and unhealthy diets (WHO, 2021).

The accumulation of hardened lipid or plaque in the heart that leads to myocardial infarction is closely related to imbalanced lipid metabolism (Schaefer *et al.*, 2016). Therefore, maintaining a reasonable regulation of lipid profile (*i.e*. total cholesterol, triglycerides, and lipoproteins) has been one of the strategies for early prevention of CVD and hyperlipidaemia treatment (Schaefer *et al.*, 2016). Diet approach, changes in physical activity, and lifestyle are usually the first approach being taken to control the lipid profile. However, particularly in chronic cases, modern medicine such as statin is needed to lower low-density lipoprotein cholesterol (LDL-C) (ACC, 2019).

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However, some countries opt for alternative therapies such as traditional medicine. Traditional medicine encompasses the knowledge and usage of materials such as plants and animals, used wholly or in parts, in treating illnesses or diseases (Fokunang *et al.*, 2011). In some instances, mixtures of both animals and plants are used in the prescription. This knowledge can come from their experience, traditional wisdom, or adequately documented such as in traditional Chinese medicine (TCM) and traditional Indian medicine (Ayurveda) (Patwardhan *et al.*, 2005), or passed from generation to generation verbally or recorded in manuscripts or journals. This knowledge can also be influenced by cultures, religions, and customs (Fokunang *et al.*, 2011).

Plants with anti-hyperlipidaemic activities

In drug development, a drug must be able to attach to its targeted site or receptor to function. Therefore, the mechanism of drugs or lead drugs has been essential in developing new medicines to overcome the side effects (Rang *et al.*, 2016). For example, in hyperlipidaemia, the main aim of the treatment is to improve lipid metabolism. The improvement in lipid metabolism can be achieved either by regulating the cholesterol synthesis, excretion of cholesterol, transport, and absorption of lipid (Ji *et al.*, 2019).

Several plants have been reported to possess anti-hyperlipidaemic or lipid-lowering activities. Their activities are usually due to the main bioactive compound or synergistic effects of the compounds in the body (Zhou *et al.*, 2016). Compounds such as alkaloids, phenolics, saponins, or carotenoids have been noted to have anti-hyperlipidaemic activities. A summary of the traditional usage, pharmacological activities, and bioactive compounds of selected lipid-lowering plants are shown in Table 1.

According to Ji *et al.* (2019), the bioactive in herbal medicines intervenes in lipid metabolism in four ways; (1) by controlling cholesterol absorption in enterocytes by reducing the cholesterol uptake or/and improving the cholesterol esterification, (2) by cholesterol synthesis suppression, (3) promotes reverse cholesterol transport, and (4) increasing and promoting cholesterol excretion by the liver (Figure 1).

Cholesterol absorption inhibition in enterocytes

According to Nakano *et al.* (2019), cholesterol absorption into circulation can be divided into two processes: uptake of cholesterol into intestinal epithelial cells, and assimilation of cholesterol, which is the transfer of cholesterol from the cell interior to the thoracic duct lymph *via* the basolateral membrane. Enterocytes are simple columnar epithelial cells that line the inner small and large intestines. The apical membrane of these enterocytes contains Niemann-Pick C1-like 1 (NPC1L1), a polytopic transmembrane protein that functions as a cholesterol transporter (Jia *et al.*, 2011).

The NPC1L1 will allow the diet and bile into the enterocytes, followed by the esterification process of free cholesterol (FC) from the diet and bile by acyl CoA: cholesterol acyltransferase-2 (ACAT)-2 enzyme. The step later produces cholesterol esters (CEs) in the endoplasmic reticulum (ER) (Lee *et al.*, 2000). Finally, the CEs will be packed into lipoprotein or undergo exocytosis, excreted, and distributed throughout the body. All of these processes will subsequently increase the level of lipids in the body (Brown and Yu, 2009). Therefore, cholesterol absorption inhibition by inhibiting the NPC1L1 or reducing the esterification process by inhibiting acyl CoA: cholesterol acyltransferase-2 (ACAT)-2 can be an excellent strategy for lowering lipid levels.

A series of bioactive polyphenols such as curcumin from turmeric (*Curcuma longa* L.), and pigments such as lycopene from tomato (*Solanum lycopersicum* L.), and monascin and ankaflavin from mould *Monascus* species have been reported to inhibit cholesterol uptake by binding on NPC1L1 or interfere with the pathways that involve NPC1L1 (Ji *et al.*, 2019). Meanwhile, compounds such as triterpenic acid from hawthorn, and berberine from Chinese goldthread (*Coptis chinensis* Franch.) have inhibited intestinal ACAT activity (Lin *et al.*, 2011; Wang *et al.*, 2014).

Cholesterol synthesis suppression

Cholesterol level reductions can be achieved by interfering with the pathways involved in cholesterol biosynthesis. These can be done by inhibiting the enzymes such as HMG-CoA reductase (HMGCR) (involves mevalonate pathway) and squalene synthase (SQS) (involved in the isoprenoid pathway), suppressing the transcription factors that regulate the enzymes (SREBP-2), or by modulating the key sensor in the lipid metabolism, AMP-activated protein kinase (AMPK) (Ji *et al.*, 2019). Examples of compounds that use this mechanism are

Table 1. Several plants with anti-hyperlipidaemic activities.

Plants	Part used	Traditional usage	Type / variety / cultivar	Pharmacological activity	Experiment model	Main active compound
Ginseng (*Panax ginseng* C.A.Mey.)	Rhizome	Used (as tonic) to invigorate, treat fatigue, improve blood circulation and breathing, haemorrhage, fever, headache, and discomfort, before or after parturition (Ong, 2004; Park *et al*., 2012).		Decreased total serum cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and plasma malondialdehyde (MDA) levels. Increased high-density lipoprotein (HDL) level, superoxide dismutase (SOD), and catalase (CAT) activity.	Human - normal subjects, 8 weeks, 6 g per day (Kim and Park, 2003).	Ginsenoside.
				Significant decrease in LDL, TC, LDL/HDL, and CHOL/HDL ratios.	Human - hypercholesterolaemic subjects, 400 mg per day (200 mg, 2 times per day), 8 weeks (Hamdan *et al*., 2013).	
			Red ginseng	Decreased hepatic triglyceride and cholesterol levels. Suppressed expression of fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.	HepG2 cells, 1 - 50 µg/ml (Lee *et al*., 2015).	
			White ginseng	No significant improvement on lipid profile, but positive effect on morphological of the aorta and antioxidant enzyme activities.	High cholesterol-fed New Zealand white rabbits, 0.05% white ginseng incorporated in high cholesterol food, 4 weeks (Lee *et al*., 2013).	
Ginger (*Zingiber officinale* Roscoe)	Rhizome	Used to relieve myalgia (muscle pain), rheumatism, pains, fever; respiratory-related sickness - flu, cough, and asthma; stomach-related problem - diarrhoea, cramps, bloated, and indigestion. It is also used to treat motion sickness, toothache, hypertension, and chills, and to improve appetite (Afzal *et al*., 2001; Semwal *et al*., 2015).		Decreased trimethylamine-N-oxide (TMAO), up-regulation the expression of hepatic cholesterol 7α-hydroxylase (CYP7A1), and promoted excretion of total acidic sterols in faecal. Improved plasma levels of pro-inflammatory markers -interleukin (IL)-1β, IL-6, tumour necrosis factor α (TNF-α), and monocyte chemotactic protein 1 (MCP-1).	In hypercholesterolaemic mice, 1% ginger extract was in the diet for 12 weeks (He *et al*., 2019).	Gingerol and shagaol.
				Significant decrease in triglyceride, cholesterol, LDL, and very low-density lipoprotein (VLDL) levels.	Hyperlipidaemic patients, 3 g/day, 45 days (Alizadeh-Navaei *et al*., 2008).	

Tea (*Camellia sinensis* (L.) Kuntze)	Leaf	Used (in infusion) to reduce heat in the body, eliminate thirst, shorten the sleeping time, maintain weight, and as an anti-inflammatory.	Pure compound, epigallocatechin-3-gallate (EGCG)	Decreased liver weight and coefficient, serum TG, TC, LDL-C, and free fatty acid (FFA) levels. Increased serum HDL-C levels in hyperlipidaemic rats. Suppressed malondialdehyde (MDA). Increased superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) contents. Activated SIRT1 and FOXO1, regulate SREBP-2 protein. Inhibit hepatic cholesterol synthesis.	SREBP-2 transgenic rats, 50 mg/kg (Li and Wu, 2018).	Epigallocatechin-3-gallate (EGCG), epicatechin (EG), and theanine.
			White tea	Decreased VLDL by regulating apolipoprotein B (APOB) and microsomal triglyceride transfer protein (MTTP) genes. In addition, activation of sterol regulatory element-binding protein 2 (SREBP2) and peroxisome proliferator-activated receptor δ (PPARδ) subsequently increased the low-density lipoprotein-cholesterol (LDL-C) uptake by low-density lipoprotein receptor (LDLR).	HepG2 cells, 100 - 300 µg/mL of 90% ethanolic extract (Luo *et al*., 2020).	
Garlic (*Allium sativum* L.)	Bulb	Used for high blood pressure treatment, antibacterial, and anti-venom for snake bites (Koch and Lawson, 1996).		Decreased body weight, blood cholesterol, TG, and glucose and significantly decreased hepatic cholesterol storage.	ICR mice, 12 weeks treatment, 20 mg/kg.	Allicin.
				Decreased TC, free cholesterol (FC), and cholesterol ester (CE) levels. Promoted cholesterol efflux by upregulating the ABCA1 expression and reduction of lipid accumulation *via* PPARγ/LXRα signalling.	THP-1 macrophages (Lin *et al*., 2017).	
Stevia (*Stevia rebaudiana* Bert.)	Leaf	Used as alternative sweeteners in cooking and herbal preparation to overcome bitter taste (Vaněk *et al*., 2001)		Decreased body weight and significantly downregulated TC, LDL, and VLDL levels. Improved HDL level as compared to control group.	Hyperlipidaemic rats, 400 mg/kg, 8 weeks (Ahmad *et al*., 2018).	Glucoside; stevioside, steviolbioside, rebaudiosides, and dulcoside.
				Downregulated the expression of peroxisome proliferator-activated receptor-gamma (PPARγ), sterol regulatory element-binding protein-1c (SREBP-1c), CCAAT/enhancer-binding protein alpha (C/EBPα), and fatty acid synthase (FAS). Increased protein expression for carnitine palmitoyltransferase 1 (CPT1), silent mating type information regulation 2 homolog 1 (SIRT1) and peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). Activated adenosine monophosphate (AMP)-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) both *in vitro* and *in vivo*.	3T3-L1 adipocyte cells, 200 µM of stevioside, for 24 and 72 h, DB/DB mice, 40 mg/kg of stevioside, and 3 weeks (Park *et al*., 2022).	

Turmeric (*Curcuma longa* L.)	Rhizome	Used (as paste) to treat eye infections, treat wounds, burns, and skin diseases (Thakur *et al.*, 1989).	Significantly decreased TC, TG, leptin, and free fatty acids in hyperlipidaemic hamsters. Increased HDL and apolipoprotein A-I (AOA-I) and paraoxonase activity in plasma, compared with the control group. In the liver, fatty acid β-oxidation activity was significantly higher, while FAS, HMG-CoA reductase, acyl CoA: cholesterol acyltransferase-2 (ACAT)-2 activities were significantly lower.	Hyperlipidaemic hamsters, 0.05 g/100 g diet (curcumin supplementation on a high-fat diet), 10 weeks (Jang *et al*., 2008).	
			Decreased LDL-C and c-reactive protein (CRP) as compared to the control group.	Human - healthy subjects with metabolic syndrome, 2.4 g/day, 8 weeks (Amin *et al*., 2015).	
Fenugreek (*Trigonella foenum-graecum* L.)	Seed, leaf	Used to control blood glucose and cholesterol. It is also prescribed to treat gastro-intestinal-related problems such as constipation, respiratory system (bronchitis), endocrine, and hepatic (Sun *et al.*, 2021).	Significant decrease in serum total cholesterol, LDL-C, and the atherogenic index, with a significant increase in the HDL-C.	Hyperlipidaemic rabbits, aqueous emulsified fenugreek seed powder (500 mg/kg body weight/day), 4 weeks (Sharma and Choudhary, 2017).	Major steroidal sapogenins: diosgenin, yamogenin, tigogenin, neotigogenin, smilagenin, and sarsasapogenin (Basu, 2006).
			TG, TC, LDL, and fasting blood glucose (FBG) levels significantly decreased in the borderline hyperlipidaemic group as compared to the placebo group.	Human - borderline hyperlipidaemic subjects, 8 g fenugreek seed powder, 8 weeks (Yousefi *et al*., 2017).	
			Significantly decreased LDL-C, TC, TG, and FBG after 12 weeks of administration. Increased in HDL-C as compared to the placebo group.	Human - hyperlipidaemic subjects, 60.0 g per day, 12 weeks (Fedacko *et al*., 2016).	

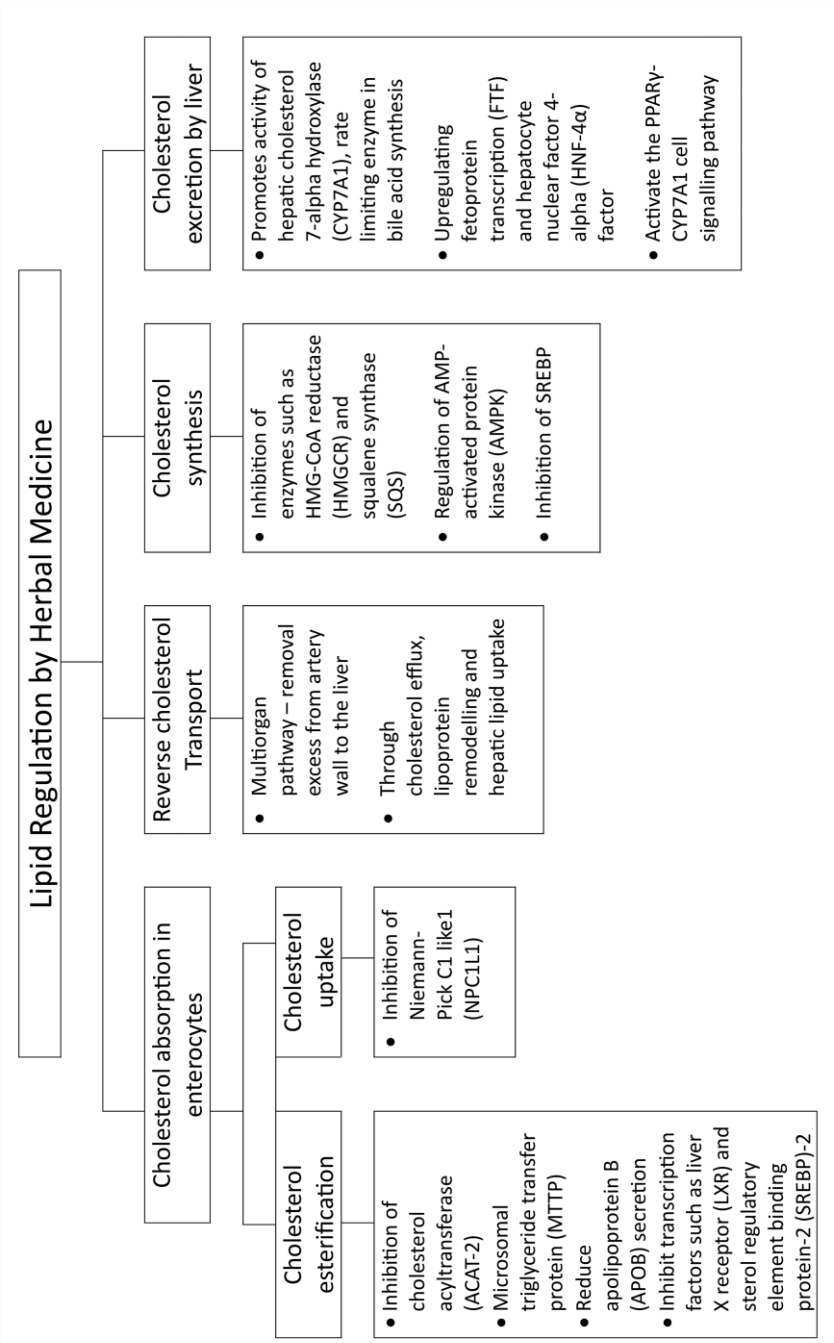

Figure 1. Lipid metabolism regulation by the bioactive compounds in herbal medicines (Ji *et al.*, 2019).

alkaloids such as ginsenosides and flavonoids epigallocatechin-3-gallate (EGCG) (Table 1). The ginsenoside isolated from ginseng suppressed the expression of fatty acid synthase (FAS) and HMGCR. Flavonoids such as epigallocatechin-3-gallate (EGCG) and epicatechin from tea leaves regulate the SREBP-2 (Lee *et al.*, 2015; Li and Wu, 2018; Luo *et al.*, 2020).

Reverse cholesterol transport

Reverse cholesterol transport (RCT) is a mechanism that involves multiple organs in the removal of excess cholesterol from peripheral tissues, and delivering them to the liver, which finally will be excreted into bile and faeces (Leaf, 2003; Chapman *et al.*, 2010). The primary lipoprotein involves in this process is HDL-C, whereby the mature HDL-C interact with scavenger receptor class B member 1 (SR-B1) in the liver to regulate the cholesterol transfer. The indirect process of transferring cholesterol from matured HDL-C to apolipoproteins B-100 (Apo B-100), primarily low-density lipoprotein (LDL), is catalysed by the cholesteryl ester transfer protein (CETP) (Xu *et al.*, 2013; Rosenson *et al.*, 2016). This process can decrease the concentration of HDL-C and apolipoprotein A-I (AOA-I) and increase CE's concentration in VLDL and remnants. Therefore, inhibiting CETP activity can decrease VLDL and LDL-C, and later translate into the reduction of atherosclerotic plaque (Shrestha *et al*., 2018). In addition, HDL cholesterol content in plasma has been noted to exert anti-inflammatory, which is crucial in the prevention and treatment of atherosclerotic plaque and cardiovascular events (Feig *et al.*, 2014). Compounds such as leoligin from roots of edelweiss (*Leontopodium nivale*) and anthocyanin has shown to inhibit CETP (Reisinger *et al*., 2009; Qin *et al*., 2009; Duwensee *et al*., 2011).

Increase cholesterol excretion by the liver

Cholesterol homeostasis is maintained by regulating cholesterol catabolism and bile acid synthesis. Rate-limiting enzyme cholesterol 7- alpha-hydroxylase (CYP7A1) in the neutral pathway of bile acid synthesis plays a significant role in this. Compounds like gingerol and shogaol from ginger have been noted to modulate CYP7A1 activity by upregulating the CYP7A1 gene expression and promoting the excretion of acidic sterol in the faeces (He *et al.*, 2019). Similarly, green tea catechin has also been noted to enhance CYP7A1 gene expression. Lee *et al.* (2008) noted that the ECGC stimulated the gene at the mRNA level and its dose-dependent activity.

Monitoring herbal quality control

One of the critical aspects of herbal products before being marketed to the public is maintaining the quality of the herbs - starting from the raw materials to the processed finished products; these include one-herb formulation or multiple mixtures of herb formulation. Chemical composition in plants is known to vary based on internal and external factors such as their botanical species and origins (*i.e*., planting conditions, weather conditions, and climates), growth stages, the parts that are used (*i.e*., aerial part, underground part or whole), and pre- and post-harvest conditions (*i.e*., storage conditions and processing methods) (Goodarzi *et al.*, 2013). In addition, certain plant species or herbs are limited in number, which can prompt counterfeit herb products or being replaced with another species within the same genus with similar morphology (Zhao *et al.*, 2015).

To overcome this, regulatory bodies such as the European Medicine Agency (EMA) and the World Health Organisation (WHO) have come out with a guideline on accessing the quality of herbal medicinal products (by EMA) and strategy for traditional medicine (by the WHO). Preparation of standardised extract, identification of active compound, and the quantity of the herbal substance is among the required information listed in these guidelines. The quality control for herbal products or formulations is done by either monitoring or quantifying one or a few compounds known for their therapeutic effect or by monitoring the chemical fingerprint (WHO, 2013; EMA, 2022).

Chemical fingerprint

Similar to fingerprint in humans, which is unique to each individual, the chemical fingerprint in plants comprises the characteristic profile or composition of chemicals inside the said plant (Alaerts *et al.*, 2012; Goodarzi *et al.*, 2013). However, instead of monitoring one or a few compounds, chemical fingerprints are intended to grasp as much information as possible by chromatographic or spectroscopic techniques (Alaerts *et al.*, 2012; Goodarzi *et al.*, 2013).

Chromatographic techniques

Between these two, chromatographic techniques are widely used and acceptable in identifying and quantifying chemical markers (Bingbing *et al.,* 2021). These include simple methods such as thin layer chromatography (TLC) and high thin layer chromatography (HPTLC), and more advanced analytical instruments such as liquid chromatography [*i.e.*, high-performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC)]. Generally, liquid chromatography (LC) is used for detecting non-volatile compounds, and gas chromatography (GC) is used for volatile compounds (Alaerts *et al.,* 2012; Goodarzi *et al.,* 2013; Sendker and Sheridan, 2017).

Mathematical data handling techniques such as similarity analysis (SA) and exploratory data analysis are used to determine the dissimilarities between the samples from the standard control or the authentic standard (Alaerts *et al.,* 2012; Sendker and Sheridan, 2017). For example, SA - samples are compared two by two using SA parameters, such as correlation coefficients (*r*). Correlations coefficients (*r*) in fingerprints have been used to detect adulterants and inter-intra batch-to-batch effects on the sample (Yang *et al.,* 2011).

While in exploratory data analysis, instead of using coefficients, the trend in the data is visually presented using principal component analysis (PCA) or hierarchical clustering analysis (HCA) (Yang *et al.,* 2011). Despite the advantages of the chromatographic techniques, the processed data usually tend to focus on several selected peaks that are the significant compounds (Sehlakgwe *et al*., 2020). However, the techniques might cause some information to miss or slip through since herbal medicine comprises a complex chemical profile. In addition, some of the minor peaks or information might be the ones that synergistically affect the bioactivity and the efficacy of herbal medicines (Yang *et al.,* 2011; Sehlakgwe *et al.,* 2020).

Spectroscopic techniques

As mentioned earlier, chemical fingerprints can also be determined by using spectroscopic techniques (van Helmond *et al.,* 2019). Advancement in technology makes it possible for more sophisticated analytical instruments such as nuclear magnetic resonance (NMR), mass spectroscopy (MS), and Raman and Fourier transform infrared (FTIR) to be applied in herbal quality control (Liu *et al.,* 2016). Among all the spectroscopy techniques, NMR and MS are the ones that are extensively used (Marshall and Powers, 2017). Analytical techniques such as NMR and MS can present the complete composition of the entire plant or herbal extract — either by working independently or complemented by chromatographic techniques such as LC and GC (*e.g*., LC-MS, GC-MS, and LC-NMR) (Liu *et al.,* 2016).

Metabolomics approach

The data generated from spectroscopic techniques such as NMR and MS are larger and more complex. Therefore, the metabolomics approach has utilised the big data generated by these analytical techniques. Metabolomics aims to identify and quantify the metabolites' response to internal and external factors (as maximum as possible)—including genetic modification and pathological stimuli (Casadei *et al.,* 2018). Metabolomics can be conducted in two approaches—targeted and untargeted approaches. The targeted approach is usually conducted when the investigator has a specific set of targeted group metabolites. While in the untargeted, the investigator will look at the metabolites wholly (Hanhineva and Pasanen, 2017).

Data from the NMR and MS will then be processed, treated, and statistically analysed using multivariate analysis (MVDA) using the unsupervised or/and supervised method (Eriksson *et al.,* 2006). Unlike univariate analysis, MVDA is a statistical technique used to analyse data generated from more than one variable. Unsupervised methods such as principal components analysis (PCA) and supervised methods such as partial least square (PLS) or orthogonal partial least square (OPLSDA) will be used to analyse the data (Eriksson *et al.,* 2006). The findings will be presented visually—the clustering among the samples can provide information on how much it deviates from the sample control and authenticated standard, and which compounds or metabolites are responsible for the separation. Since any changes in metabolome can affect the clustering—sample preparation, and pre- and post-processing of the data is crucial. Therefore, thorough preparation and planning must be done before the experiment (Eriksson *et al.,* 2006).

When selecting analytical instruments for the metabolomics approach, both NMR and MS had advantages and disadvantages. For instance, samples analysed using NMR is recoverable, which is helpful, especially when dealing with a limited amount of

sample, and the samples can be used for other tests. On the other hand, MS has higher sensitivity than NMR—it can detect up to nano- and picomolar compounds or metabolites, which makes it ideal for detecting low concentration biomarkers, chemical markers, and many more. A summary of advantages and disadvantages of these two analytical techniques is given in Table 2.

Nevertheless, despite the minor shortcomings, the metabolomics approach is widely used in other fields such as ecology, forensics, healthcare, and nutrition (Xiao *et al*., 2022). The disadvantages of both analytical instruments can be reduced either by combining these instruments for analysis or by adapting to the current methods or protocol (Sehlakgwe *et al.*, 2020).

Table 2. Comparison between NMR and MS spectroscopy for methods in metabolomics analysis (Kikuchi and Hirayama, 2006; Khoo and Al-Rubeai, 2007; Teng, 2013; Emwas *et al.*, 2013).

Characteristic	NMR spectroscopy	MS Spectroscopy
Sensitivity	Low, however, can be improved with higher field strength (500 - 700 MHz), with additional micro- and cryo-, and dynamic nuclear polarisation probe. NMR can detect compounds up to micromolar (μM).	High detection can detect up to nano- and picomolar metabolites. However, the result can suffer from ion suppression in complexes and salty mixtures.
Selectivity	Usually used for non-targeted metabolites.	Used for both targeted and non-targeted metabolites.
Reproducibility	High.	Low.
Tissue sample	Can be analysed directly by using high magnetic magic angle spinning, NMR-MAS.	Tissue needs to be extracted prior to analysis.
Sample preparation and measurement	With little to no preparation, all metabolites can be analysed simultaneously.	Different column is needed for different class or group detection; the need to optimise the method based on the sample is time-consuming. Preparation in GC-MS is more tedious and complex compared to LC-MS.
Sample recovery	Recoverable.	Non-recoverable (destructive) due to derivatisation.
Experiment time	About 5 -15 min per sample, depending on the type of experiment conducted, proton NMR requires a shorter time, while pre-saturation experiments such as water suppression might take up to 10 - 15 min. Experiment 2-dimensional NMR might take up to 16 - 24 hours.	Less than 3 min for direct infusion, more than 10 min.
Molecular dynamics and diffusion	Applicable.	Not applicable.
Target analysis	Not good.	Good.
Number of detectable metabolite	40 to 200 metabolites.	Can be up to more than 500 on various MS techniques
Specific advantage	Non-destructive, good replication, and can provide structured information.	GC-MS: high sensitivity, and the standard library is available for identification. LC-MS: high sensitivity, high detectable of metabolites.
Specific disadvantage	Low sensitivity and not good in targeted analysis.	GCMS: Complex sample preparation, destructive (sample non-recoverable), not suitable for metabolites that are heat labile and have a high boiling point. LC-MS: Ion depression effect, lack/no structure information, destructive detection.

Metabolomics application in quality control - ginseng and others as an example

Among the plants that possess hypolipemic activities, ginseng is the plant that is extensively researched and studied. Ginseng (*Panax ginseng* C.A.Mey.), also known as Asian ginseng, is highly sought after in Asia, especially in TCM. It was estimated that the herbs are consumed in more than 35 countries worldwide, with the industry expected to be 43.07 million dollars by 2027 (Park *et al.,* 2014; Market Data Forecast, 2022). Besides being used for health purposes, herbs are also used for beauty industries and even incorporated into foods to increase food functionality. Two ginsengs are commonly available in the market: white ginseng (WG) and red ginseng (RG). The differences between these two types are based on their post-harvest processing method. The fresh ginseng will either be dried to produce the WG, or steamed for 2 - 3 h at 95 - 100°C and left to dry for RG production (Zhang *et al.,* 2012; He *et al.,* 2018).

Ginsenosides are the main active components in *P. ginseng.* There are about 24 types of naturally occurring ginsenosides in *P. ginseng*, whereby ginsenosides such as Rg_3, Rg_5, Rg_6, Rh_1, Rh_2, Rk_1, Rs_3, and F_4 are known to be specific to RG. Ginsenosides Rb_1, Rg_1, and Re are used as the standard by the Chinese Pharmacopoeia to determine the quality of ginseng (Zhang *et al.,* 2012). However, these three ginsenosides are major to both RG and WG. Therefore, it is difficult to differentiate them, and use them as quality control markers.

A similar problem is also encountered when distinguishing different species within the same genus, or different cultivars within the same species. In the case of *P. ginseng*, it is usually substituted with another ginseng species such as *P. quinquefolius* (American ginseng). The differences in morphology between these two ginsengs are limited since they are from the same genus. Ginsenosides Rf and F_{11} have been selected as the biomarkers using LC-MS/MS (Li *et al.,* 2000). However, this method still cannot fully discriminate between the two species (Park *et al.,* 2014). In addition, other factors such as plant parts or growth stages, and the condition of plants used in herbal medicine can also influence the activity, efficacy, and human safety (Lee *et al.,* 2019).

Therefore, several studies have used either NMR-based or MS-based metabolomics approaches to solve these problems. For example, in the case of *P. ginseng*, the chemical marker that distinguished the processed *P. ginseng* - WG and RG were determined. By using LC-MS-based metabolomics, a total of 43 compounds were identified, including three sulphur compounds. The ginsenoside R_3 was found to be the chemical marker of RG, and in WG, the primary chemical marker was malonyl ginsenoside Rb_1/isomer and malonyl ginsenoside Rg_1/isomer (Zhang *et al.,* 2012). Zhang *et al.* (2012) also highlighted that specific quality issues in post-harvest treatment affect the quality and produce inconsistencies, such as heat treatment and sulphur fumigation in WG, and non-standardised processing procedure in RG.

The metabolomics approach also managed to distinguish different types *of Panax* species. For example, Park *et al.* (2014) used an optimised ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS) method to confirm Rf as the chemical marker with additional ginsenosides Ra_1 and F2 as a potential biomarkers for discriminating *P. ginseng* and *P. quinquefolius*. A clear separation also being achieved by Zhao *et al.* (2015), which used an untargeted proton NMR (^{1}H NMR) metabolomics approach as quality control in distinguishing commercial *P. ginseng* and *P. quinquefolius* (cultivated and wild types).

In addition, the metabolomics approach is also being applied to determine the effect of external factors such as growing conditions, and internal factors such as different ginseng parts usage - which can also affect the quality of the ginseng (In *et al.,* 2017; Lee *et al.,* 2019). In a study by In *et al.* (2017), the different parts of roots, main, lateral, and fine roots showed that protopanaxadiol (PPD), highest in fine roots, was responsible for the discrimination of the different parts. The effect of the growing season on ginseng was studied by Lee *et al.* (2019) using ^{1}H NMR. Lee *et al.* (2019) highlighted the changes in the primary and secondary metabolites throughout the seasons. The result is essential, primarily to determine the optimum growing stages for harvesting ginseng, and the suitable season for planting. Therefore, the quality of the ginseng being harvested is premium.

Aside from ginseng, the metabolomics approach is also being applied to several other lipid-lowering plants for quality control. Gingerol is the primary compound in ginger (*Zingiber officinale* Roscoe) Like ginseng, the availabilities of gingerol

and the other active compounds depend on the ginger varieties or cultivar, and pre- and post-harvest treatments can also affect the active components. A study conducted by Tanaka *et al.* (2015) using LC-MS managed to distinguish five types of *Z. officinale* (Table 3). They noted that acetylated derivatives in gingerol was the important markers in distinguishing the ginger types instead of gingerol. In this study also, they managed to discriminate between the dried rhizome of "Tokyo" and steamed and dried rhizome of "Kankyo" of *Z. officinale* var. *Rubens.*

In tea (*Camellia sinensis* (L.) Kuntze) leaves, post-processing plays a significant part in the tea produced. Among processed tea, white tea is considered the least processed form of tea. Unlike black tea or green tea, which involves fermentation or frying, white tea only involves drying the leaves under the sunlight or at a controlled room temperature (Dai *et al.*, 2017). A study by Dai *et al.* (2017) highlighted the differences between the tea types based on their metabolites, which were mainly contributed by the treatment process (Table 3).

Garlic (*Allium sativum* L.) is a vegetable known for its aroma and flavour which are mainly contributed by organosulphur and nitrogen-containing metabolites inside the bulb. A few studies have been conducted to detect the changes in the sulphur and nitrogen metabolites since most of these metabolites are considered volatile compounds prone to post-harvest treatments, environmental factors, and growth stages. Therefore, the metabolomics approach has been used by several researchers in determining the quality of garlic (Table 3).

In summary, the metabolomics approach manages to distinguish and identify the differences in herbal plant species within the same genus, different cultivars within the same species, or the effect of external factors that influence the availability of the metabolites in the plant metabolome (Dai *et al.*, 2017; Lee *et al.*, 2019).

Table 3. Metabolomic approach application in herbal plants.

Plant	Experiment	Result
Ginger	Chemical properties comparison of *Z. officinale* var. *Rubens* "Shokyo and Kankyo" (processed and non-processed), *Z. officinale* var. *Rubra*, *Z. officinale* var. *amarum*, and Z. officinale cv. Ogawa Umare using LC-MS (Tanaka *et al*., 2015).	Acetylated derivatives of gingerol distinguished important chemical markers that distinguished the different ginger types.
Tea leaves	Comparison of white tea metabolome with green tea and black tea using LC-MS (Dai *et al*., 2017).	Different levels of amino acids, catechins, dimeric catechins, flavonol glycosides, flavone glycosides, and aroma precursors. Amino acids (*i.e*., proline and valine), kaempferol glycosides, and galactosylated quercetin compounds were high in white tea.
Garlic	Quality of garlic in six growth stages (Liu *et al*., 2020).	A total of 91 metabolites were identified. Different patterns in growth stages from different places. Metabolites with "γ-glutamyl-" increased dramatically during growth.
	Metabolites differences in dark and light purple garlic (Leite *et al*., 2021).	Organic acid and fatty acids such as malonic acid and docosanoic acid were the metabolites that differentiate between dark and light purple garlics.
	Authenticating the geographic origin of garlic using high-resolution mass spectrometry (HRMS) (Hrbek *et al*., 2018).	Alliin, phosphatidylcholine (16:0/18:2), arginine, dehydroalanine, phosphatidylethanolamine (16:0/22:6), L-γ-Glutamyl-S-allyl-L-cysteine, and choline glycerolphosphate were the compounds that contributed to the classification of the samples.

Conclusion

Metabolomics approach can be one alternative for quality control of herbal and herbal-related products in the market. This approach is essential to maintain the herbal quality, safety, and efficacy. In addition, more studies and data are needed to establish a metabolite databank. Consequently, identifying and establishing chemical markers for quality purposes would then be easier.

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