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MINI REVIEW

Current authentication methods of herbs and herbal products: a systematic review

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Abstract

The authentication of herbal species is of great concern for the quality control of herbal products. Hence, many authentication methods have been developed for the accurate identification of herbal species. This systematic review aimed to gather information and provide evidence about the current authentication methods for several herbs and herbal products. In this study, the methodological quality was also evaluated using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist method. As a result, a total of seventeen articles were found to suit the inclusion criteria of this study, which include molecular-based analysis, chemical fingerprinting, as well as macroscopic and analogical analysis. All of these methods have different approaches to the authentication of herbal-related products, and it also depends on the specific needs. This review also asserts that the combination methods might be a useful alternative for authentication purposes that produce better results. The multivariate analysis combined with analytical methods could be a great combination due to its great performance in authentication and suitability for routine screening.

1. Introduction

Herbs can be defined as plant materials derived from parts of a plant, which might be entire, fragmented, or in powdered form (WHO, 2011). Most herbs are considered an important source of nutrition due to their therapeutic values (Yudharaj *et al*., 2016). Currently, the Malaysian herbal industry is facing rapid development due to the overwhelming demand for herbal-based products (Ahmed, 2021). Since this industry has a great potential for being one of Malaysia's main agriculture industries (Nordin *et al*., 2008), this situation might, therefore, contribute to the increased demand for the supply of herb materials.

However, the lack of adequate supply due to such high demand has created a crisis that fuels the potential for product adulteration due to the absence of effective regulation, quality control, or standardization of herbal products (Hassali *et al*., 2013). Hence, this has resulted in undue reliance on foreign-market raw materials such as China, Indonesia, Thailand, India, and many other nations. Nevertheless, imported raw materials sometimes come with many unacceptable problems such as poor quality, adulterated issues, and unreasonable prices (Zaki and Rani, 2013). Consequently, the lack of herbal materials supply led to arbitrary substitution and adulteration in the raw drug market (Sagar *et al*., 2014).

Herbal authentication is a quality assurance process that ensures the correct herbal species and plant parts are used as raw materials for herbal products (Sued *et al.,* 2016). Besides, correct identification of herbal plants forming the herbal products is a prerequisite and fundamental to the whole realm of medicine and science (Ganie *et al*., 2015). Moreover, identifying herbal plants as the raw materials should be performed to ensure that the raw materials used in finished products are suitable for their intended use (Osathanunkul *et al*., 2018). In addition, it is crucial to utilize a precise authentication method to ensure that the herbs used as materials are correct and authentic prior to any processes in order to ensure the quality and the safety of finished herbal products (Leonti, 2013; Mishra *et al*., 2016). Thus, authentication of herbal plants taken up for research or medicinal use is a necessity to achieve satisfactory results and also to maintain the efficacy and therapeutic property of the preparations in which these plants are used (Ganie *et al*., 2015).

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Authentication tests usually involve applicable analytical techniques in dealing with particular samples (Hargin, 1996). This is useful in the cases of finished herbal products that are frequently substituted or adulterated with other morphologically and chemically indistinguishable varieties (Revathy *et al*., 2012). Commonly, the methods used to assess the authenticity of herbs depend on morphological and analogical analysis, organoleptic characters, deoxyribonucleic acid (DNA)-based, chemical fingerprinting and many others (de Boer *et al*., 2015; Parveen *et al*., 2016). However, different methods will have different roles and limitations for authentication purposes. Therefore, this systematic review aimed to gather information and provide evidence about authentication methods used for herbs and finished herbal products. Correspondingly, a systematic review of existing single authentication and combination authentication methods is performed, in order to know the current research that has been done in this area. This information could be useful for individuals or agencies in selecting the most adequate authentication of herbal-related products.

2. Methodology

This review was performed by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement checklist method that involves four steps, namely identification, screening, eligibility, and inclusion as shown in Figure 1. These steps are also based on the previous systematic reviews by Zakaria *et al*. (2020) and Wiseman and Harris (2015).

2.1 Identification

The search activity started at the beginning of October to November 2020. The first process in the systematic review was the identification of keywords. There were several main keywords used to search appropriate studies including "authentication", "authenticate", "method", "herbal product" and "herbs". The literature related to the authentication of herbs or herbal products by several methods was identified through only one electronic database called Scopus. This database was selected because it delivers a comprehensive overview of various scientific research outputs and provides reliable information required for this review. The database search was also limited to articles in the English language and published between 2016 and 2020. Titles and abstracts were scanned for relevance by one author and the reference lists of the selected articles were also reviewed.

2.2 Screening

The screening process was done by assessing the selected studies to determine whether they met the inclusion criteria as stated in Figure 1. This enables the authors to identify any disagreements pertaining to the suitability of this study. During this process, articles that did not align with the inclusion criteria were removed and the reason for the exclusion was recorded.

2.3 Eligibility

The full-text articles were reviewed by the authors to determine the inter-rater reliability for the inclusion and exclusion criteria. The criteria for eligibility captured studies on the various authentication methods related to herbs; besides, only journal articles and open access journals in Scopus were included in this review.

2.4 Inclusion

The inclusion criteria were included to assess the relevance of each study: (1) herbal authentication by identifying the herbal species, (2) the method used to authenticate the herbal species, and (3) plant parts such as leaves, flowers, fruit, seed, bark, root, stem, rhizomes and herbal materials such as fresh juice, gums, fixed oils, essential oils, resins; and herbal preparations such as extracts or tinctures.

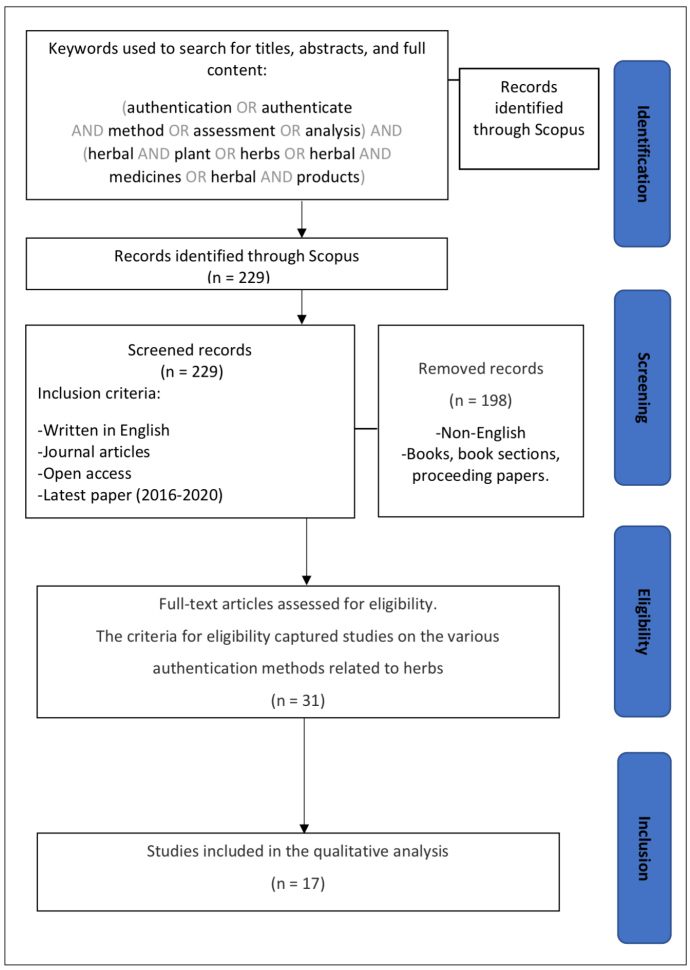

Figure 1. The PRISMA flow diagram

3. Results

Our systematic search found 229 studies on the authentication of herbal products from the Scopus database. After excluding the needful by reading abstracts and titles, 198 articles did not meet the inclusion criteria. The exclusion reasons along with the flow diagram are presented in Figure 1. Subsequently, 31 articles were selected to be evaluated in detail. However, 14 articles were excluded because there was no clear explanation about the authentication methods of herbal products. Finally, 17 studies were selected for this systematic review and the methods used in each study are summarized in Table 1. In addition, previous studies have also shown a balanced selection of methods, specifically the sole methods and combination methods for authentication purposes. Hence, this review describes authentication through the combination (n = 11) and single methods (n = 6).

4. Discussion

Most of the included studies involve the identification and discrimination of plant species from their adulterants by which the quality of the herbal-related products is of concern. A wide variety of methods have also been developed and used in authentication studies for identification and discrimination purposes (Böhme *et al*., 2019). In this review, several commonly used methods in the authentication of herbal-related products are summarized, which involve both single and combination methods.

4.1 Herbal authentication by single methods

Various methods are often used for authentication, identification, and discrimination purposes. These methods involve the single methods of molecular-based, chemical fingerprinting, and morphological and anatomical analysis. Overall, all of these methods have different approaches to the authentication of herbal-related products and it also depends on the specific needs. Thus, the advantages and disadvantages of the method in the authentication of herbs had been summarized in Table 2.

Table 1. Methods used by the included studies

Author	Analytical methods	Multivariate analysis
Raclariu *et al*. (2017)	DNA metabarcoding, thin layer chromatography, and high-performance liquid chromatography-mass spectrometry.	None
Seethapathy *et al*. (2019)	DNA metabarcoding	None
Kim *et al*. (2017)	Reverse transcription- Polymerase chain reaction melting array	None
Aladdin *et al*. (2015)	Microscopy evaluation, high-performance thin -layer chromatography, and high-performance liquid chromatography.	None
Bekbolatova *et al*. (2018)	Macroscopic and microscopic evaluation	None
Lestari *et al*. (2019)	Macroscopic and microscopic evaluation	None
Liu *et al*. (2018)	Recombinase polymerase amplification and lateral flow strips	None
Osathanunkul *et al*. (2018)	DNA barcoding and high-resolution melting	None
Duan *et al*. (2018)	DNA barcoding and high-resolution melting	None
Li *et al*. (2018)	DNA barcoding and high-resolution melting	None
Mishra *et al*. (2018)	DNA barcoding and high-resolution melting	None
Fadzil *et al*. (2018)	DNA barcoding and high-resolution melting	None
Seethapathy *et al*. (2018)	DNA barcoding and nuclear magnetic resonance spectroscopy	None
Žiarovská *et al.* (2016)	Direct analysis in real-time, and time-of-flight mass spectrometer	Principal component analysis
Yuk *et al*. (2016)	Ultra-performance liquid chromatography and quadrupole time of flight mass spectrometer	Principal component analysis
Huang *et al*. (2019)	Matrix-assisted laser desorption ionization-time of flight mass spectrometry	Principal component analysis, hierarchical cluster analysis, partial least squares discriminant analysis, k-nearest neighbour, classification and regression tree, and soft independent modelling of class analogies.
Sima *et al*. (2018)	High-performance liquid chromatography and micellar electrokinetic chromatography	Principal component analysis, clustering analysis, and linear discriminant analysis.

Table 2. Advantages and disadvantages of method in the authentication of herbs

	Advantages	Disadvantages
DNA-based method	• Extremely sensitive and specific tool for authentication	• Time-consuming • Expensive large instruments and equipment • Not suitable for rapid analysis
Chemical fingerprinting	• Provide effective information about qualitative and quantitative information on the characteristic components • Able to explore chemically the complexity of herbal-related products	• Cannot distinguish between closely related species effectively • Depending on the chemical marker or target compound
Macroscopic and microscopic evaluation	• Can be done effectively on fresh parts of herbs	• Need experienced skills from a professional taxonomist • Tedious and time-consuming

4.1.1 Molecular-based analysis

Molecular-based analysis helps identify the biological composition of living species. Previously, various molecular-based analysis methods have been introduced for different purposes (Dorigo *et al*., 2002; Szabó *et al*., 2008). Meanwhile, deoxyribonucleic acid (DNA)-based techniques have been widely used for the authentication of plant species. DNA-based techniques such as the classical DNA-based method and DNA barcoding, are targeted approaches aiming to detect the presence of certain species (Mezzasalma *et al*., 2017). Interestingly, DNA metabarcoding, the combination of high-throughput sequencing (HTS) and DNA barcoding, enables untargeted approaches to detect species from complex mixtures and matrices (Raclariu *et al*., 2018). This is particularly useful for species that are often substituted or adulterated with other different species. This method also allows for the identification of unlabelled species with unsafe characteristics and adverse interactions with human consumption (Newmaster *et al*., 2013; Biswas and Biswas, 2014; Speranskaya *et al*., 2018). In this review, three studies have used molecular-based analysis for authentication assessment and performed a different type of analysis to investigate the authenticity of various herbal species and detect adulteration from closely related species that usually have been substituted or mixed up together in the preparation of herbal products.

Raclariu *et al*. (2017) used the amplicon metabarcoding (AMB) method and nuclear ribosomal Internal Transcribed Spacer (nrITS) as the primers to authenticate 78 herbal products containing herbal materials from *Hypericum perforatum*. As the result, this method could detect the presence of *H. perforatum* and some of its closely-related species that consist of *H. humifusum, H. tetrapterum* and *H. hirsutum* in the analyzed products by investigating the biological composition that contains a mixture of DNA from different species, with the relative success rate of 49 %. The finding of this study confirmed that AMB can be used to identify the presence of *H. perforatum* and simultaneously detect the substitution and adulteration from other species. Meanwhile, Seethapathy *et al*. (2019) investigated several Ayuverdic herbal products containing species that were not listed on the product labels. Several products sold as tablets, capsules, powders, and extracts were tested via DNA metabarcoding. Based on the results from both studies, the authors claimed that DNA-metabarcoding is one of the effective methods to be applied for quality control of herbal products because it can successfully detect the correct species as one of the materials in the herbal products.

This statement was refuted by Indrayanto (2018), who stated that the application of DNA profiling alone as a quality control tool is not recommended because the secondary metabolites of herbs might be affected by many external factors. The false negatives can also be expected because the DNA of herbs might be degraded during the manufacturing or post-harvest process (de Boer *et al*., 2015). Therefore, this method has the potential to be one of the reliable authentication methods if the external factors affecting its effectiveness could be controlled.

In contrast with the method used by Raclariu *et al*. (2017) and Seethapathy *et al*. (2019), Kim *et al*. (2017) used the reverse transcription-polymerase chain reaction (RT-PCR) melting curve assay to identify four medicinal *Paeonia* species and peptide nucleic acid (PNA) probes were used in this analysis. RT-PCR is a quantitative method for determining the copy number of PCR templates, such as DNA or cDNA, and consists of two types: probe-based and intercalator-based (Mo *et al.,* 2012). According to several studies, PNA probes are very efficient in detecting and identifying the taxonomic origins of various species (Hur *et al*., 2015; Kim *et al*., 2015; Han *et al*., 2016). Hence, this approach successfully discriminated against the four species and detected adulterants in the commercial herbal products. Mo *et al*. (2012) claimed RT-PCR is a relatively simple, inexpensive, extremely sensitive and specific tool to determine the expression level of target genes. Even

though this method is useful for quality control and standardization of *Paeonia*-based products, this approach is rather time-consuming because it took more than an hour to establish the PNA probe melting array. Similarly, Jia *et al*. (2020) found that most polymerase chain reaction (PCR)-based methods utilize expensive large instruments and equipment as well as take a long time to perform the analysis. Hence, these limitations make the field diagnosis of samples impossible and not suitable for rapid analysis.

4.1.2 Chemical fingerprinting

A chemical fingerprint is a unique pattern that indicates the presence of multiple biochemical markers within a sample (Li *et al*., 2008). Due to its advantages, this approach is one of the standard choices for species authentication and quality control of herbal products instead of molecular-based analysis (Pandithurai *et al*., 2015). In this review, two studies obtained from the Scopus database have evidently used chemical fingerprinting as the authentication tool for herbal products (Aladdin *et al*., 2015; Raclariu *et al*., 2017).

Chromatographic fingerprinting (CF), one of the chemical fingerprint approaches, is a technique used for the separation of a mixture into its constituent component (Mohammed Abubakar *et al*., 2017). The complex mixtures are separated due to the difference in the time taken for each component to travel through a system that contains a stationary phase and a mobile phase (Majors and Carr, 2001). CF technique has proved to be a comprehensive strategy for assessing the intact quality and exploring the complexity of herbal medicines (Wang *et al.,* 2012; Yin *et al*., 2013). Also, the determination of common peaks in a set of chromatographic fingerprints could provide effective information about qualitative and quantitative information on the characteristic components of herbal medicines investigated (Lalit *et al.,* 2010).

Raclariu *et al*. (2017) have also demonstrated the application of thin-layer chromatography (TLC) and high -performance liquid chromatography-mass spectrometry (HPLC-MS) in their study in order to authenticate the sample and the results showed that both of these methods have shortcomings in detecting the presence of adulterant or species substitution in *H. perforatum* herbal products. In the HPLC-MS method, hypericin and hyperforin were identified using as the compound markers. As the result, the chromatograms of *H. olympicum*, *H. patulum* and *H. polyphyllum* were indistinguishable from that of *H. perforatum* (Raclariu *et al.*, 2017). This shows that the evaluation of chromatograms of closely related- species by visual observation alone could be impossible and very subjective. This statement was supported by Indrayanto (2018) who suggested that, in order to have better herbal material identification results, the application of direct methods such as Fourier transform infrared (FTIR), near-infrared (N-IR), and Raman spectroscopy should be included. Therefore, there is a need to combine any suitable method with chromatography for proper herbal authentication.

Next, Aladdin *et al*. (2015) demonstrated the application of high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) methods in conducting the authentication assessment of the leaf and stem extracts of *Marantodes pumilum* varieties. Based on their study, it was found that HPTLC and HPLC fingerprint profile for the leaf extract of *M. pumilum var. alata* was distinguished from the stem and other two varieties, *M. pumilum var. pumila* and *M. pumilum var. lanceolata*. However, in HPTLC analysis, the stem extract could only be differentiated after the derivation of p-anisaldehyde. This might be due to the differences in the phytochemicals content in the leaf and stem. While the availability might be similar, the concentration of phytochemicals might affect the detection process in HPTLC. The findings of this study also reported that HPLC is better than HPTLC due to its ability to distinguish between the leaf and stem extracts of *M. pumilum* varieties. Thus, the chemical marker or target compound of the herbal products is crucial in this type of chemical fingerprinting. Subjectively, this could either serve as an implication or limitation of this approach.

4.1.3 Macroscopic and microscopic evaluation

Traditionally, herbal plants are usually authenticated based on their phenotype, which involves the experienced skills of a professional taxonomist. Identification methods employed in the authentication of herbal materials are macroscopic and microscopic evaluation. The macroscopic examination involves the comparison of morphological characters that are visible with the naked eye or under low magnification with descriptions of the plant or botanical drug in floras or monographs (Sued *et al*., 2016). These techniques are easily applied to fresh whole plants or plant parts are generally difficult to identify, as many useful diagnostic characteristics are lost during dehydration (Joharchi and Amiri, 2012). In addition, some researchers claimed that this analysis has many disadvantages in terms of its accuracy and effectiveness for authentication assessment, especially of plant species (Indrayanto, 2018; Osathanunkul e*t al.*, 2018). In addition, performing macroscopy and microscopy of each plant part in every consignment in the industry is a tedious and time-

consuming job (Singh *et al.,* 2018). On the other hand, three studies obtained from the Scopus database have used this approach to aid the identification and authentication of herbal products.

Based on Bekbolatova *et al*. (2018), the macroscopic and microscopic evaluation of *Crataegus almaatensis l*eaves, flowers, and fruits was done as part of pharmacognostic studies. Besides, this has also been used to set up the standardization criteria of the herbal products containing *C. almaatensis*. Since this evaluation is part of the standardization process, it is crucial for ensuring product safety and quality as well as the effectiveness of raw materials and finished products. Based on the results, all morphological features of *C. almaatensis* such as height, leaf characteristics, the intensity of leaf colour, flowers, and fruits were identified by macroscopic evaluation, while the anatomical features of *C. almaatensis* were determined by microscopic evaluation.

In a similar study by Aladdin *et al*. (2015), the authors reported that the transverse section of the fresh part of *Marantodes pumilum* would give the most accurate identification of the three varieties of the species. The identification was based on anatomical features that consist of stomata and trichomes, outline structure of leaf margin, petiole and midrib, organization of a vascular system, areolar venation, pattern of anticlinal walls of the epidermis, and the distribution of secretory canals and cell inclusion of the stem. Additionally, the results of a study done by Lestari *et al*. (2019) demonstrated that the leaves of *Polyscias guilfoylei* had a smooth surface with green colour, pinnate venation, elongated to a lanceolate shape, cuspidate apex, serrated margin, and broad base stem. Moreover, microscopy of the leaves also indicated the presence of anisocytic and paracytic stomata. It can be deduced that macroscopic and microscopic evaluation can be done effectively while using only fresh plant parts instead of processed materials. As such, this evaluation should be done prior to any other processes at the level of the identification of raw materials and not the finished products.

4.2 Herbal authentication through combination methods

Current development in the identification and authentication of plant species and products in the herbal marketplace is moving toward the use of combination methods (Mishra *et al*., 2018). Ten previous studies were found to use combination methods for the authentication of herbal products. In general, these studies support the notion that combination methods are more preferred for application in herbal authentication compared to single methods.

4.2.1 Molecular-based combination method

Recent years have witnessed an increased use of the molecular-based method for species discrimination (Leliaert *et al*., 2014). Earlier, the effectiveness of the PCR-based method application was well discussed by Kim *et al*. (2017). However, Liu *et al*. (2018) provided a new combination method that can overcome the disadvantage of the PCR-based method, which requires advanced equipment or complicated professional skills. The authors recommended a combination of recombinase polymerase amplification with lateral flow strips (RPA-LFS) assay in order to identify *Gingko biloba* and its closely related species, *Sophora japonica* in herbal products. This combination method overcomes the limitation of chemical analysis in detecting different species with similar chemical profiles. In the context of rapid analysis, this method could also replace the common PCR-based method (Liu *et al*., 2018). Besides, Ma *et al*. (2019) found that this combination method has a shorter detection time with higher sensitivity to temperatures. However, high sensitivity might also lead to a higher false-positive rate (Altman and Bland, 1994; Simon, 2015). Nonetheless, there is still an undeniable limitation to this method such as the costs incurred, which tend to be relatively higher than other molecular detection methods (Ma *et al*., 2019). Overall, RPA and LFS are good combination methods in terms of rapid analysis and sensitivity in authenticating herbal products.

Other than that, most of the selected journal articles for this review studied the combination of DNA barcoding and high-resolution melting (Bar-HRM). HRM analysis is a method that measures the dissociation rates of double-stranded DNA into single-stranded DNA at increasing temperatures (Reed and Wittwer, 2004). Five previous studies highlighted the application of Bar-HRM analysis to control the quality of various herbal products by ensuring that the correct species is used as the raw material for the particular herbal products or discriminating the authentic plant species from its adulterants. According to Osathanunkul *et al*. (2018), this analysis has the ability to prove over 50% of the tested samples sold in the market as *Tinospora crispa* was adulterated and contained other *Tinospora* or other plant species, indicating that this analysis method is highly sensitive in detecting the plant species. In addition, Duan *et al*. (2018) and Li *et al*. (2018) agreed that the combination of Bar-HRM is an accurate, rapid, reliable, and cost-effective method. This combination may, thus, facilitate DNA barcoding as a method for identifying closely related species in herbal medicine products. These methods can also differentiate several closely related plant species from their common adulterants at the species level.

Mishra *et al*. (2018) found a distinct finding compared to other studies in which the effectiveness of this method depends on the use of DNA barcodes such as internal transcribed spacer 1 (ITS1), internal transcribed spacer 2 (ITS2), maturase K (matK), ribulose bisphosphate carboxylase large chain (rbcL), and psbA-tmH. Evidently, a good DNA barcode should have low intra-specific and high inter-specific variability (Hebert *et al*., 2003). In addition, Kress *et al*. (2002) alleged that the best discrimination between plant species was achieved when using two or more chloroplast barcodes. Meanwhile, the feasibility of the two DNA barcodes of rbcL and ITS2 were compared in a study done by Fadzil *et al*. (2018). With the aid of the difference curve from the HRM analysis, any samples with close or similar melting temperatures could be differentiated with high resolution and power compared to the conventional melting curve by analyzing both the melting curve shape and temperature at the same time. Hence, the use of ITS2 as a DNA barcode was more reliable than rbcL due to the high discrimination power in both post-Bar-HRM analyses.

To conclude, in the context of sensitivity and robust tool, Bar-HRM is an acceptable combination method for authentication purposes. The selection of DNA barcodes prior to analysis is also important in order to achieve a clear identification.

4.2.2 Molecular and spectroscopy

Seethapathy *et al*. (2018) reported that DNA barcoding and nuclear magnetic resonance (NMR) could be effectively used as a regulatory tool to authenticate *Garcinia*-based food supplements. In this study, they assessed the adulteration of the morphologically similar samples of *Garcinia* using DNA barcoding and used NMR to quantify the content of (−)-hydroxycitric acid and (−)-hydroxycitric acid lactone representing the marker compounds of *Garcinia* species in raw herbal drugs and *Garcinia* food supplements. In this regard, a metabolomic approach using NMR would be useful for large-scale analysis of *Garcinia* foods and this study has shown that quantitative NMR is a fast and sufficiently sensitive method for the quantification of (−)-hydroxycitric acid content. The results showed the usefulness of DNA barcoding and NMR spectroscopy in complementing traditional methods such as morphological and microscopic evaluation, for quality control and the authentication of herbal products.

Žiarovská *et al.* (2016) reported the identification of *Smallanthus sonchifolius* in tea mixtures through direct analysis in real-time (DART) coupled with the time-of-flight mass spectrometer (TOF-MS) method. In this study, the combination of restriction cleavage and spectroscopy approach was developed for the authentication assessment of the tea mixtures. The restriction profiles were analyzed using both agarose gel electrophoresis and capillary electrophoresis. Based on the results, capillary electrophoresis was found to be more sensitive than agarose gel electrophoresis due to its ability to detect weak fragments on the generated virtual electropherogram. Meanwhile, in DART analysis, the spectra obtained were used for the principal component analysis (PCA) analysis, which clearly visualizes data in the form of graphs and aids in the discrimination between the measured samples. Both methods were also well-developed for the identification process individually. However, this study did not mention any correlation or significance between them. Thus, there is no strong evidence to claim that this combination in the authentication assessment was good.

4.2.3 Analytical methods and multivariate analysis

Next, chromatography and spectroscopy can also be paired up for authentication assessment similar to the study done by Yuk *et al*. (2016). Generally, the application of chemical profiling using ultra-performance liquid chromatography (UPLC) coupled with quadrupole time of flight mass spectrometer (QTOF -MS) on three ginseng species (*Panax quinquefolius, Panax ginseng,* and *Panax notoginseng*) and two commercial ginseng products containing *P. quinquefolius* and red *P. ginseng* was examined. Subsequently, the data obtained from the analytical method was used to compare the authenticity of herbal products with standard references using multivariate analysis. In line with Huang *et al*. (2019), multivariate analysis that consists of PCA, hierarchical cluster analysis (HCA), partial least squares-discriminant analysis (PLS-DA), k-nearest neighbour (KNN), classification and regression tree (CART), and soft independent modelling of class analogies (SIMCA) was combined with matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI) and TOF-MS in order to differentiate *Radix Astragali* and *Radix Hedysarum*. Both studies found that the combination of analytical methods with multivariate analysis could be used and suitable for routine screening of the authentication of herbs based on their accuracy to identify plant species in a shorter time.

Next, Sima *et al*. (2018) demonstrated that HPLC and micellar electrokinetic chromatography (MEKC) combined with chemometric was successfully done for the authentication assessment of several fruits based herbal medicines that consist of cranberry, bilberry and sea-buckthorn extracts. The results did not show significant differences in using the two separation

techniques. However, the authors stated that it is suitable to use either of the techniques for similar experiments with respect to their advantages and disadvantages. Nonetheless, the results indicated that the reproducibility of HPLC is better. Besides, the authors suggested that a combination of the analytical method with PCA and linear discriminant analysis (LDA) leads to a more powerful classification and discrimination of samples. Hence, this proves that the combination of the two types of chemometrics can produce clearer and more successful authentication output.

4. Conclusion

Authentication of herbs is a very crucial step in order to ensure the safety and effectiveness of herbal-related products. Regardless of either the combination or single methods, the appropriate method must be used to perform the authentication assessment of herbs and herbal products. Based on this review, the authentication works by single methods show less effectiveness than combined methods. Thus, the combination methods might be a useful alternative for authentication purposes. Interestingly, multivariate analysis is one of the great combination techniques with some of the analytical methods in order to authenticate herbs and herbal products. This combination can produce clearer and more successful authentication output. Also, this combination produces more successful and rapid authentication results as well as suitable for routine screening. It could be implemented in any field, especially in quality control laboratories. Herewith, the safety and quality of the finished products will contribute to the stability of the herbal industry economy.

Conflict of interest

The authors declare no conflict of interest.

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