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Review Article

Quality Assessment methods for Ginger (*Zingiber officinale*): A review

Mai T. Abdo^{*}, Haidy A. Gad, Sherweit H. El-Ahmady, Mohamed M. Al-Azizi

Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt

ABSTRACT

The rhizome of ginger (*Zingiber officinale* Roscoe) is a commonly used edible vegetable, condiment, and spice that are used worldwide for culinary, nutritional, medicinal and industrial purposes. Its great medicinal and economical value provide a great possibility for contamination and adulteration. Closely related species are usually used mistakenly as ginger and are hardly differentiated. A proper method of quality control is required for its authentication and analysis. This review discusses various techniques employed for the quality control of ginger including different chromatographic and spectroscopic methods, DNA profiling in addition to the application of multivariate analysis. The advantages and drawbacks of each method are presented as well as recommendations for improved quality assessment.

Keywords: *Quality control; Ginger; Zingiber officinale.*

*Correspondence | Mai T. Abdo; Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt. Email: mai.tawfik@pharma.asu.edu.eg

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1. INTRODUCTION

Herbs and spices have been shown to alter our biological functioning, as methods of treatment for various ailments. Many medicinal plants have social and spiritual importance in different cultures and have played a role in ethnic beliefs throughout history. Plants have also shown pivotal significance in economic terms as important ingredients in foodstuff, medication, and cosmetics.

The rhizome of ginger (*Zingiber officinale*), a popular member of family Zingiberaceae has

been widely used since ancient times. It is cultivated worldwide in many tropical and subtropical regions. Ginger has been extensively used as a spice in various cuisines like Chinese, Indian, Thai, and others, due to its aromatic and pungent characteristics. In addition, it is used as a flavor in different beverages and as a preservative in many marketed products. Furthermore, it has been regarded as an imperative medicinal plant in Ayurveda, Chinese, and Tibb-Unani traditional medication. Traditionally, ginger has been used to treat different ailments including nausea, sea or motion sickness, nausea correlated to pregnancy,

vomiting, anorexia, gastrointestinal spasms, acidity, cramp, flatulence, diarrhea, dyspepsia, common cold, influenza, cough, osteoarthritis, inflammatory disorders, migraines, headaches, hypertension, cardiac palpitations and impotence [1]. Ginger (*Zangabil* in Arabic), has religious importance in the Middle East; mentioned in the holy Quran. Recent studies have renewed interest in ginger mainly due to its nonvolatile pungent constituents showing remarkable biological activities including anti-inflammatory, antiplatelet aggregation [2], antioxidant, immunomodulatory, anti-tumor, anti-apoptotic, hypoglycemic, hypolipidemic, anti-emetic actions [3], antihypertensive, antimicrobial and others [4].

Ginger is listed as “generally recognized as safe” (GRAS) by the Food and Drug Administration (FDA), USA [1]. The importance of ginger as a medicinal herb and a worldwide used spice makes it liable to adulteration as high-quality ginger is mostly mixed with lower quality or exhausted samples to add volume or with lower price pungent materials, such as galangal, grains of paradise or capsicum to increase its pungency [5]. It is worth noting that in certain clinical trials investigating the activity of ginger as a crude extract or powder for the treatment of arthritis [6] or pregnancy-related nausea, [7, 8] have provided mixed and variable results and this may be attributed to the limited enforcement for standardization of ginger food supplements. Moreover, the quantity of ginger rhizome in each capsule (by weight) is only mentioned on the labels of these food supplements. Chemical standardization of these products to determine and label the precise active constituents is rarely achieved [9]. Thus, effective measures are required for the authentication and quality control of ginger to assure its safety and purity. The aim of this review is to present the reported methods for the quality control of ginger including

different chromatographic and spectroscopic methods, DNA markers in addition to different hyphenated techniques in combination with chemometrics and provide insight on optimum methods of its quality control.

The data presented in this review were collected up to 2018 from various databases including SciFinder (<https://scifinder.cas.org/SciFinder/login>), Web of Knowledge (<http://www.webofknowledge.com>) and PubMed (<http://www.ncbi.nlm.nih.gov/PubMed>).

2. QUALITY CONTROL METHODS

2.1. Chromatographic techniques

Chromatography is a common name for the techniques used for separation of molecules based on their structure and/or composition. Early chromatographic non-instrumental techniques as paper and column chromatography are still used till today for preliminary investigations and simple laboratory work. However, there is a great focus on the use of instrumental techniques as high-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) for the reliable separation of different components of the analyzed mixtures. This is followed by structure elucidation of the separated components using different spectroscopic techniques, allowing for the qualitative and quantitative analysis of complex mixtures. Advantages of chromatography include operating at low temperature, the possibility of large scale or continuous operation, separation of molecules according to size and/or chemical characteristics, separation of sensitive and thermo-labile compounds and high purity of the separated compounds. Hence, chromatography is greatly exploited in food, nutrition and phytochemical studies [10]. Different chromatographic techniques have been utilized for the quality control of ginger such as thin layer chromatography (TLC), HPTLC, HPLC and gas

chromatography (GC). Cited examples for the application of different chromatographic

techniques for the quality control of ginger are summarized in **Table 1**.

Table 1. Application of different chromatographic techniques for the quality control of ginger

Extraction	Method of analysis	Purpose of the study	Reference
Samples (20-50µg) in 4% ether solution were used for spotting Mobile phase: Anhydrous diethyl ether: n-hexane (4:1, v/v)	TLC	Quality control of ginger	[11]
Methanol extract Mobile phase: n-hexane : ethyl ether (4:6, v/v)	TLC densitometric method	Detection of 6-gingerol and 6-shogaol in some marketed gingers	[12]
Methanol extract Mobile phase: Toluene : ethyl acetate (3:1, v/v)	HPTLC	Determination of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol in some commercial gingers	[14]
Methanol extract Mobile phase: n-hexane : ethyl acetate(5:5, v/v)	HPTLC	Determination of the quantity of 10-gingerol in ginger extract and ginger-containing dietary supplements, teas and commercial creams	[15]
Methanol extract Mobile phase:	HPTLC	Determination of the quantity of 6-gingerol in ginger extract and ginger-containing dietary supplements, teas and	[16]

n-hexane: ethyl acetate (4:6, v/v)		commercial creams	
Methanol extract Mobile phase: Acetonitrile : water : formic acid (7:2:1, v/v/v)	RP_HPTLC	Quantitative analysis of 6, 8 and 10-gingerols from the methanol extract of fresh ginger rhizomes collected from the northwestern Himalayas	[17]
Methanol extract Mobile phase: Hexane: ethyl acetate: formic acid (5.5:4:0.5, v/v/v)	HPTLC HPLC	Chemical profiling of four Jamaican ginger cultivars, comparison of the composition of oleoresin of ginger collected at different aging stages	[18]
Ethanol extract Isocratic elution Mobile phase: Acetonitrile : 2% aq. acetic acid (2:3, v/v)	HPLC-UV	Direct detection of the major ginger pungency compounds	[19]
Acetone extract Linear gradient elution Mobile phase: Methanol: water (6.5:3.5, v/v)	HPLC-UV	Determination of pungent gingerol compounds in green and dry ginger	[20]
Methanol extract Gradient elution Mobile phase:	HPLC-UV- ES/MS	Identify the individual constituents responsible for the pungency of fresh ginger	[21]

Water: Acetonitrile			
Ethanol extract Isocratic elution Mobile phase: Methanol: Water (7:3, v/v)	HPLC-UV	Detection of the pungency of fresh, sun-dried and sun-dried/steam distilled ginger rhizomes in a relation to the content of 6-gingerol	[22]
Methanol extract Mobile phase: Superheated deuterium oxide	HPLC-NMR	Separation and analysis of the constituents of ginger extract.	[23]
Ethyl acetate and ethanolic extracts Mobile phase: Methanol: Water (6.5:3.5, v/v)	HPLC-UV	Analysis of 6-, 8-, 10-gingerols and 6-shagoal in ethyl acetate and ethanolic extracts of ginger-containing dietary supplement, teas, spices, and drinks.	(24)
Methanol extract Linear gradient elution Mobile phase: Acetonitrile: Water	HPLC-UV	Simultaneous determination of 6-, 8- and 10-gingerols in three medicinal gingers	[25]
Methanol extract Chloroform extract Linear Gradient elution Mobile phase: Acetonitrile: Water with 1% formic acid	HPLC-MS/MS	Identification and quantification of gingerols and other compounds in ginger-containing dietary supplements	[9]

<p>Methanol extract</p> <p>Gradient elution</p> <p>Mobile phase:</p> <p>Solvent A: sodium phosphate buffer</p> <p>Solvent B: sodium phosphate buffer containing 58.5% acetonitrile and 12.5% tetrahydrofuran</p>	<p>HPLC-ECD</p>	<p>Quantification of 8 known ginger components (6-, 8-, and 10-gingerol, 6-, 8- and 10-shogaol, 6-paradol, and 1-dehydrogingerdione) in 11 ginger-containing marketable products</p>	<p>[26]</p>
<p>Ethyl acetate extract</p> <p>Hot water extract</p> <p>Gradient elution</p> <p>Mobile phase:</p> <p>Acetonitrile: Water with 0.5mM ammonium formate and 0.1% formic acid</p>	<p>HPLC-TOF/MS</p>	<p>Instantaneous isolation, detection, and quantitative analysis of gingerol-related compounds in ginger products</p>	<p>[27]</p>
<p>Liquid carbon dioxide ginger oil</p>	<p>GC-MS</p>	<p>Analysis of volatile constituents of ginger oil</p>	<p>[28]</p>
<p>n-hexane</p>	<p>GC-MS</p>	<p>Determining the compounds responsible for the characteristic odor of Japanese ginger</p>	<p>[29]</p>
<p>SPME</p>	<p>2D GC-MS</p>	<p>Identification and qualitative comparison of the volatile constituents of fresh and crystallized sweet ginger</p>	<p>[30]</p>

MD-SPME	GC-MS	Analysis of volatile oil compounds in fresh ginger and its comparison with conventional SPME	[31]
SDE, PEE and HS-SPME	GC-MS	Identification of volatile components extracted by three extraction methods	[32]
Hydrodistillation	GC-FID GC-MS	Comparing the chemical composition of fresh and dried ginger	[33]

2.1.1. Thin layer chromatography

TLC is considered the earliest utilized method for the quality control of most herbal extracts for its ease of use, simple and low cost. In 1972, Connell assigned a TLC method for the quality control of ginger. The results showed limited use of this method in the qualitative analysis due to the variability of results and lack reproducibility [11].

In 2007, Melianita *et al.* settled a TLC densitometric method for the detection of 6-gingerol and 6-shogaol in some marketed gingers. Quantitative analysis of the methanolic extract of ginger was carried out by measuring the UV absorbance of the analytic spots at wavelength $\lambda=577$ nm. However, this method detected only 6-shogaol and 6-gingerol neglecting the rest of the significant constituents in ginger [12].

2.1.2. High-performance thin layer chromatography

Recently, increased level of sophistication and modifications have been implemented to TLC including higher quality silica plates with much smaller particle and pore size, higher packing density and smoother surface providing superior separation power, better sample diffusion, increased analysis speed and enhanced sensitivity

of detection known as HPTLC (high performance liquid chromatography) [13]. Thus, it became a common method of analysis with the advantages of being reliable, sensitive, relatively low price and could be used for quantitative analysis using small amounts of sample and mobile phase thus being eco-friendly. In addition, it allowed for simultaneous assay of several components in multicomponent formulations and utilizing diverse methods of detection, which provided a multidimensional plant profiling, in comparison to the usual single-dimensional chromatogram of HPLC. However, the disadvantages included its inability to identify foreign organic or inorganic matter and afford a non-chemical assessment of quality [13].

In 2009, Melianita *et al.* modified the method performed in 2007 to apply HPTLC. This method was reported to be less expensive when compared to gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry and even with high-performance liquid chromatography equipped with UV diode array detector [14]. A similar method was utilized for the quantitative detection of 6-gingerol and 10-gingerol in ginger extract and ginger-containing commercial products and dietary supplements from local markets of Riyadh, Saudi Arabia [15, 16].

In 2010, Khan *et al.* adopted an RP-HPTLC method for quantitative analysis of 6, 8 and 10-gingerols from the methanol extract of fresh ginger rhizomes collected from northwestern Himalayas [17].

In 2011, Salmon *et al.* used HPTLC for fingerprinting analysis of Jamaican ginger and to chemically profile four Jamaican ginger cultivars. In addition, this developed method was applied for comparing the composition of oleoresins of ginger collected at different aging stages (7, 8 and 9 months) from eight geographical regions of Jamaica. Furthermore, stability investigation showed a noticeable change of the chemical constituents of ginger samples stored at 70°C, which was observed by apparent differences in the HPTLC profile. This analysis revealed that the oleoresins stored at 4 °C and 25 °C were stable for a minimum of 6 months. In addition, the study identified 6, 8, 10-gingerols and 6-shogaol as reference markers that could be used for the standardization of ginger products. The results indicated chemical homogeneity of the four cultivars together with aging samples collected from different parts of Jamaica. As a conclusion, the study presented this method is suitable for rapidly detecting the authenticity and quality of the ginger products. However, HPTLC showed a major disadvantage of low reproducibility as well as the chemical resemblance among the four cultivars. On the other hand, the HPLC investigation (discussed later) showed significant variance in total pungency levels and content of the essential oil [18].

2.1.3. High-performance liquid chromatography

HPLC is considered one of the primary tools of quantitative analysis. It has been applied either for obtaining characteristic chemical fingerprints and chemical profiling for quantitative purposes.

Regarding its use in the quality control of ginger, HPLC was shown to be the most commonly used technique, however; quantification requires authentic reference markers including gingerols and shogaols.

In 1985, Baranowski developed the first HPLC method for the quantitative analysis of ginger using the spectra of authentic 6-gingerol for identification. The results showed that this method could be used for rapid analysis of ginger pungency, detecting changes during drying, processing and possible detection of adulteration [19].

Chen *et al.* (1986) designed an HPLC method for the analysis of acetone extracts of both green (4-5 months after planting) and dry ginger (8-9 months after planting). The gradient elution programming used in this study showed a better separation than the isocratic elution suggested by Baranowski. The results showed that different concentration ranges of pungent gingerol compounds offered a possible reference to differentiate or evaluate the maturity of raw materials [20].

In 1998, He *et al.* utilized electrospray mass spectrometry for the first time with HPLC-UV to identify the individual constituents responsible for the pungency of fresh ginger methanolic extract. Electrospray mass spectrometry provides an advantage of analysis of thermo-labile compounds including gingerols. The results showed that UV at 280 nm was more selective for the detection of gingerols and shogaols rather than 230nm as previously reported [21].

Later on, Balladin *et al.* (1998) adopted an HPLC method for the detection of the pungency of fresh, sun-dried and sun-dried/steam distilled ginger rhizomes in a relation to the content of 6-gingerol. Reference standards of synthetic [n]-gingerols, [n]-shogaols synthesized using regio-selective aldol condensation of zingerone and

corresponding aldehyde (verified by NMR spectroscopy) were used. The method was established to detect the level of the pungency of extracted ginger oleoresin, detection of any contaminants and stability studies of marketed ginger oleoresins [22].

The first report of utilizing nuclear magnetic resonance spectroscopy coupled with high-performance liquid chromatography (HPLC-NMR) was by Saha *et al.* (2003). Through NMR, unidentified peaks were detected without the need for referenced standards as necessitated by previous methods. Moreover, this study involved the use of superheated water chromatography with deuterium dioxide (D₂O) as an eluent for the separation of ginger extract, avoiding interfering signals with the spectra of the analytes. The analysis showed spectra for vanillin, dihydroferulic acid, zingerone and ferulic acid [23].

Later, in 2007, Schwertner *et al.* adopted HPLC for the analysis of 6-,8-,10-gingerols and 6-shogaol in ethyl acetate and ethanolic extracts of ginger-containing dietary supplement, teas, spices, and drinks. It was found the amounts of 6-gingerol and 6-shogaol obtained from both extracts were similar. However, ethyl acetate was selected as the extraction solvent due to its immiscibility with water and thus could be used to extract ginger-containing drinks. Furthermore, ethyl acetate having lower polarity than ethanol would likely cause minor co-extraction interference. The study also compared the use of C-8 against C-18 reverse phase columns and concluded C-8 to give better resolution for quantification of 6-, 8-, 10-gingerols and 6-shogaol using external standards rather than the C-18 column [24].

In 2008, Li *et al.* used an HPLC-DAD method for simultaneous determination of 6-, 8- and 10-gingerols in three gingers samples. The developed method involved using C-18 column

and 6-, 8- and 10-gingerols as reference standards for their detection in dried, baked and fresh ginger [25].

Tandem mass spectrometry along with HPLC (LC-MS/MS) was adopted by Tao *et al.* (2009) for the identification and quantitative analysis of gingerols, shogaols, parasols, and gingerdiones in the chloroform extract of ginger-containing dietary supplements. This method was also used for the quantification of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, and 10-shogaol with selected reaction monitoring in ginger dietary supplements. In this study, constant neutral loss scanning in the range m/z 80-500 was investigated for the detection of selective neutral loss for gingerols. Results showed that neutral loss scanning of 194 or 136 u can be used to detect specifically most gingerol-related compounds (except for gingerols), that was in spite of the complex nature of ginger extracts [9].

In 2010, another electrochemical array detector (ECD) coupled with HPLC method was reported by Shao *et al.* for quantification of 8 known ginger components (6-, 8-, and 10-gingerol, 6-, 8-, and 10-shogaol, 6-paradol, and 1-dehydrogingerdione) in 11 ginger-containing marketed products. This study showed that the use of ECD was advantageous over the use of electrospray or UV detectors due to its high sensitivity for redox-sensitive components as found in ginger. However, there was one disadvantage of requiring a constant level of electrolyte within a suitable range in the mobile phases [26].

In 2012, Time-of-flight mass spectrometry (TOF) coupled with HPLC was first used and reported by Park *et al.* for instantaneous isolation, detection, and quantitative analysis of gingerol-related compounds in ginger products. Ethyl acetate extracts of fresh and powdered dry gingers, hot water ginger extracts, and ginger teas

were the topic of investigation. This method showed 70-100 times higher sensitivity when compared to the ordinary HPLC-UV detection method. However, this study could not compare the sensitivity of the LC-TOF/MS method directly with that of LC-MS/MS. Furthermore, this method identified 19-gingerol-related compounds (4-, 6-, 8-, 10-, and 12-gingerols, methyl-6-, methyl-8-, and dehydro-6-, dehydro-8-, dehydro-10-, and dehydro-12-gingerols, dehydro-6-, dehydro-8-, dehydro-10-, dehydro-12-, dehydro-14-gingerdione, and 6-, 8-, and 10-shogaols), which is much more than any other previously reported method. This study also supported the previous hypothesis suggesting that shogaols are not intrinsic components of fresh authentic ginger but are considered chemical degradation products of gingerols during ginger processing and exposure to heat [27]. The use of LC-TOF/MS allowed for the ability of untargeted detection with the all-time recorded full spectrum, which was not possible with LC-tandem MS. Thus, this method could be used for the detection of low levels of gingerols and shogaols in ginger-containing commercial products [27].

2.1.4. Gas chromatography (GC)

Gas chromatography has been widely used for the detection of volatile constituents and is considered the first choice in the quantitative analysis of essential oils and volatile substances. Regarding ginger, GC has been used mainly for the analysis and identification of its volatile components rather than its quality control. This is mainly due to the inability to detect pungent components of ginger due to their thermal instability [26]. Studies using gas chromatography were performed for either overcoming this problem or investigating a better extraction method for the volatile constituents of ginger.

In 1988, Chen *et al.* used GC-MS for the analysis of volatile constituents of ginger oil extracted with liquid carbon dioxide used to overcome the thermal instability that might occur due to steam distillation [28].

In 1995, Nishimura utilized a modified multidimensional GC-MS for determining the compounds responsible for the characteristic odor of Japanese ginger. Aroma extraction dilution analysis was used for the detection of the flavor dilution factor (FD). Compounds with high FD appeared to have the most noticeable flavor in the fresh rhizomes of ginger. These compounds included geraniol, geranyl, linalool, neral, borneol, and isoborneol. In addition new compounds including (E)-2-alkenals, 2-octyl acetate, 2-pinen-5-ol, 2-(2',3'-epoxy-3'-methyl butyl)-3-methylfuran, and (E)- and (Z)-3,7-dimethyl-3,6-octadienal were also identified. This study showed that the characteristic flavor of ginger was not related particularly to one compound but a mixture of highly flavored oxygenated compounds (monoterpenoids and (E)-2-alkenals) [29].

Solid phase micro-extraction (SPME) was used by Shao *et al.* in 2003 for the extraction of the volatile oil from both fresh ginger and crystallized ginger sweets. Two-dimensional gas chromatography was used for the identification and qualitative comparison of their volatile constituents. Both fresh and sugar crystallized ginger, showed the same heavy compounds, however, sugar crystallized ginger sweet showed lower content of components of higher volatility. Concerning pungent principles, this method could detect neither gingerols nor shogaols, as gingerols are thermally unstable and shogaols are rarely found in fresh ginger [30].

In 2007, Yu *et al.* used GC-MS following microwave distillation and solid-phase microextraction (MD-SPME) for the analysis of volatile oil compounds in fresh ginger. MD-

SPME allowed for instantaneous isolation, concentration, and extraction of volatile constituents, where, 54 components were identified including geranial, zingiberene, β -sesquiphellandrene, and β -phellandrene. However, conventional SPME resulted in the identification of only 34 components. In addition, MD-SPME provided additive advantages of requiring less amount of sample, without the need for organic solvent nor water for the analysis [31].

In 2009, Yang *et al.* compared between three different extraction methods for extracting the volatile components of ginger: steam distillation extraction (SDE), petrol ether extraction (PEE) and headspace solid-phase microextraction (HS-SPME). GS-MS was used for the identification of volatile components extracted by these methods. HS-SPME method was concluded as simple, fast and efficient for extraction and semi-quantitation of volatile constituents of ginger rhizome [32].

For comparing the chemical composition of fresh and dried ginger in 2010, Sasidharan *et al.* used GC-FID and GC-MS. Zingiberene appears to be the major compound in both oils but with different ratios. Dried ginger oil showed lower content of sesquiterpene alcohols, oxygenated compounds, and monoterpene content except for myrcene [33].

2.2. Spectroscopic techniques

3.2.1. Ultraviolet spectroscopy

In 2012, Shinde *et al.* developed a UV spectroscopic method for quick detection of gingerol in the ginger extract. The method showed the λ max of ginger as 281.40 nm. The developed method was capable to quickly quantify ginger extract in a variety of products. However, this method lacked sensitivity and could not be used for the definitive detection of individual components of ginger [34].

2.3. DNA-based markers

Despite the various methods previously described for the quality control of ginger, limitation in the differentiation of ginger (*Zingiber officinale*) from closely related species as bitter or pinecone ginger (*Zingiber zerumbet*) and Thai or Cassumunar ginger (*Zingiber cassumunar*) remains a problem. The similarity of the rhizome morphology of these two species to that of *Zingiber officinale* allows for their use as adulterants. Recent genetic tools that applied polymerase chain reaction (PCR), cloning, sequencing and hybridization procedures provided more accurate and satisfying methods for authenticating medicinal herbs [35]. Supplementary methods for definitive detection and authentication of ginger are useful including DNA-based molecular markers methods [36].

In 2006, Jiang *et al.* used phylogenetic analysis based on molecular data to study the genetic variability of ginger obtained from different geographical sources. In addition, differences between ginger (*Zingiber officinale*) and other medicinally used *Zingiber* species were investigated. *Z. zerumbet*, *Z. montanum*, *Z. mioga*, *Z. spectabile*, and *Alpinia galangal* were selected due to their common use as adulterants of ginger and/or their use as medicinal plants. In addition, *galangal* was used as the outgroup (non-ginger) for the phylogenetic analysis. Even though ginger obtained from different geographical sources showed similar phylogenetic analysis, however, differentiation between ginger and other *Zingiber* species based on the sequence data was achieved. The drawback for this method was the difficulty to obtain DNA from commercial ginger-containing products as it is often sold as a ground powder or as non-polar solvent extract [37].

Later on, the authentication of ginger in ginger-containing marketed products was accomplished by Chavan *et al.* (2008) where a DNA-based molecular markers method was developed using SCAR (sequence-characterized amplified region). Screening of DNA from several parts of *Z. officinale*, *Z. zerumbet* and *Z. cassumunar* were performed using RAPD (random amplification of polymorphic DNA) primers followed by cloning and sequencing of

the RAPD bands unique to *Z. officinale*. Longer species-specific PCR primers were developed using this sequence information to amplify specific SCAR markers for distinguishing *Z. officinale* from the other selected *Zingiber* species, as well as commonly used medicinal plants in ginger-containing commercial products. Furthermore, these SCAR markers were tested in Trikatu [a mixture of dried, semi-processed powders of *Z. officinale*, *Pi. nigrum* (Black pepper) and *Pi. longum* (Long pepper)] as well as individual powders of each of the three medicinal plants to ensure that it specifically amplifies the desired marker in *Z. officinale*. In addition, SCAR primers were run on DNA samples of some other commonly used Ayurvedic medicinal plant species that are likely to be found with ginger in multicomponent formulations. The developed method was proposed as a complementary tool for differentiating *Z. officinale* from other *Zingiber* species [36]. The disadvantages of this method included the use of sophisticated instrumentation as PCR, gel analysis and long analysis time.

Recently, Chaudhary *et al.* (2014) tried to overcome the disadvantages of the previous method by developing a loop-mediated isothermal amplification (LAMP)-based marker method for DNA authentication of *Zingiber officinale* and studied their suitability for the analysis of fresh, dried powder and multicomponent ginger-containing formulations. The method involved twelve rhizome samples of ginger and closely related species screened with randomly amplified polymorphic DNA (RAPD). The procedure involved eluting, cloning and sequencing of the common noticeable DNA fragment in all samples. This developed method was recommended for use when botanical authentication of ginger from closely morphologically related species was difficult, such as in the case of incomplete or damaged samples and in dried herbal products [38]. Cited examples for the application of different DNA-based markers for the quality control of ginger are summarized in **Table 2**.

Table 2. Application of DNA-based marker for the quality control of ginger

Method of analysis	Purpose of the study	Reference
Phylogenetic analysis based on molecular data	Study the genetic variability of ginger obtained from different geographical sources and differentiation between ginger (<i>Zingiber officinale</i>) and other medicinally used <i>Zingiber</i> species	[37]
DNA-based molecular markers method using SCAR	Authentication of ginger in ginger-containing market products and identification of <i>Zingiber officinale</i> from multicomponent formulations.	[36]
DNA-based molecular markers method using (LAMP)	Rapid and easy molecular authentication of the medicinal plant <i>Zingiber officinale</i> Roscoe	[38]

2.4. Different chromatographic techniques coupled to Chemometrics (Multivariate analysis)

As previously discussed, most analytical methods used till now for the quality control of ginger and even other herbal extracts depended on the use of one or few markers present in the sample of pharmacologically activity and/or significant concentration. This is acceptable and successful if the target was the assessment of certain components. However, this strategy neglects other components present in the extract, which might add to the therapeutic activity of the herb. In addition, these other components may vary considerably depending on collection season, drying process, geographical origin, storage and other factors, which may affect the activity and stability of the plant extract. Hence, these methods do not provide a complete picture of the sample in question and are probably one of the reasons for the variability of results of clinical trials and investigations involving the use of herbal extracts. To overcome this problem, the necessity to develop a complete profile of the phytochemical constituents of the plant sample in question (fingerprint) arises in order to guarantee the consistency and reproducibility of pharmacological and clinical studies. This will also allow for the understanding of their biological activity, pharmacokinetics and possible side effects and to assure authenticity and quality of the product. This is the so-called fingerprint analysis. Chromatographic fingerprint techniques can be used to analyze both the marker compounds and the unknown constituents in multicomponent herbal formulations, which is recommended by the US Food and Drug Administration, the European Medicines Evaluation Agency, and State Food and Drug Administration of China (SFDA) to judge the quality, authenticity, and stability of botanical products (39). Fingerprinting analysis using the

commonly used analytical tools as (HPLC, GC, and HPTLC) together with different spectroscopic methods as (UV, IR and mass spectroscopy) reflects the complex chemical composition of herbal extracts. Despite the possibility of differentiating the different extract chromatograms visually, to rely on this process is usually inaccurate, biased and non-quantitative. In addition, missing minor differences between very closely related species might occur. These disadvantages are tackled by combining fingerprinting analysis together with chemometric methods (multivariate analysis). Recent coupling of chromatographic techniques and fast-developing chemometrics analysis methods offers dominant tools to analyze the overlapping peaks of complex multicomponent herbal formulations [40].

As an application of chemometrics for fingerprinting analysis of Chinese herbs in 2001, Chau *et al.* used 2D GC-MS coupled with chemometrics for producing a chemical fingerprint for fresh and dried ginger samples. This study showed that steam distillation showed more extraction efficiency of volatile components from both fresh and dried ginger than Ultra-sonic or Soxhlet extraction. The obtained chromatograms can be further analyzed using window factor analysis (WFA), Evolving factor analysis (EFA) and heuristic evolving latent projections (HELP). The study didn't specify which programs were exactly used. However, it concluded that these chemometric techniques can greatly reduce the complexity of the produced chromatograms and enhance its resolution for better-fingerprinting analysis. In addition, this study reported great differences between chemical fingerprints of volatile components between fresh and dried ginger samples. However, details were not presented in this work [41].

In 2004, Gong *et al.* used two-dimensional GC-MS coupled with chemometric analysis for the determination of volatile components in both fresh and dried ginger. Chemometric analyses were used to correct the baseline drifting, detect the purity of the obtained peaks, identify the number of the chemical constituents present and moreover, enhance the resolution of the produced chromatograms and mass spectral data. The results showed that most of the volatile constituents of fresh and dried ginger are similar despite their different pharmacological activities [42].

Later on, He *et al.* (2010) used the same previous approach (42) for the detection of differences between volatile constituents of the essential oil of fresh ginger (*Zingiber officinale* Roscoe), dried ginger (Rhizome Zingibers) and scarfskin of fresh ginger (Ginger peel). A total of 85, 81 and 80 volatile constituents were determined from the three essential oils, respectively with 52 volatile components were common between the three oils. In conclusion, the study proved this method as effective for the analysis of complex systems as Chinese traditional medicine and can be used successfully for the quality assessment of products in the food industry [43].

In 2013, Rafi *et al.* used reverse phase capillary liquid chromatography (RP-CLC) for instantaneous determination of four components (6-, 8-, 10-gingerol and 6-shogaol) found in ginger. Thirty-seven samples of three varieties of ginger growing in Indonesia (*Z. officinale* var. amarum), (*Z. Officinale* var. office-Narum) and (*Z. officinale* var. rubrum) were studied. The results showed that the four compounds were not enough to distinguish between the three varieties. By applying discriminant analysis (DA), 100% correct classification of each analyzed group (three varieties) was achieved. Cross-validation was used for evaluation of the predictive ability

of the classifying model and it was found that 94% of all the samples were correctly classified [44].

Feng *et al.* (2014) used HPLC-PDA for fingerprint analysis of 10 batches of ginger from different markets in China coupled with the chemometric techniques including similarity analysis, hierarchical clustering analysis and principal component analysis of the ginger samples. The results showed that chemometric analyses classified 10 batches of ginger to two groups reflecting quality differences between these samples [39].

Later on, Yudthavorasit *et al.* (2014) adopted an HPCL-DAD method combined with chemometrics for classification of ginger (*Zingiber officinale*) according to geographical origins including five different producing countries. This method involved the use of 6-, 8-, 10-gingerols as the reference standard. The chromatographic peaks were identified using mass spectrometry and eight particular peaks identified by mass spectrometry were used for further discriminative analysis. Chemometric analyses were performed using similarity analysis, HCA, PCA, and linear discriminant analysis (LDA). The method showed to be a practical tool that was suitable for originating and authentication of unclassified ginger samples. In addition, it could be used to detect quality control and food authenticity concerns regarding ginger origin by analyzing unknown ginger samples and processing the produced data with chemometrics. Furthermore, this study showed that different markers could be used for specifying of ginger from different geographical origin, where 8-gingerol and methyl- 6-gingerol are selective markers for detecting ginger from India and Thailand, respectively while methyl diacetox-8-gingerdiol, 10-gingerol, and diacetox-8-gingerdiol can be specifically used as markers for Chinese ginger [45].

Recently, Maiset *al.* (2018) used UPLC-Q/TOF-MS coupled with Metabolomics as an approach for discrimination between two ginger cultivars (China) and (Ghana). This study applied the use of identified metabolites of ginger, isolated using UPLC, together with multivariate analysis for the discrimination between two ginger cultivars. Principle component analysis (PCA), Orthogonal Partial least square regression and Soft independent modeling by class analogy (SIMCA) were used for data analysis. The results

showed identification of sixteen metabolites were identified, from which six metabolites were assigned as marker compounds for differentiation between Ghanaian and Chinese ginger. As a conclusion, this method was reported as a reliable and successful method for interspecies differentiation between ginger cultivars. However, more samples are needed for wide coverage of different ginger cultivars for accurate detection of the exact metabolites that can be used in its discrimination (**Table 3**) [46].

Table 3. Quality control of ginger using chromatographic techniques coupled with chemometrics

Extraction	Method of analysis	Purpose of the study	Reference
Steam distillation Ultrasonic Extraction Soxhlet Extraction	2D GC-MS With WFA, EFA, and HELP	Fingerprinting analysis of fresh and dried ginger	[41]
Steam distillation	2D GC-MS With ELPG and Eigenstructure tracking analysis	Determination of volatile components in both fresh and dried ginger	[42]
Standard extraction method for volatile oil described by Chinese pharmacopeia 2005	2D GC-MS With heuristic evolving latent projections (HELP), ELPG and Eigenstructure tracking analysis	Detection of difference between volatile constituents of the essential oil of fresh ginger dried ginger and scarfskin of fresh ginger (Ginger peel)	[43]

<p>Methanol extract</p> <p>Isocratic elution</p> <p>Mobile phase:</p> <p>60% acetonitrile</p>	<p>RP_CLC-UV with DA</p>	<p>Instantaneous determination of four components (6-, 8-, 10-gingerol and 6-shogaol) in three varieties of ginger (<i>Z. officinale</i> var. <i>amarum</i>), (<i>Z. Officinale</i> var. <i>office-Narum</i>) and (<i>Z. officinale</i> var. <i>rubrum</i>)</p>	<p>[44]</p>
<p>Methanol extract</p> <p>Linear gradient elution</p> <p>Mobile phase:</p> <p>Acetonitrile and 0.1% phosphate in water</p>	<p>HPLC-PDA with HCA and PCA</p>	<p>To establish an efficacious and combinational method and pattern recognition technique for quality control of ginger.</p>	<p>[39]</p>
<p>Methanol extract</p> <p>Binary gradient elution</p> <p>Mobile phase:</p> <p>Water: Acetonitrile</p>	<p>HPLC-DAD with SA, HCA, PCA, and LDA</p>	<p>Classification of ginger according to geographical origin including five different producing countries.</p>	<p>[45]</p>
<p>Methanol extract</p> <p>Gradient elution</p> <p>Mobile phase:</p> <p>Water + 0.1% formic acid : Acetonitrile</p>	<p>UPLC-Q/TOF-MS with PCA, OPLS, and SIMCA</p>	<p>Discrimination between fresh ginger of two different geographical origins China and Ghana</p>	<p>[46]</p>

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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