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RESEARCH ARTICLE

Using DNA Barcoding to Detect Adulteration in Different Herbal Plant-Based Products in the United Arab Emirates: Proof of Concept and Validation

Kareem A. Mosa[1,2,*], Sameh Soliman[3,4], Ali El-Keblawy[1], Muna A. Ali[1], Hessa A. Hassan[1], Aysha A.B. Tamim[1] and Moza M. Al-Ali[1]

[1]*Department of Applied Biology, College of Sciences, University of Sharjah, P.O. Box 27272, Sharjah, UAE;* [2]*Department of Biotechnology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt;* [3]*Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, P.O. Box 27272, Sharjah, UAE;* [4]*Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*

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Abstract: ***Background*:** Commercially available herbal and medicinal plants-based products are susceptible to substitution or contamination with other unlabeled or undesired materials. This will reduce the quality of the product, and may lead to intoxication and allergy.

***Methods*:** DNA barcoding is a molecular technology that allows the identification of plant materials at the species level, by sequencing short stretches of standardized gene sequences from nuclear or organelle genome in an easy, rapid, accurate and cost-effective manner. The aim of this research is to apply DNA barcoding to investigate the authenticity of commercially available herbal and medicinal plant-based products within the UAE markets. A total of 30 samples were analyzed, covering six different herbal products (thyme, cardamom, anise, basil, turmeric, and ginger), obtained in fresh and dried forms. DNA was extracted and three barcode loci including (rbcL), (matK) and (ITS) were amplified, sequenced and analyzed by BLAST.

***Results*:** In terms of amplification efficiency, the results suggest that rbcL is the most suitable marker for species identification giving 75% of successful amplification, followed by ITS with 66.67%, whereas matK had the lowest with 18.52%. Adulteration was detected in two samples, ginger powder and dry thyme leave samples. The adulterants were from *Triticum* and *Oryza* genera.

***Conclusion*:** Clearly, the results from this report provide evidence that DNA barcoding technique is efficient in the recognition of commercial plant products. Thus, it can be considered as a fast, effective, and reliable method to detect adulteration in plant-based products in the UAE market.

Keywords: DNA barcoding, adulteration, market fraud, authentication, plant-based products, herbal plants.

1. INTRODUCTION

Medicinal plants and herbs have been used for thousands of years to heal and treat various diseases. These plants are not only important in traditional medicine, but also in the discovery of new potential drugs by using defined active compounds. Thyme, cardamom, anise, basil, turmeric, and ginger are among the commonly consumed herbal and medicinal plant-based products by a broad range of people for the management of different types of diseases and/ or as condiments for confectionery purposes. For example, turmeric is a well-known plant that is used for several purposes, such as spice, food coloring agent, cosmetic agent, and as a medicinal herb for its anti-inflammatory and anti-microbial properties [1].

The commercial demand for plant-based products is growing in particular for their medicinal values. However, the use of herbal drugs is susceptible to either unintended contamination by not expert personnel or intended contamination and adulteration for gaining more money [1, 2]. These adulterated products may reduce the effectiveness of their biological activity, or might lead to detrimental health implications and adverse effects, especially if mixed with toxic species or allergens [3, 4]. Thus, a reliable approach is needed for accurate identification and authentication of medicinal plants and herbal drugs.

The conventional identification methods of plant products and herbal drugs involve the use of organoleptic characterization, macroscopic, microscopic, and analytical

*Address correspondence to this author at the Department of Applied Biology, College of Sciences, University of Sharjah, UAE; Tel: 00971-6-505 3838; Fax: 00971-6-505 3814; E-mail: kmosa@sharjah.ac.ae

techniques that rely mainly on protein, metabolite and gene analysis of the plant samples [5, 6]. These techniques use sophisticated equipment and require well-trained personnel. For example, the metabolite analysis is based mainly on HPLC (High-Performance Liquid Chromatography), NMR (Nuclear Magnetic Resonance) and MS (Mass Spectrometry), which might not be available, especially in the labs of many of the developing countries [5]. In addition, the macroscopic and microscopic examinations require well-trained personnel. Furthermore, the morphology studies are not suitable for the fragmented or powdered plant materials. Moreover, the chemical analyses and the metabolic profiles could be affected by physiological, genetic, environmental factors, storage conditions, and processing methods, which lead to inconsistencies and unreliable results [7]. Most of these techniques require fresh samples for the analysis, which might not be available in most of the used products [8]. Typically, plant-based adulterants and fillers are present in low quantities and sometimes it is difficult to detect their composition [1, 9]. Therefore, highly sensitive and reliable approach is required for identification, discrimination, and authentication of medicinal plants.

DNA-based approaches have been developed in the early 1990's, and are widely used for the identification of medicinal plants [10]. Unlike the chemical approaches, these methods are not affected by age, environmental factors or physiological conditions, not tissue-specific and need small samples that could be used dry [11]. DNA-based methods are basically dependent on DNA markers, including Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), and Inter-Simple Sequence Repeats (ISSR) [12]. These methods have the disadvantages of being expensive, laborious and time-consuming, sometimes require the involvement of harmful radioactive materials, lack of uniformity, and inadequate standardization [12, 13]. DNA Barcoding is one of the recent DNA-based identification methods in which short stretches of standardized gene sequences either from nuclear or organelle genome is used. It is an easy, rapid, accurate and cost-effective technique [14, 15]. DNA Barcoding was first proposed in 2003 by Paul Hebert for animal identification using the 5' end of cytochrome c oxidase 1 (CO1) gene present in the mitochondrial genome [15]. However, the use of CO1 as a universal plant barcode or finding a plant equivalent was not possible due to the low rate of nucleotide substitution in plant mitochondrial genomes [16]. Thus, the search for plant barcodes was shifted to the chloroplast and nuclear genomes that are with high substitution rates; to enable the discrimination between species [17]. In this context, the use of DNA barcoding can aid in verifying the presence or absence of the original medicinal plant species and identifying the replaced species in the case of adulteration [11]. A total of 17 different barcode regions that could be used in medicinal plants identification have been reported; among them, the Internal Transcribed Spacer (ITS), maturase K (matK), and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) are commonly used [6]. *MatK* has a high evolutionary rate among all the plastid regions used. It also has a suitable length and interspecific divergence along with low transition/transversion rate [18, 19]. *RbcL* codes for RuBisCO enzyme [20] and is widely used in phylogenetic analysis. In contrast to matK, rbcL evolves slowly and has a moderate discriminatory power. Thus, it can be considered as one of the best candidates and can be used in combination with various loci to achieve accurate plant identifications [19]. ITS is a non-coding spacer region that is considered a powerful phylogenetic marker with high levels of interspecific divergence [19]. Although none of the proposed markers has been proven to be perfect as a universal plant barcode [21], the use of a combination of different barcodes exhibited a very high discrimination capacity [22]. There are some patents on the application of DNA barcoding on the identification of herbal and medicinal plants [23-25]. Another challenge that faces DNA barcoding in plants is the presence of secondary metabolites (such as polysaccharides and polyphenols) that may inhibit the PCR reaction which eventually may lead to a false negative result [26].

The objective of this research is to validate the efficacy and applicability of a DNA barcoding approach as a tool for the detection of market fraud and adulteration in commercially available medicinal plants and herbal products in the UAE markets. In this study, the barcoding loci *rbcL, matK* and *ITS* are used based on earlier reports of their effectiveness for testing the DNA extracted from commonly used plant materials. Among the most commonly used herbal products in the UAE markets are turmeric, ginger, anise, thyme, and cardamom. These products are used fresh, dry, and in powdered forms. The results from this research may contribute to increasing the accuracy in detecting market fraud, and in minimizing the time required for the identification, which consequently would lead to the reduction of medicinal plants adulteration.

2. MATERIAL AND METHODS

2.1. Samples Collection

Labeled medicinal plant-based samples including Thyme (Th), Turmeric (Tr), Basil (B), Ginger (G), Cardamom (C), Anise (A) were collected from different herbal markets in the UAE. The collected samples were fresh or dried leaves, rhizomes and seeds. Thirty representative samples were chosen from different brand companies and given codes (Table **1**). The fresh samples were stored at -80^{o}C, while dried forms were stored at room temperatures.

2.2. Samples Preparation and DNA Extraction

Samples other than the fine powders (*e.g.* dry tea bags, dry leaves, fresh leaves, fresh rhizomes, and fresh seeds) were ground with liquid nitrogen to a fine powder, using an autoclaved mortar and pestle. Samples were then stored at -80°C until their use in the analyses. DNA extraction was performed for all samples using NORGEN BIOTEK CROP plant/ fungi DNA isolation kit, following the manufacturer's instructions. The extracted genomic DNA was run on 1.5% agarose gel to check the quality of the extracted DNA.

2.3. PCR Amplification

The extracted DNA was used to amplify matK, rbcL, and ITS regions from the tested samples using different universal primers [27-30] (Table **S1**). PCR reactions were

Table 1. Medicinal plant based samples collected from different UAE markets. Sample coded with serial number (Sample ID) for further references.

Sample ID	Plant Sample	Sample Type	Product Source
1-Th	Thyme	Dry leaves - packed	Local
2-Th	Thyme	Dry leaves- packed	Local
3-Th	Thyme	Dry leaves- packed	Imported
4-Th	Thyme	Dry tea bags- packed	Imported
5-Th	Thyme	Dry tea bags- packed	Imported
6-Th	Thyme	Dry leaves- packed	Local
7-Th	Thyme	Fresh leaves- loose	Local
8-Th	Thyme	Fresh leaves- loose	Local
9-Tr	Turmeric	Dry powder- packed	Local
10-Tr	Turmeric	Fresh rhizome- loose	Local
11-Tr	Turmeric	Dry powder- loose	Local
12-Tr	Turmeric	Dry powder- packed	Imported
13-B	Basil	Dry leaves- packed	Local
14-B	Basil	Dry leaves- packed	Imported
15-B	Basil	Dry leaves- packed	Local
16-B	Basil	Dry leaves- packed	Imported
17-B	Basil	Dry leaves- packed	Local
18-B	Basil	Fresh leaves- loose	Local
19-G	Ginger	Dry powder- packed	Local
20-G	Ginger	Dry powder- loose	Local herbal market
21-G	Ginger	Dry powder- packed	Local
22-G	Ginger	Fresh rhizome- loose	Imported
23-C	Cardamom	Dry powder- packed	Local
24-C	Cardamom	Dry powder- loose	Local herbal market
25-C	Cardamom	Fresh seeds- loose	Local herbal market
26-A	Anise	Dry powder- loose	Local herbal market
27-A	Anise	Dry tea bags- packed	Imported
28-A	Anise	Fresh seeds- packed	Local
29-A	Anise	Fresh seeds- loose	Local herbal market
30-A	Anise	Dry tea bags- packed	Imported

prepared using NORGEN BIOTEK CROP master mix kit according to the manufacturer's instruction. Different PCR programs were used for each primer (Table **S2**). Each PCR reaction was followed by gel electrophoresis and purification either by DNA gel extraction or enzymatic extraction.

2.4. DNA Gel Extraction, Enzymatic Extraction and Purification

PCR products were run on 1.5% agarose gel and then DNA gel extraction was performed using NORGEN BIOTEK CROP DNA purification kit following the manufacturer's procedure. Exonuclease I (Exo I) at 0.5µl or 1µl and Thermo Scientific™ FastAP™ Thermosensitive Alkaline

Phosphatase at 1µl or 2µl were added (according to the quality of the bands obtained from the purified PCR products identified on 1% agarose gel) to the PCR product in 1.5mL microcentrifuge tube to clean up the PCR products. Enzymatic hydrolysis was carried out for 15 min at 37°C, followed by 15min incubation at 80°C for enzymes inactivation. To test the quality of the template, the purified products were run on 1.5% agarose gel.

2.5. Sequencing, Bioinformatics, and Data Analysis

This step was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Thermo Fisher Scientific, USA) using a BioRad S1000 thermal cycler. The products were purified using ethanol/EDTA/sodium acetate precipitation according to the manufacturers' instructions. Capillary sequencing was performed using the Genetic Analyzer 3500 (Applied Biosystems, Thermo Fisher Scientific, USA).

The resulting sequences were trimmed using FinchTV (version 1.4.0), assembled by BioEdit (version 7.2.5) and compared to National Center for Biotechnology Information (NCBI) database sequences by running Basic Local Alignment Search Tool (BLAST) alignment with the default parameters. Finally, the highest sequence identity was chosen with respect to the highest identity percentage and query coverage.

3. RESULTS

3.1. PCR Amplification of The Tested Barcodes

Even though the genomic DNA of some samples was clearly detected on 1.5% agarose gel, other samples (*e.g.* cardamom seeds, cardamom powder, powdered turmeric, powdered ginger, and dry leaves of Thyme) were not detected. However, PCR amplification of rbcL, ITS, and matK barcodes was successful (Table **2**). PCR amplification using rbcL locus showed the best results since it was able to produce positive bands with all tested samples, except basil dry leaves, and thyme tea bag Fig. (**1**). All the samples showed a single clear band between 500bp and 600bp. On the other hand, the matK locus was amplified only from the ginger powder, thyme dry leaves, and three samples of anise (seeds, tea bags, and powder) (Table **2**). Amplification of ITS barcode successfully produced bands close to 700bp from all tested samples of ginger, cardamom, and turmeric Fig. (**2**). Moreover, ITS locus was successfully amplified from all tested anise samples, except tea bag sample (30-A), and from all tested fresh samples of thyme and basil, except the dry thyme leaves, all tested thyme tea bag, and all tested basil dry leaves (Table **2**).

3.2. Sequences Analysis and Validation

Following gel extraction or enzymatic treatment, PCR amplified bands were sequenced. The successfully sequenced samples are listed in Table **3**. The forward and the reverse sequences were assembled to form a long contiguous sequence. The sequence of ITS locus revealed that all the tested anise samples showed 100% identity to *Pimpinella anisum*, and sequences of rbcL locus showed total identity to the same *P. anisum* for anise tea bag, and virtual identity (99% identity) for anise seeds and powder samples. ITS locus was sequenced successfully from 25-C cardamom seeds and 23-C cardamom powder. The sequences matched *Aframomum polyanthum* with ~ 95% identity for both samples. However, the rbcL locus of 25-C and 23-C aligned with *Alpinia zerumbet* and *Elettaria cardamomum* respectively, and were given 99% identity for both samples.

Table 2. PCR amplification of matK, ITS, and rbcL loci in the tested fresh and processed plant-based products (+: successful amplification, - : unsuccessful amplification, NA: not available result).

ID	Sample Type	matK	ITS	rbcL
1-Th	Dry leaves	-	+	+
2-Th	Dry leaves	-	-	NA
3-Th	Dry leaves	NA	-	NA
4-Th	Dry tea bags	-	-	NA
5-Th	Dry tea bags	-	-	-
6-Th	Dry leaves	+	+	+
7-Th	Fresh leaves	-	+	+
8-Th	Fresh leaves	-	+	+
9-Tr	Dry powder	-	+	+
10-Tr	Fresh rhizome	-	+	+
11-Tr	Dry powder	-	+	NA
12-Tr	Dry powder	-	+	+
13-B	Dry leaves	-	-	-

(Table 2) Contd…

ID	Sample Type	matK	ITS	rbcL
14-B	Dry leaves	-	-	-
15-B	Dry leaves	-	-	-
16-B	Dry leaves	-	-	-
17-B	Dry leaves	-	-	-
18-B	Fresh leaves	-	+	+
19-G	Dry powder	-	+	+
20-G	Dry powder	+	+	+
21-G	Dry powder	NA	+	NA
22-G	Fresh rhizome	-	+	+
23-C	Dry powder	NA	+	+
24-C	Dry powder	-	+	+
25-C	Fresh seeds	-	+	+
26-A	Dry powder	+	+	+
27-A	Dry tea bags	+	+	+
28-A	Fresh seeds	-	+	+
29-A	Fresh seeds	+	+	+
30-A	Dry tea bags	-	-	NA
Percentage of successfully amplified samples		18.52%	66.67%	75.00%

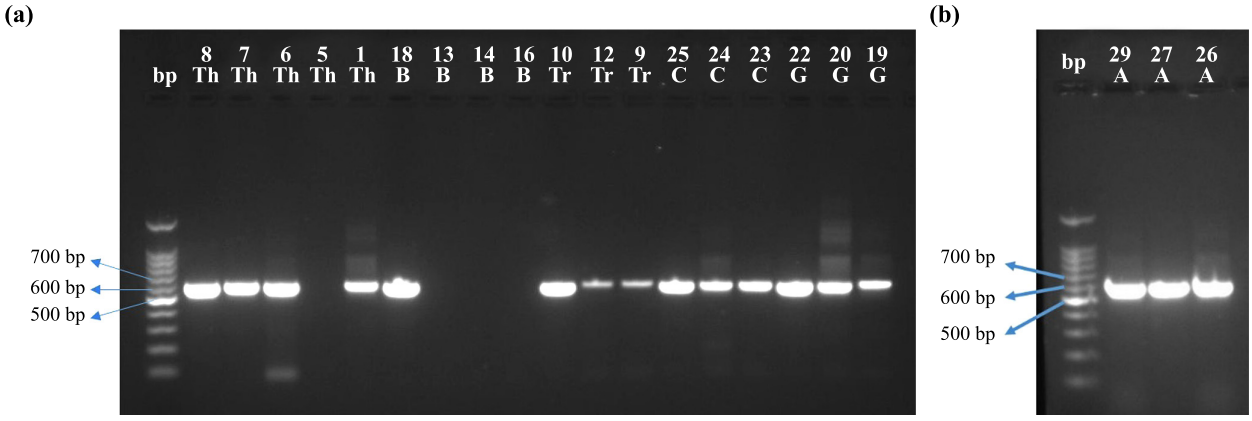

Fig. (1). 1.5% agarose gels showing the results of PCR done for fresh and dry samples of plant-based products using rbcL barcode primers. (**A**) Th: thyme, B: basil, Tr: turmeric, C: cardamom, G: ginger, and (**B**) A: anise.

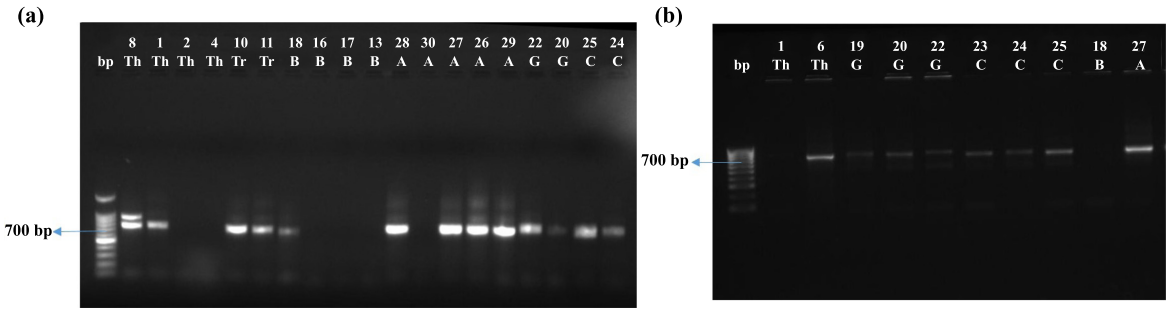

Fig. (2). 1.5% agarose gels showing the results of PCR done for fresh and dry samples of plant-based products using ITS barcode primers. Two gradient PCR programs were used: (**a**) with 45°C annealing temperature, and (**b**) with 50°C annealing temperature. Th: thyme, B: basil, A: anise, G: ginger, C: cardamom.

Table 3. BLAST results of ITS, matK and rbcL loci from fresh and processed plant based products. The mislabeled products are shaded.

ID	Plant Sample	Barcode Locus	Sequencing and Contig Assembly	GenBank_BLAST (identity %)
6-Th	Jattar (Thyme)	ITS	Unsuccessful	—
		matK	Successful	*Triticum urartu* (100%)
				Triticum aestivum (100%)
				Triticum turgidum (100%)
				Triticum macha (100%)
		rbcL	Successful	*Triticum urartu* (100%)
				Triticum aestivum (100%)
				Triticum macha (100%)
				Triticum dicoccon (100%)
				Triticum turgidum (100%)
				Triticum timopheevii (100%)
				Aegilops speltoides (100%)
7-Th	Fresh thyme	rbcL	Successful	*Thymus sibthorpii* (100%)
				Thymus serpyllum (100%)
				Thymus odoratissimus (100%)
				Thymus striatus (100%)
				T.alsinoides (100%)
8-Th	Fresh thyme	ITS	Unsuccessful	—
		rbcL	Successful	*Satureja pilosa* (100%)
9-Tr	Turmeric powder	rbcL	Successful	*Curcuma phaeocaulis* (100%)
10-Tr	Fresh turmeric	ITS	Unsuccessful	—
		rbcL	Successful	*Curcuma phaeocaulis* (99%)
11-Tr	Turmeric powder	ITS	Unsuccessful	—
12-Tr	Turmeric powder	rbcL	Successful	*Curcuma longa* (100%)
				Curcuma amada (100%)
				Curcuma roscoeana (100%)
				Curcuma chuanhuangjiang (100%)
				Curcuma phaeocaulis (100%)
				Curcuma sichuanensis (100%)
				Curcuma aromatica (100%)
				Curcuma kwangsiensis (100%)
18-B	Fresh basil	ITS	Unsuccessful	—
		rbcL	Successful	*Ocimum basilicum* (100%)
19-G	Ginger powder	ITS	Unsuccessful	—
		rbcL	Successful	*Zingiber officinale* (100%)

(Table 3) Contd…

ID	Plant Sample	Barcode Locus	Sequencing and Contig Assembly	GenBank_BLAST (identity %)
20-G	Ginger powder	ITS	Unsuccessful	—
		matK	Successful	*Triticum urartu* (99%)
				Triticum aestivum (99%)
				Triticum turgidum (99%)
				Triticum macha (99%)
		rbcL	Successful	*Oryza australiensis* (99%)
				Oryza minuta (99%)
				Oryza punctata (99%)
				Oryza glumipatula (99%)
				Oryza coarctata (99%)
22-G	Fresh ginger	ITS	Unsuccessful	—
		rbcL	Successful	*Zingiber officinale* (100%)
23-C	Cardamom powder	ITS	Successful	*Aframomum polyanthum* (95%)
				Aframomum letestuanum (95%)
		rbcL	Successful	*Elettaria cardamomum* (99%)
24-C	Cardamom powder	ITS	Unsuccessful	—
		rbcL	Successful	*Alpinia zerumbet* (99%)
25-C	Cardamom seeds	ITS	Successful	*Aframomum polyanthum* (95%)
				Aframomum letestuanum (95%)
		rbcL	Successful	*Alpinia zerumbet* (99%)
26-A	Anise	ITS	Successful	*Pimpinella anisum* (100%)
		rbcL	Successful	*Pimpinella anisum* (99%)
		matK	Unsuccessful	—
27-A	Anise	ITS	Successful	*Pimpinella anisum* (100%)
		rbcL	Successful	*Pimpinella anisum* (100%)
		matK	Unsuccessful	—
28-A	Anise	ITS	Unsuccessful	—
29-A	Anise	ITS	Successful	*Pimpinella anisum* (100%)
		rbcL	Successful	*Pimpinella anisum* (99%)
		matK	Unsuccessful	—

In the case of 24-C powdered cardamom, only rbcL locus was amplified and the sequence successfully provided 99% identity to *Alpinia zerumbet.* The sequences of rbcL locus of fresh ginger and 19-G powdered ginger samples were identical to *Zingiber officinale*. Interestingly, the rbcL locus of 20-G powdered ginger was aligned to many species from *Oryza* genus, and the matK locus was aligned to several species in *Triticum* genus, with 99% identity for both loci, indicating that the product was mislabeled and contained *Oryza* and *Triticum* as fillers. The sequences of rbcL locus of fresh turmeric were aligned to *Curcuma phaeocaulis* with 99% identity. Likewise, 9-Tr powdered turmeric was totally identical to *C. phaeocaulis*. The 12-Tr powdered turmeric sample aligned to several species of *Curcuma* genus. Additionally, basil rbcL locus showed total identity to *Ocimum basilicum*. The rbcL locus of 8-Th fresh thyme showed total identity to *Satureja pilosa*. However, the tested fresh thyme sample, 7-Th, aligned to several species from the genus *Thymus*. Both rbcL and matK loci of 6-Th thyme dry leaves showed complete identity to several species of *Triticum* genus, indicating that the product was mislabeled and contained *Triticum* as fillers.

4. DISCUSSION

Substitution of herbal ingredients and incorporation of unlabelled fillers can be considered as major problems in the manufacture and commercialization of herbal medicines and consequently can reduce their therapeutic potential, which in turn can represent a serious risk to the health of the consumers. The traditional taxonomic system is based on diagnostic morphological features of the plant species which cannot be used to identify processed or powdered plant materials [7]. Thus, DNA barcoding has been considered as a taxonomic method to identify plant materials based on the use of short DNA sequences from specified regions of the genome of a particular plant species. Furthermore, it represents a critical tool for quality controls of food products, to safeguard public health, guarantee food traceability, minimize food piracy, and to establish a level of quality assurance in the market [14, 26].

Authentication at the DNA level provides more reliability, compared to proteins and RNA since DNA is more stable, found in all tissues and is not affected by external factors. Consequently, robust DNA-based markers can be used for plant identification and authentication at the commercial level. The major advantages of DNA barcoding include identification of plants at any life cycle stage, and to distinguish even small pieces of a single species that co-exist with a mixture of species [14]. Other advantages of using DNA barcoding is that it is reproducible and testable as long as the link between DNA test sequences and reference specimens is supported. In addition, DNA barcoding can be verified at any time. Furthermore, it represents a universally applicable method that can be linked to any kind of biological or biodiversity information [31]. However, the DNA barcoding technique is associated with some limitations when it is used in identification and validation of herbal-based products as it is restricted to authentication of single-ingredient based samples. Moreover, it does not provide quantitative data regarding the relative abundance of species in mixed samples [32]. Another limitation is the quality of the DNA template. DNA in herbal products is damaged due to the various stages of plant material processing. Thus, it may be difficult to amplify long barcodes (300-1000bp), leading to false negative results. To overcome this problem short barcodes of 100-200 bp might be used [17]. Furthermore, in the case of spices, the absence of a reference database library of barcodes has limited the reliability of DNA barcoding in the detection of adulterations [1]. In this research, the DNA barcoding of different organs of six plant species well known as herbal products was studied. The plant organs included leaves (thyme and basil), rhizomes (ginger and turmeric), and seeds (anise and cardamom). Furthermore, different plant forms were used, including dry leaves, powders, and tea bags. Two major limitations of the DNA extraction from the aforementioned plant forms is the presence of phytochemicals that interfere with the process of DNA extraction, such as polysaccharides, phenolics and alkaloids [6]. In addition, the dry forms of the used tissues affect the integrity of DNA. However, we successfully extracted the genomic DNA from all tested plant samples, although the DNA quantities of most of the samples were very low and fragmented particularly with dried seeds samples such as anise and cardamom. Thus, the concentrated stock DNA was used directly for PCR without dilution.

The three barcode loci used in this research were ITS, matK, and rbcL. In agreement with Consortium for Barcode of Life (CBOL), MatK and rbcL were the ideal loci used in our study since they were able to amplify most of the plant samples. However, ITS has been suggested as a proper locus by several studies for its high variability [1, 9, 33]. The greatest amplification was produced by rbcL (amplified in 75% of the tested samples), followed by ITS (amplified in 66.67% of the samples). The lowest percentage of amplification was observed in matK, with only 18.52% of the tested samples. A recent report regarding the detection of adulterants in turmeric also encountered a 100% amplification of rbcL and ITS, but not matK [1].

Two fresh thyme samples with different morphologies (7-Th and 8-Th) were used in this research. The rbcL locus of 7-Th was aligned to multiple species in *Thymus* genus, including *T. serpyllum* which is described as wild thyme [34]. The rbcL locus of 8-Th was identical to *Satureja pilosa*, which is a Turkish thyme-like spice [35]. However, the rbcL and matK loci of 6-Th dry thyme leaves aligned differentially to several species of *Triticum* genus, indicating the adulteration of the 6-Th sample, although it was clearly written on the packed label “100% pure thyme" with no additives.

The fresh and powdered turmeric samples aligned to *C. phaeocaulis*. 12-Tr aligned to multiple species of *Curcuma* genus, which is one of the genera in *Zingiberaceae* family that is known as spices [36]. Cardamom is the common name of several species in *Zingiberaceae* family, including *A. zerumbet, E. cardamomum,* and others [37]. Many species in this family have the same common name because it is very hard to distinguish them based on the morphological features. The rbcL locus in 25-C cardamom seeds sample and 24-C powdered cardamom aligned to *A. zerumbet,* and 23-C powdered cardamom was aligned to *E. cardamomum*. It has been reported that *Aframomum* genus is separate from the *Amomum* genus, which is commonly known as cardamom [38]. The ITS locus of 25-C and 23-C aligned with two species of *Aframomum* genus, although with low identity percentages (95%), compared to 99% identity for rbcL. Thus, the ITS results for these two samples are less reliable than the results obtained with rbcL locus. rbcL locus of the fresh ginger sample and 19-G powdered ginger sample were identical to only one species of ginger, namely *Z. officinale*. This supports the reliability of rbcL locus in identifying *Z. officinale* species. However, the rbcL locus of the 20-G powdered ginger sample which was obtained from a local herbal market aligned to several species of *Oryza* genus. Moreover, the matk locus of the 20-G sample was aligned to several species of *Triticum* genus. The results from authentication of 20-G powdered ginger indicating that the sample is mislabeled and contains fillers from *Triticum* and *Oryza* species.

In spite of the high amplification percentage of ITS, it was successfully sequenced only in anise and cardamom samples. ITS locus showed high discriminatory efficiency for *P. anisum*. Furthermore, rbcL barcode had high discrimination efficiency for *S. pilosa*, *C. phaeocaulis*, *O, basilicum*,

Z. officinale, *E. cardamomum*, *A. zerumbet*, and *P. anisum*. rbcL barcode was not efficient in discriminating the species in *Triticum*, *Oryza*, and *Thymus* genera. Moreover, matK had the lowest discrimination efficiency of the species in *Triticum* genus. The low discrimination efficiency of matK and rbcL represents a limitation when using DNA barcoding as a taxonomical tool. However, when applying DNA barcoding to detect mislabeled products, this will not affect the results as long as the adulterants are not from the same species of the main ingredient.

CONCLUSION

To conclude, DNA barcoding is generating a global, open access library of reference barcode sequences, which enables non-taxonomists to identify specimens even if they are in powdered forms or mixed with other species. In the present study, ITS, matK, and rbcL barcode markers were used efficiently to identify and evaluate several medicinal plant-based products in different forms (fresh, dry powder and tea bags) from several UAE markets. The results showed that the aforementioned genes were different in quality where rbcL has the highest amplification efficiency, while matK has shown the lowest amplification efficiency. Both rbcL and matK can identify plant samples at the genus level and hence any possible adulteration. Both were able to identify adulteration of 6-TH powder thyme and matk was able to identify 20-G powder ginger. On the other hand, ITS showed high amplification efficiency however the sequencing results were not successful except in two samples only, 25-C cardamom seeds and 23-C cardamom powder.

CURRENT & FUTURE DEVELOPMENTS

Overall, DNA barcoding appeared to be a promising tool in the identification of plant species and herbal medicines but it remains to be fully exploited. Furthermore, food piracies lead to economic and nutritional losses as well as food safety and health issues. Therefore, the established DNA barcoding protocol is a rapid and relatively inexpensive for genetic traceability, taxonomic identification and genetic authentication of crop plant sources.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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