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Field Identification of *Matricaria chamomilla* using a Portable qPCR System

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Abstract

Quality control in botanical products begins with the raw material supply. Traditionally, botanical identification is performed through morphological assessment and chemical analytical methods. However, the lack of availability of botanists, especially in recent years, coupled with the need to enhance quality control to combat the stresses on the supply chain brought by increasing consumer demand and climate change, necessitates alternative approaches. The goal of this protocol is to facilitate botanical species identification using a portable qPCR system on the field or in any setting, where access to laboratory equipment and expertise is limited. Target DNA is amplified using dye-based qPCR, with DNA extracted from botanical reference materials serving as a positive control. The target DNA is identified by its specific amplification and matching its melting peak against the positive control. A detailed description of the steps and parameters, from hands-on field sample collection, to DNA extraction, PCR amplification, followed by data interpretation, has been included to ensure that readers can replicate this protocol. The results produced align with traditional laboratory botanical identification methods. The protocol is easy to perform and cost-effective, enabling quality testing on raw materials as close to the point of origin of the supply chain as possible.

Introduction

The practice of using botanicals to maintain and improve health dates back to thousands of years. Due to stresses on the supply chain brought by increased consumer demand[1], unsustainable harvesting practices and climate change[2], botanical adulteration is becoming a growing concern in the food and dietary supplement industry[3]. The presence of undeclared or misidentified botanical species may lead to reduced efficacy, or even safety issues. For example, black cohosh (*Actaea racemosa*), used for treating premenstrual discomfort, may be substituted with a low-priced Asian species with limited clinical data support for its efficacy[4]. In a more serious case, substitution of *Aristolochia fangchi* for

Stephania terandra in a clinical study for weight loss using Chinese herbs led to severe nephrotoxicity and renal failure in some participants[5,6]. The two different species shared a Chinese common name “Fang Ji”. These cases highlight the need for more stringent quality control, starting with the identification of raw materials[7], preferably as close to the point of origin of the supply chain as possible, so resources can be efficiently allocated to the material of correct identity.

A number of orthogonal approaches can be used for botanical identification. Traditionally, botanical identification is performed through morphological assessment[8,9] and chemical analytical methods[10,11,12,13]. Morphological identification is based on differences in macroscopic and microscopic features of plant materials if differences exist (**Figure 1**). However, the lack of training programs on classical botany in the recent years has resulted in a shortage of experts[14], making this approach impractical for routine quality control. Its application in powdered botanical materials is also limited. Chemical analytical methods are widely used in pharmacopoeias and laboratories, but are not ideal for field testing due to the size of instruments such as High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC), and Nuclear Magnetic Resonance Spectroscopy (NMR) (**Figure 2**), and environment requirements. Recently, genomic methods have emerged as an alternative technique for botanical species' authentication and substitution detection and has proven to be efficient and precise. Genomic methods exploit the high fidelity and specificity of genetic information in plant materials[15,16,17,18,19]. Molecular diagnostic tools are available in the form of portable devices, and often include automated data interpretation tools that lower the barrier to technology use, making this approach ideal for field identification[20,21,22,23,24]. Once the molecular analysis method has been designed and validated[25,26,27], it can be performed by any personnel with basic molecular biology training. Among the different portable tools available, real-time PCR on DNA sequences is one of the cost-effective choices[28]. The combination of a portable device, together with customized and validated molecular analysis, allows verification of botanical species and ingredients outside the laboratory, such as in farms and botanical material warehouses, reducing the time and costs associated with traditional methods.

The goal of this protocol is to introduce a method for botanical identification in situations where access to laboratory equipment and expertise is limited or unavailable, using a portable qPCR system. The method is demonstrated on a field of *Matricaria chamomilla* (**Figure 3A**), commonly known as German chamomile, widely used for its anti-inflammatory and antioxidant properties[29]. It can be confused with related species of similar appearance or odor, especially from the genera *Chamaemelum*, *Tanacetum*, and *Chrysanthemum*[30,31,32]. Among the related species, *Chamaemelum nobile*, also known as Roman chamomile, is a noticeable one with comparable production levels in commerce (**Figure 3B**). The method demonstrated was designed to not only identify the target botanical species *M. chamomilla*, but also detect its close relative, *C. nobile*, based on specific amplification of DNA sequences.

This article explains, in detail, how to perform field botanical identification of *M. chamomilla* using intercalating dye-based qPCR and melt curve analysis on a portable device. The protocol includes the collection of botanical samples from the field, on-site DNA extraction, and set up of real-time PCR reaction. To ensure a valid conclusion, target botanical *M. chamomilla* and non-target botanical *C. nobile* genomic DNA,

pre-extracted from certified botanical reference materials, are used as positive control. The specificity of this method is demonstrated by performing both *M. chamomilla* and *C. nobile* identification tests individually on samples and controls. Non-template negative control is used to exclude false positive results caused by PCR contamination.

Protocol

1. Sample collection

1. Set up a testing area in the field with a flat and horizontal surface.
2. Identify a representative plant that reflects the characteristics of majority of the plants in the chamomile flower field (**Figure 4**).
3. Pick a flower head from the representative plant using sterile gloves.
4. Place the sample into a 2.0 mL collection tube.
5. Repeat steps 1.3 to 1.4 and collect a leaflet (approximately 0.5–0.7 cm long) from the same plant.
   NOTE: *M. chamomilla* flower and leaf are small enough to sit at the bottom of a 2.0 mL collection tube. For other botanicals with larger surface area, a paper punch or scissors (rinsed in 70% ethanol prior to use) can be used to isolate tissue samples for testing. When multiple sampling is required, rinse the paper punch or scissors between handling of different samples.

2. DNA extraction

1. Preheat the dry bath incubator to 95 °C.
2. To each collection tube, add 100 µL of the extraction solution from the plant DNA extraction kit (listed in **Table of Materials**). For better DNA extraction efficiency, grind the tissue sample in the extraction solution using a tissue pestle.
3. Close the tube. Ensure that the botanical tissue is covered with the extraction solution throughout the extraction process.
4. Place the collection tubes in a preheated dry bath incubator and incubate the collection tubes at 95 °C for 10 min.
5. After 10 min, take the tubes out of the dry bath incubator.
6. Add 100 µL of the dilution solution from the same DNA extraction kit and mix the solution by pipetting up and down several times.
7. Repeat the same step for leaflet extraction.
8. Shake to mix the solution further. The plant tissue usually does not appear to be degraded after this treatment. The liquid color may change and become cloudy.
   NOTE: The diluted solution can be stored at room temperature overnight if not proceeding immediately. It is not necessary to remove the cellular debris from plant tissue before storage. The liquid in the tubes holds the DNA templates for downstream PCR amplification.

3. PCR reaction setup

1. Configure the qPCR instrument thermocycling conditions according to the manufacturer's specifications. Apply the PCR thermocycling profile listed in (**Table 1**), which starts with a constant temperature step for initial denaturation, followed by 25 cycles of amplification, and ends with temperature ramping to obtain a high-resolution melting curve.
2. Thaw the qPCR Master Mix and primers (**Table 2**) at room temperature prior to use.

3. Plan the reaction that will be loaded in each well: wells containing positive control with target species, positive control with non-target species, samples, and negative control (**Figure 5**).
    1. In this example, ten wells are used – five for the German chamomile identification test and the remaining five for the Roman chamomile identification test. For each type of species identification test, one well contains positive control with DNA extracted from targeted species' reference material, one well contains positive control with DNA extracted from non-targeted species' reference material, two wells are filled with flower and leaf DNA samples extracted from the field, and one well is allocated for a negative control. **Table 3** describes each well type.
4. Prepare a reaction master-mix according to the manual for each botanical species identification test. A typical reaction master-mix contains universal qPCR Master Mix (2x), forward and reverse species-specific primers, and nuclease-free water. **Table 4** lists the reaction system composition.
    NOTE: If not using immediately, store the qPCR reaction master-mix at +2 °C to +8 °C in a cooler or mini-fridge.
5. Thoroughly mix the reaction master-mix by pipetting before use.
6. Place the qPCR cartridge face-up on a flat and stable surface.
7. Load 18 µL of the reaction master-mix configured in step 3.4 into the cartridge wells according to the wells defined in step 3.3. For this demonstration, add the German chamomile identification test reaction master mix into wells labeled for GC test (GCT in wells 1, 3, 5, 7, 9) and the Roman chamomile identification test reaction master-mix into wells labeled for RC test (RCT in wells 2, 4, 6, 8, 10) (see **Figure 5**).
8. Transfer 2 µL of sample DNA from the supernatant of DNA extraction tubes and pre-extracted DNA positive controls into cartridge wells preloaded with qPCR master mix. After adding each DNA template to the qPCR master mix, gently mix the solution by pipetting.
    NOTE: Avoid floating cellular debris when transferring DNA from the DNA extraction tube. Use minicentrifuge to separate the supernatant and cellular debris, if necessary.
9. Carefully seal the cartridge with adhesive film. Load the cartridge onto the thermocycling chamber and close it.
10. Set the qPCR instrument to run.

Representative Results

Following the protocol described in section 1, botanical DNA from flower head and leaf were extracted into the supernatant after heat incubation of the collection tube at 95 °C for 10 min. In the current study, the supernatant showed a yellow and greenish color for both flower and leaf, indicating that a variety of natural compounds were released into the supernatant with botanical DNA (**Figure 6**). Although reliable PCR amplification was achieved later in triplicate for all field extracted DNA template, DNA quality assessment was performed in the laboratory as reference. The concentration of flower head DNA extract, determined by fluorometry, ranged from 3.69–5.36 ng/µL, while the concentration of leaf DNA extract ranged from 6.42–9.29 ng/µL. The A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios of flower and leaf DNA extracts were measured by spectrophotometry. However, due to the overlap between DNA and phytochemical UV absorption spectrum, these ratios could not be reliability measured (data not shown).

Intercalating fluorescent dye was used to monitor the amplification of target fragments in real-time. Since both the specific primers *M. chamomilla* and *C. nobile* target the internal transcribed spacer 2 (ITS2) region, which has tens to hundreds of copies in the plant genome, 25 PCR amplification cycles are sufficient to generate enough amplicons for the identification of chamomile species. In **Figure 7**, the Ct value for *M. chamomilla* positive control in *M. chamomilla* identification test was less than 25 (GCP_GCT), while after 25 amplification cycles, the fluorescence of the same control in *C. nobile* identification test was below the detection threshold (GCP_RCT). On the other hand, after 25 cycles, the fluorescence for *C. nobile* positive control in *M. chamomilla* identification test was below the detection threshold (RCP_GCT), while the Ct value for the same control in *C. nobile* identification test was less than 25 (RCP_RCT). The amplification of target and non-target positive controls in their respective assays demonstrate the specificity of the *M. chamomilla* identification assay. For sample DNA, field flower head and leaf DNA extract yielded Ct values of 15.18 and 19.41 in *M. chamomilla* identification test, respectively (Sample1(FLOWER)_GCT and Sample2(LEAF)_GCT). Both of these samples were not amplified in *C. nobile* identification test (Sample1(FLOWER)_RCT and Sample2(LEAF)_RCT). The amplification patterns of both the samples matched the amplification pattern of *M. chamomilla* positive control. Negative controls were not amplified in both *M. chamomilla* and *C. nobile* identification tests (NC_GCT and NC_RCT), excluding the possibility of false positives caused by PCR contamination. To further confirm specific amplification in positive controls and samples, fractions of PCR end product from each well were run on 2% agarose gel in the laboratory (**Figure 8**). For *M. chamomilla* identification test, both field samples yielded amplicons running at the same position as the *M. chamomilla* positive control with an estimated size slightly above 100 bp (theoretical size 102 bp). For *C. nobile* identification test, non-target species *C. nobile* positive control yielded a band between 50 and 100 bp, fitting the theoretical size of 65 bp. The rest of the lanes showed no specific amplification product, which was in agreement with the absence of fluorescent signal for these wells, as observed in field testing.

Following PCR amplification, a melting curve analysis was performed to assess the dissociation characteristics of double-stranded DNA (dsDNA) during heating. As the temperature ramped up during the final cycle for melt curve analysis, increases in temperature caused the double-strand amplicons to dissociate. The intercalating fluorescent dye was gradually released into the solution, decreasing fluorescence intensity (**Figure 9A**). The inflection point of the first derivative curve was used to determine the melting temperature (Tm) (**Figure 9B**), which depends mainly on DNA fragment length and GC content. Combining Ct value with melting temperature can increase the specificity of qPCR analysis. In the current study, the melting temperature peak of *M. chamomilla* positive control PCR amplicon occurred at 85.6 °C (GCP_GCT) and it was distinct from the melting temperature peak of *C. nobile* positive control PCR amplicon at 79.1 °C (RCP_RCT). The PCR amplicon from field flower head and leaf produced melting temperature peaks at 85.2 °C and 84.8 °C, respectively (Sample1(FLOWER)_GCT and Sample2(LEAF)_GCT). To assess melting temperature variations measured by the portable qPCR system, additional datapoints were collected to confirm that sample melting temperatures were always close to the melting temperature obtained from *M. chamomilla* positive control (within 2 °C) and were far away from the melting temperature of *C. nobile* positive control amplicon (**Figure 10**). Melting temperature peaks were sometimes reported in other wells. However, their

Ct values were not less than 25 and melting temperature peaks were not close to *M. chamomilla* or *C. nobile* positive control (more than 2 °C apart).

In summary, field *M. chamomilla* identification test can be interpreted based on decision rules summarized in **Table 5**. With all the positive controls testing positive for the putative botanical species, negative for the other species, and negative controls testing negative, both field samples were determined to contain *M. chamomilla* but not *C. nobile*. In addition, to align field testing results with other analytical techniques, field conclusions were further confirmed by a previously validated DNA barcoding method[25] (data not shown).

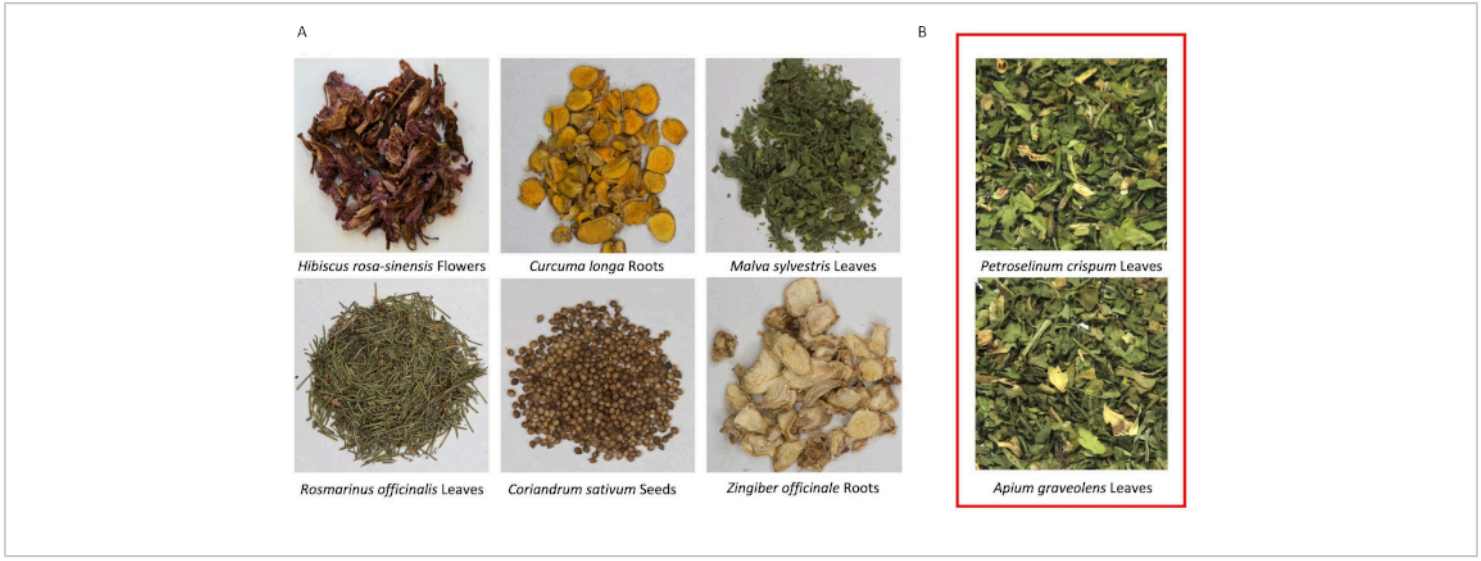

Figure 1: Morphological identification of botanical materials. (**A**) *Hibiscus rosa-sinensis* flowers, *Curcuma longa* roots, *Malva Sylvestris* leaves, *Rosmarinus officinalis* leaves, *Coriandrum sativum* seeds, *Zingiber officinale* roots. (**B**) *Petroselinum crispum* and *Apium graveolens* flakes are difficult to differentiate. Please click here to view a larger version of this figure.

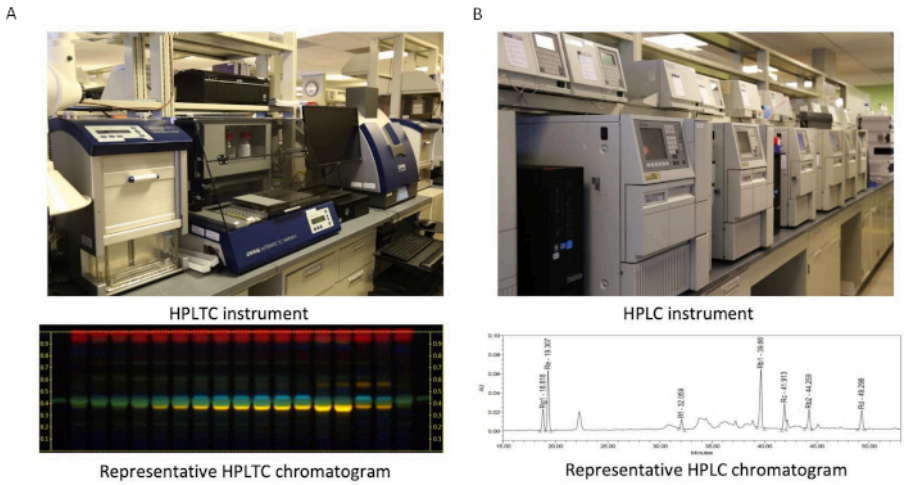

Figure 2: Chemical identification of botanical materials. (**A**) HPTLC instrument and a representative HPTLC chromatogram. (**B**) HPLC instrument and a representative HPLC chromatogram. Please click here to view a larger version of this figure.

Figure 3: *Matricaria chamomilla* and *Chamaemelum nobile* in the field. (**A**) *Matricaria chamomilla,* adapted from Wikipedia under CC BY-SA 3.0, https://en.wikipedia.org/wiki/Matricaria_chamomilla#/media/File:Matricaria_February_2008-1.jpg. (**B**) *Chamaemelum nobile,* adapted from Wikipedia under CC BY-SA 3.0, https://en.wikipedia.org/wiki/Chamomile#/media/File:Chamaemelum_nobile_001.JPG. Please click here to view a larger version of this figure.

Figure 4: Collecting *M. chamomilla* plant parts from the field. Please click here to view a larger version of this figure.

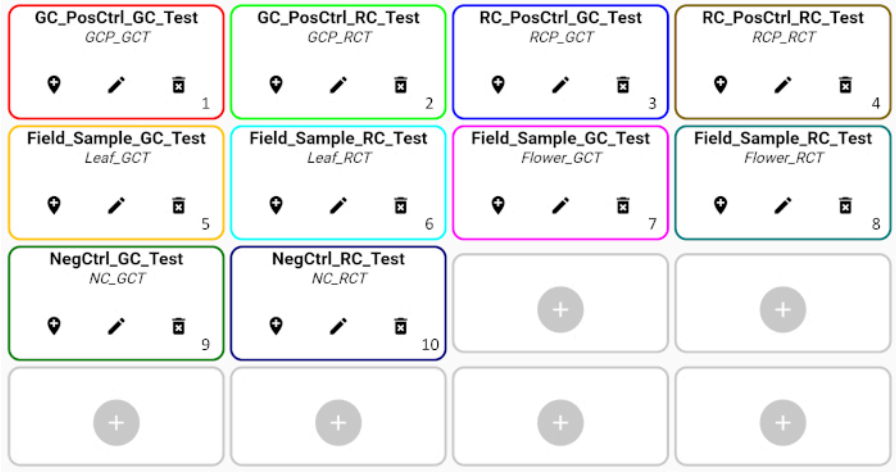

Figure 5: Layout of testing wells in the demonstration. Please click here to view a larger version of this figure.

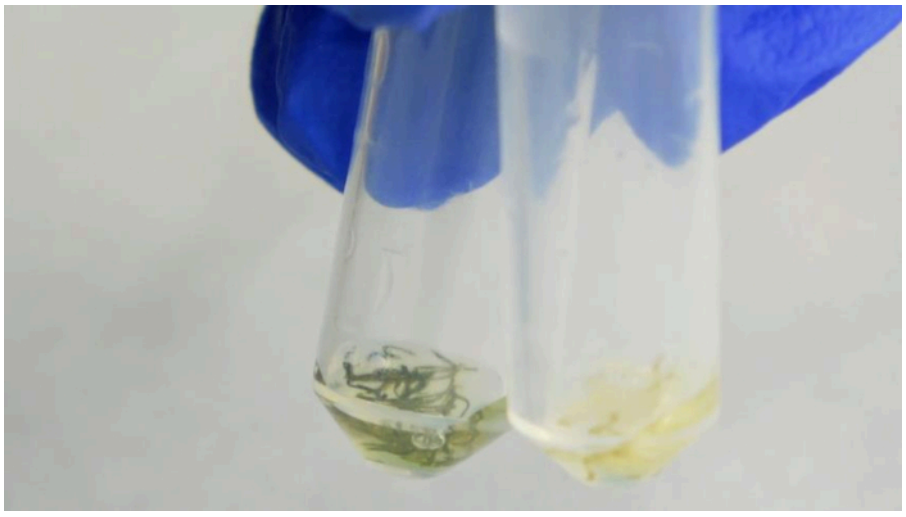

Figure 6: Field DNA extract in collection tubes. Botanical tissue remains in the original tube and is covered by yellowish DNA extract. Please click here to view a larger version of this figure.

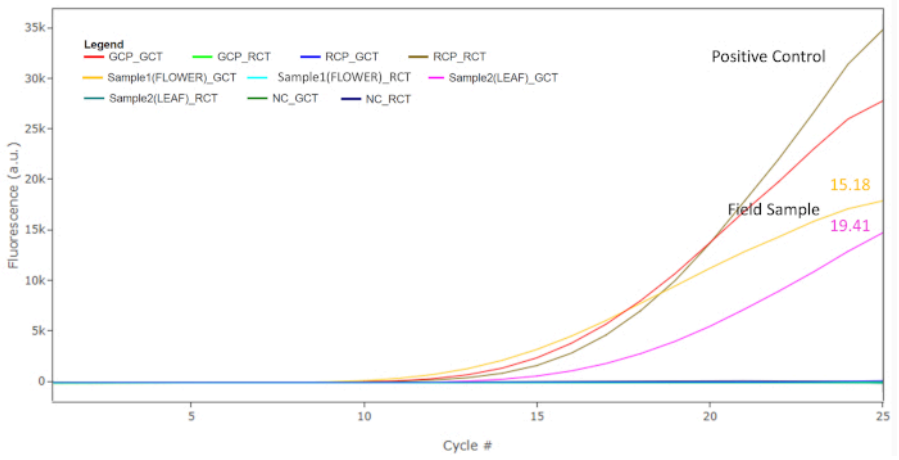

Figure 7: Fluorescence plot showing the accumulation of PCR products over 25 cycles of thermocycling. *M. chamomilla* positive control and *C. nobile* positive control show Ct values less than 25 in *M. chamomilla* and *C. nobile* identification tests, respectively. The field flower and leaf samples were amplified by *M. chamomilla* identification test with Ct values of 15.18 and 19.41. The rest of the wells were not amplified. Please click here to view a larger version of this figure.

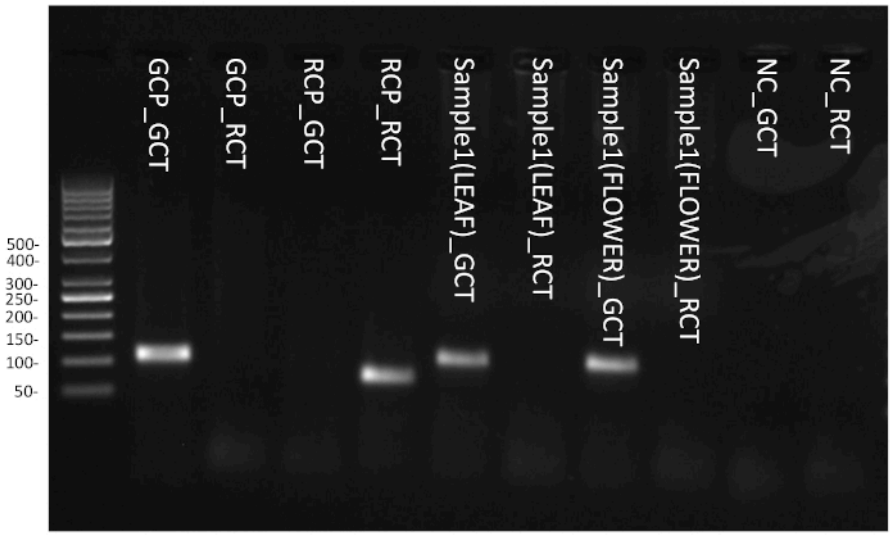

Figure 8: Gel electrophoresis of field PCR amplification products. Please click here to view a larger version of this figure.

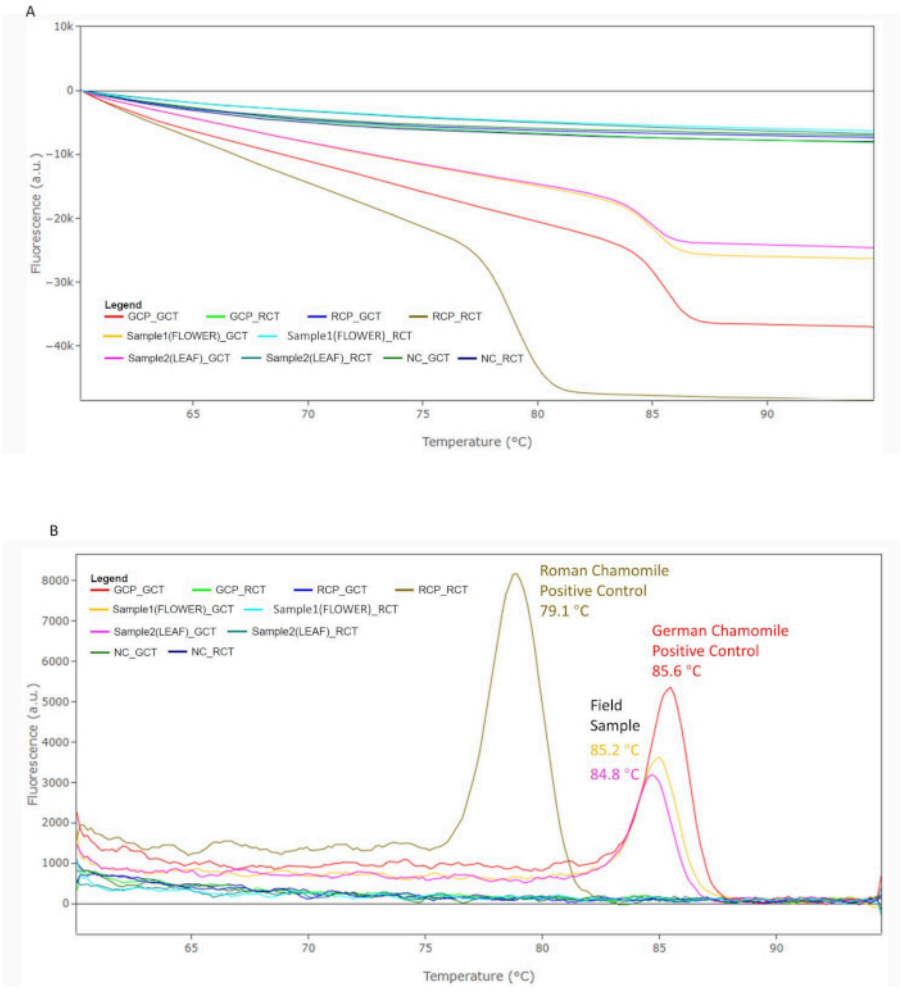

Figure 9: Melting temperature analysis. (**A**) The fluorescence signal in each well decreases with the increasing temperature. (**B**) The identity of the PCR products was confirmed by the melting temperature peak in melting curve analysis. The field flower and leaf samples show peaks at 85.2 °C and 84.8 °C. These are close to the peak produced by *M. chamomilla* positive control. The *C. nobile* positive control produced a peak at 79.1 °C, which is different from the other three samples. Please click here to view a larger version of this figure.

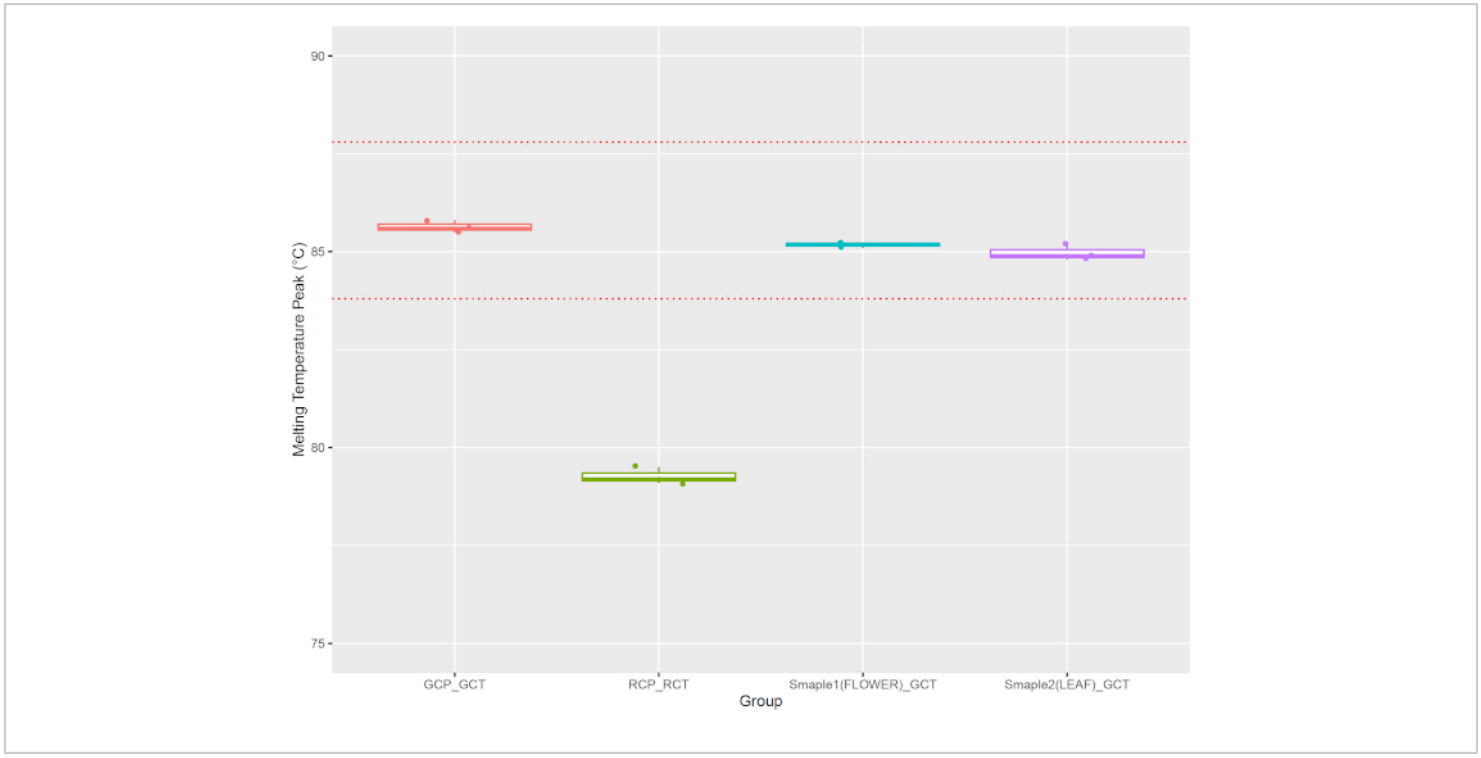

Figure 10: Melting temperature peak variation between positive control and field samples. Please click here to view a larger version of this figure.

Stage	Cycle	Temperature	Time
Constant Temperature	1	95 °C	60s
Amplification	25	95 °C	30s
		60 °C	30s
Melting Curve	1	60 °C	Ramp 0.05 °C/s
		95 °C	

Table 1: qPCR thermocycling conditions for *M. chamomilla and C. nobile* identification tests.

Assay	Primer name	Sequence 5'-3'	Position	Region	Amplicon Size
Matricaria recutita	ZL3	TCGTCGGTCGCAAGGATAAG	Forward	ITS2	102 bp
	ZL4	TAAACTCAGCGGGTAGTCCC	Reverse		
Chamaemelum nobile	ZL11	TGTCGCACGTTGCTAGGAAGCA	Forward	ITS2	65 bp
	ZL12	TCGAAGCGTCATCCTAAGACAAC	Reverse		

Table 2: Primer pairs for *M. chamomilla* and *C. nobile* identification tests.

Well position	Well name	Description
1	GC_PosCtrl_GC_Test	German chamomile positive control under GC Test
2	GC_PosCtrl_RC_Test	German chamomile positive control under RC Test
3	RC_PosCtrl_GC_Test	Roman chamomile positive control under GC Test
4	RC_PosCtrl_RC_Test	Roman chamomile positive control under RC Test
5	Field_Sample_GC_Test	Sample of leaf tissue under GC Test
6	Field_Sample_RC_Test	Sample of leaf tissue under RC Test
7	Field_Sample_GC_Test	Sample of flower tissue under GC Test
8	Field_Sample_RC_Test	Sample of flower tissue under RC Test
9	NegCtrl_GC_Test	Negative control sample under GC Test
10	NegCtrl_RC_Test	Negative control sample under RC Test

Table 3: Well types and descriptions for *M. chamomilla* and *C. nobile* identification tests.

Reagent	GC_Test	RC_Test
Universal qPCR Mix*	10 µL	10 µL
ZL3 primer (10 µM)	0.4 µL	NA
ZL4 primer (10 µM)	0.4 µL	NA
ZL11 primer (10 µM)	NA	0.4 µL
ZL12 primer (10 µM)	NA	0.4 µL
H_2O (Nuclease-free)	7.2 µL	7.2 µL
* contains Hot Start Taq DNA Polymerase		

Table 4: Master-mix composition for *M. chamomilla* and *C. nobile* identification tests.

Well Name	Expected Result	Positive Result Criteria	Negative Result Criteria
		Detected / Present	Not Detected / Absent
GC_PosCtrl_GC_Test	Detected	Ct < 25 and 84 <= Tm <= 86	-
GC_PosCtrl_RC_Test	Not Detected	-	No Ct value within 25 cycles
RC_PosCtrl_GC_Test	Not Detected	-	No Ct value within 25 cycles
RC_PosCtrl_RC_Test	Detected	Ct < 25 and 79 <= Tm <= 81	-
Field_Sample_Leaf_GC_Test	Present	Ct < 25 and 84 <= Tm <= 86	No Ct value within 25 cycles
Field_Sample_Leaf_RC_Test	Absent	-	No Ct value within 25 cycles
Field_Sample_Flower_GC_Test	Present	Ct < 25 and 84 <= Tm <= 86	No Ct value within 25 cycles
Field_Sample_Flower_RC_Test	Absent	-	No Ct value within 25 cycles
NegCtrl_GC_Test	Not Detected	-	No Ct value within 25 cycles
NegCtrl_RC_Test	Not Detected	-	No Ct value within 25 cycles

Table 5: Rules for qPCR result interpretation.

Discussion

The design of primers and template selection are the crucial steps in obtaining an efficient and specific qPCR amplification. After identifying a suitable template, primer design software is typically used to aid selection of primers based on design variables such as primer length, melting temperature, and GC content[33,34]. Optimization and validation can be performed under the expected experimental

conditions of the assay to ensure specificity, sensitivity, and robustness of a PCR reaction[35]. Sub-optimal primer design may result in primer-dimer formation, wherein primer interactions produce non-specific products[36].

The no template controls (NTC) used in this study check for both DNA contamination and the presence of primer-dimers that could affect the assay. Results showed no amplification, a good indication that both DNA contamination and primer-dimers are not a concern. DNA contamination and primer-dimers are manifested in melt curves through no template controls, and as extra peaks in melt curves of positive controls. Typically, the melt curve of a positive control is expected to contain a single peak, unless AT-rich subdomains in the template cause uneven melting. Double peaks could be predicted by simulating melting assays using the uMELT software[37]. In this study, the gold standard of running the PCR product on agarose gel was used to confirm the presence of target PCR product and absence of contamination and primer-dimers.

A considerable challenge in botanical material molecular analysis is obtaining good-quality DNA following the botanical DNA extraction process. Botanical materials are traded and consumed for the active chemical compounds that are associated with health benefits. In the process of DNA extraction, these chemical compounds will also be released into the DNA extraction solution, potentially causing PCR inhibition, thereby resulting in failures in PCR amplification. Various plant DNA purification kits using organic solvents and columns have been developed to remove chemical compounds derived from botanicals[38]. However, fume hood and high-speed centrifuge required to assist these kits are not available in the field.

In the current protocol, the simplified DNA extraction method uses a commercial plant DNA extraction kit (see **Table of Materials** for details). It had the ability to neutralize common inhibitory substances for reproducible results and produced consistent results for *M. chamomilla* and *C. nobile.* Both *M. chamomilla* and *C. nobile* flower heads and leaves yielded PCR amplicons with specific melt peaks, indicating that the presence of PCR inhibitors was not a concern. For other plants with higher levels of PCR inhibitors, amplifying DNA in their original extraction may be less efficient. To reduce inhibition and improve amplification efficiency, with access to the whole plant, other plant parts with lower polysaccharide and polyphenol content can also be used for identification purposes. If there is limited access to different plant parts, younger leaves or petals dissected from flower heads, which typically have lower phenolic content[39], may offer a better chance of success. Since DNA sequences are consistent across the whole plant, any plant part may be used to confirm species identity. If PCR amplification is still suboptimal, the original DNA extract can be further diluted before PCR, or more sophisticated laboratory purification protocols can be used.

Another challenge for PCR analysis is false positive results caused by DNA contamination, which can negatively impact data interpretation. It is usually controlled by active housekeeping, using dedicated equipment, and restricting work to designated areas. Using qPCR, all PCR analysis can be accomplished in a closed system, which greatly reduces the chance of PCR amplicon contamination in an environment that is not well controlled. Besides, environmental DNA should also not show a false positive due to the specificity of the assay, according to a previous validation study[40].

There is room for improvement. In the protocol presented here, intercalating dye was used to show target fragment amplification in real-time. The specificity of the method is further confirmed by the characteristic melting temperature, which is distinct between *M. chamomilla* and *C. nobile* amplicons. Therefore, intercalating dye-based PCR can efficiently answer the question "What is this plant species?" in the field. However, in addition to the need of performing botanical identification on a single plant isolated from the field, in many circumstances, botanical powders or extracts in the warehouse will also benefit from an on-site rapid identity assessment. For these types of materials, additional questions may need to be addressed, such as "What is in this material?", "Does it contain the botanical species I am looking for?", "Does it contain adulterants I want to avoid?", and "Is it substituted wholly or partially by other botanical species that are harmful?". Instead of using intercalating dye, different qPCR probes can be designed to target amplicons from different botanicals in one reaction system, while maintain high specificity and efficiency of the assay. Development of probe-based qPCR and utilization of a portable qPCR system that offers multiple channel detection can further extend the application of field testing as a fit-for-purpose assay to a broader environment setting, such as botanical material warehouses and distribution centers to answer more complicated questions. In addition, using multiple probes also allows the user to include internal amplification in each reaction system, so that more information will be available when PCR inhibition is suspected.

The protocol presented here has the following advantages compared to existing technologies used for the same purpose. First, for traditional morphological and chemical identification methods, the procedure and its results need to be conducted and interpreted by experts. qPCR-based identification tests can be conducted by people with basic molecular biology training and interpreted in a more standardized manner. Second, compared to qPCR-based species identification and differentiation normally performed in the laboratory, the field identification protocol using a portable instrument does not require instruments with a large footprint, such as a high-speed centrifuge, DNA quality evaluation equipment, thermal cycler with fluorescence detector, and a computer running a special software. Thus, DNA-based species identification can be performed in any setting without delay. Third, searching for botanical materials is a task that requires a global operation. With advancements in cloud services and artificial intelligence, a portable device can potentially receive methods developed and validated by experts in the laboratory, be operated by non-experts in remote areas, and produce objective certifications from third parties. Therefore, this option is more compelling than ever with remote work becoming the trend.

In summary, the protocol here demonstrated field identification of *M. chamomilla* using a portable qPCR system. The successful application of this technique will generate highly accurate results on botanical identification and help botanical manufacturers and suppliers qualify botanical materials in a timely and cost-efficient manner.

Disclosures

We certify that Zhengxiu Yang, Zheng Quan, Tiffany Chua, Leo Li, Yanjun Zhang, Silva Babajanian, Tricia Chua, Peter Chang, Gary Swanson, Zhengfei Lu are employees of Herbalife International of America, Inc. We certify that Francesco Buongiorno, Isabella Della Noce, and Lorenzo Colombo are employees of Hyris Ltd. that produces the portable qPCR instrument used in this article.

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