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Comparing DNA Extraction Methods for Analysis of Botanical Materials Found in Anti-Diabetic Supplements

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Abstract A comparative performance evaluation of DNA extraction methods from anti-diabetic botanical supplements using various commercial kits was conducted, to determine which produces the best quality DNA suitable for PCR amplification, sequencing and species identification. All plant materials involved were of suboptimal quality showing various levels of degradation and therefore representing real conditions for testing herbal supplements. Eight different DNA extraction methods were used to isolate genomic DNA from 13 medicinal plant products. Two methods for evaluation, DNA concentration measurements that included absorbance ratios as well as PCR amplifiability, were used to determine quantity and quality of extracted DNA. We found that neither DNA concentrations nor commonly used UV absorbance ratio measurements at A_{260}/A_{280} between 1.7 and 1.9 are suitable for globally predicting PCR success in these plant samples, and that PCR amplifiablity itself was the best indicator of extracted product quality. However, our results suggest that A_{260}/A_{280} ratios below about 1.3 and above 2.3 indicated a DNA quality too poor to amplify. Therefore, A_{260}/A_{280} measurements are not useful to identify samples that likely will amplify but can be used to exclude samples that likely will not amplify reducing the cost for unnecessarily subjecting samples to PCR. The two Nucleospin® plant II kit extraction methods produced the most pure and amplifiable genomic DNA extracts. Our results suggest that there are clear, discernable differences between extraction methods for low quality plant samples in terms of producing contamination-free, high-quality genomic DNA to be used for further analysis.

Keywords Medicinal plant · DNA extraction methods · DNA quality measures absorbance ratio · Botanical supplements · A_{260}/A_{280}

Introduction

The World Health Organization estimates that 80% of the world population uses traditional medicines for coping with disease [1]. Those with type 2 diabetes mellitus (DM2) are 1.6 times as likely to use complementary medicine treatment modalities, such as dietary supplements with botanical origins, to treat DM2 and its comorbidities, and that number is only expected to rise [2]. Since many important anti-diabetic compounds have been developed from traditional folk medicines (such as metformin, the anti-diabetic biguanide that was derived from *Galega officinalis*) [3], other traditionally used plants that exhibit pharmacological and anti-diabetic activity may provide valuable new sources for anti-diabetic medications [3, 4].

The first step leading to further testing of medicinally active plants is composed of collecting and identifying samples from the traditional healers of a society or through acquisition of materials sold through multiple venues, anywhere from local markets to the WWW. However, confirmation of the scientific name and identity of the material is not a trivial task. Botanical supplements are typically made from plants that have been dried, mixed, or shredded for use in teas or pills which prohibits the use of

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traditional taxonomic keys designed to use relatively complete vegetative and reproductive plant parts for quick and accurate identification. Recently, new molecular analysis techniques, such as DNA barcoding [1, 5–7] or various DNA fingerprinting techniques, have provided additional tools to supplement traditional identification methods based on morphology [8]. As a first step, DNA must be extracted in order to use the aforementioned analytical methods. However, depending on storage and processing of the plant materials for supplement production, DNA might be potentially highly degraded or otherwise compromised. Furthermore, essential oils and other secondary metabolite contaminations may lower the amount of DNA yields and its integrity and generate recalcitrant products upon extraction [9]. Polyphenols, alkaloids and terpenoids, all of which are responsible for the valuable pharmacological properties of medicinal plants, will often co-precipitate with DNA, interact irreversibly with proteins and nucleic acids, and inhibit enzymatic processes necessary for further amplification [10, 11]. Moreover, large amounts of complex polysaccharides present in plants can also make it impossible to extract useable DNA by producing a thick and sticky elution product and by binding tightly to the extracted DNA. As a result, this may inhibit enzymatic manipulation by many commonly used molecular biological enzymes, such as polymerases [10, 11]. For this reason, a number of different plant DNA isolation methods have been developed and manufactured to selectively precipitate or inactivate contaminating substances [8–19]. Unfortunately, no one method has proven universally applicable and it is still debatable whether one DNA extraction protocol will be suitable for all plants, as differing phytochemical compositions might require specific customizations within isolation protocols [8, 15].

In an effort to rank the performance of different DNA extraction methods and evaluate the quantity and quality of DNA amenable to amplification specifically when plant sample quality is poor, five DNA extraction kits available on the market (equating a total of eight extraction protocols due to multiple protocols in some kits) were tested against 13 commonly used anti-diabetic medicinal plant supplements from northern Mexico and south Texas. Previous studies have used less comprehensive approaches using 1–3 commercial kits comparing performance [20]. Other authors have focused on either a small, specific taxonomic group (typically genus) of medicinal plants [8, 15–17], or on non-medicinal herbarium specimens that are similarly difficult to extract, but sometimes for different reasons [21, 22]. Furthermore, we evaluated and compared two different DNA quality assessment methods in order to see whether they would provide a reliable measure to predict sequencing success: (1) PCR amplification of all samples, to distinguish between good and poor DNA samples and (2) measurement of absorbance ratios (A_{260}/A_{280}) that have typically been used to assess quality of both animal and plant DNA extracts. If absorbance ratios could produce reliable predictors of DNA quality and therefore PCR performance and sequencing success, as it has been assumed in other studies [9, 12, 14–17, 19, 23], the number of expensive PCR reactions for DNA quality testing could be reduced, especially when large sample sizes have to be processed.

Materials and Methods

Materials Used

Thirteen plant samples, used as alternative medicines by the Hispanic population of southern Texas and northeastern Mexico for the treatment of type 2 diabetes, were collected for our study. All plants are outlined in Table 1 with their respective abbreviations and scientific classifications. Two of our supplements were obtained with both a common and scientific name label (*Aloe vera* and *Gymnema sylvestre*), and the *Tournefortia* sample was provided to us already identified by Richardson and King [24]. All other samples were purchased at local herbal stores, and common Spanish name labels were translated into scientific names using species lists assembled as part of ethnobotanical and anthropological works of the region [25–28]. Plant samples were separated into 25 mg dry samples originating from leaf, stem, bark, cone, and pill. Supplements that contained more than one tissue type, e.g., stems and leaves, were used accordingly for all types. All samples were obtained in dry format or as pressed pills. After being frozen at −80 °C overnight in order to aide break-up, plant samples were ground in the TissueLyser II (Qiagen, Valencia, CA) using two tungsten carbide beads (Qiagen, Valencia, CA) of 3 mm diameter and immediately processed for DNA extraction.

DNA Extraction and Measurements

The DNA extraction methods used for our study are summarized in Table 2, with manufacturers and catalog numbers provided. All DNA extractions were carried out according to the manufacturer's instructions. If the kit provided more than one lysis buffer, each one was used for our study and denoted as a different method. All final DNA was eluted with 50 μL of elution buffer provided in each kit. The DNA concentration was measured by absorbance at 260 nm using a spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies, USA), and DNA purity was analyzed using the UV absorbance ratio (A_{260}/A_{280}) measurements. Concentrations and absorbance ratios were

Table 1 Collected plant samples and species identification

Common name	Abbreviation	Scientific name	Family
Gobernadora	GB	*Larrea tridentata*	Zygophyllaceae
Chaya	CH	*Cnidoscolus aconitifolius*	Euphorbiaceae
Tronadora	TD	*Tecoma stans*	Bignoniaceae
Prodigiosa	PD	*Brickellia cavanillesii*	Asteraceae
Damiana	DM	*Turnera diffusa*	Passifloraceae
Diente de leon	DDL	*Taraxacum officinale*	Asteraceae
Retama China	RC	*Senna multiglandulosa*	Fabaceae
Aloe	AL	*Aloe vera*	Xanthorrhoeaceae
Tournefortia	TF	*Tournefortia hirsutissima*	Boraginaceae
Gymnema	GS	*Gymnema sylvestre*	Asclepiadaceae
Huereque	HQ	*Ibervillea sonorae*	Cucurbitaceae
Enebro	JB	*Juniperus communis*	Cupressaceae
Valeriana	VR	*Valeriana edulis*	Valerianaceae

Table 2 List of methods used for DNA purification

Method code	Extraction kit (lysis buffer)	Supplier (catalog no.)
Qiag	DNeasy plant mini kit	Qiagen (69106)
Nucl	Nucleospin® plant II (PL1)	MACHEREY–NAGEL (740770.50)
Nucl2	Nucleospin® plant II (PL2/PL3)	MACHEREY–NAGEL (740770.50)
Gdmk	IBI genomic DNA mini kit (plant, GP1)	IBI (IB47230)
Gdmk2	IBI genomic DNA mini kit (plant, GPX1)	IBI (IB47230)
Epic	QuickExtract™ plant DNA extraction solution	Epicentre biotechnologies (QEP80705)
Epic2	QuickExtract™ seed DNA Extraction solution	Epicentre biotechnologies (QES08095T)
Mobi	PowerPlant® DNA isolation kit	MO BIO (13200-50)

measured in triplicates using 2.0 μL of eluted DNA for each measurement, and results for both are expressed as mean ± S.D. We also ran total DNA extracts on an agarose gel to evaluate level of degradation based on the size of fragments obtained. This was done to ensure that DNA was not degraded to fragments too small to be amplified by our chosen primer pairs.

PCR Amplification and Sequencing

Two standard DNA regions were amplified using PCR, the nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) and the chloroplast DNA (cpDNA) intergenic region trnL-trnF. The primers used to amplify those regions were ITSA, ITSB [29] and uniE, uniF [30]. PCR reactions for the ITS region consisted of: DNA extract and dilutions in elution buffer (1:10 and 1:20), 1X standard Taq buffer (New England Biolabs, MA), 0.2 mM of dNTPs, 0.5 μM of each primer, and 0.08 units/μL of Taq DNA polymerase (New England Biolabs, MA). PCR reactions for trnL-trnF region consisted of: DNA extract and dilutions (1:10 and 1:20), 1X ammonium PCR buffer (Midwest Scientific™, St. Louis, MO), 0.4 mM of dNTPs, 0.5 μM of each primer, and 0.05 units/μL of PR DNA Polymerase (Midwest

Scientific™, St. Louis, MO). PCRs were also attempted with the Phusion high-fidelity DNA polymerase (New England Biolabs, MA). The PCR reactions were done in a C1000 Thermal Cycler (Bio-Rad, Hercules, CA). For the ITS region, the temperature profile was 94 °C for 1 min, 37 cycles of 94 °C for 1 min, 50 °C for 45 s and 72 °C for 2 min, and ending with one extension cycle at 72 °C for 10 min. For trnL-trnF, the temperature profile was 94 °C for 1 min, 35 cycles of 94 °C for 10 s, 50 °C for 30 s and 72 °C for 2.45 min, and ending with one extension cycle at 72 °C for 5 min. PCR products were confirmed using 1.5% agarose gels and then purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA, Cat No. 28706). PCR results presented here represent data from at least three replicate experiments.

The sequencing was outsourced to Molecular Cloning Laboratories in South San Francisco, CA. (www.mclab. com). The nucleotide sequences were edited and assembled with Sequencher 4.9 (Gene Code Corporation, Ann Arbor, MI), and ITS and trnL-trnF amplicons were confirmed by Basic local alignment search tool (BLAST) search (http://blast.ncbi.nlm.nih.gov) on the NCBI website. This allowed us to distinguish between plant sequences and sequences that might have come from contaminations of

fungi, bacteria, or other organisms contained in the medicinal herb preparation.

Results

Comparing DNA Concentration and PCR Amplification for Different DNA Extraction Methods

The eight different DNA extraction methods utilized for this study all yielded varying amounts of DNA, as determined by spectrophotometric analysis of nucleic acids (Table 3). Nucl, Nucl2, Qiag, Gdmk, and Gdmk2 all produced elutions with little to no brownish tint; Epic, Epic2, and Mobi produced elutions with either significant brownish color or crude flow-through. We found that DNA concentration was not a good predictor for PCR amplifiability (Fig. 1). In the case of Nucl, Nucl2, and Qiag, PCR products with the standard polymerase produced relatively sharp bands at the correct fragment size ($\sim$700 bp) for our ITS primers. In all other cases, extracts that did amplify by PCR also produced smears across agarose gels (data not shown). When PCR was attempted with the high-fidelity polymerase, the smear problem seemed to be exacerbated in all cases.

Both Nucl and Nucl2 resulted in the highest DNA concentration (ng/μL) for the largest amount of samples, and similarly gave elutions that produced the highest number of PCR amplicons using the ITS region (15 for each method). Qiag produced ten amplicons, Gdmk2 produced six, Gdmk produced three, Mobi produced one, and the remaining Epic and Epic2 elutions could not be amplified using PCR. In addition, no extraction method was able to produce DNA that would successfully amplify either JB or VR, the *Juniperus* cone and *Valeriana* root samples, respectively.

Due to the nature of the Epic and Epic2 extraction protocols, both methods yielded visibly dirty plant extracts. Therefore, 1:100 dilutions were used for both DNA concentration measurements and PCR amplification, as crude extracts as well as 1:10 and 1:20 dilutions had already been attempted unsuccessfully. Epic and Epic2 1:100 dilutions, along with a few Mobi extracts, had tinted, impure elutions that produced exceptionally high A_{260} measurements, as noted in Table 3. Overall, both Nucl and Nucl2 gave the best quantitative and qualitative results. In general, leaf samples, pills (which were shredded/pressed leaf materials) and bark worked better than whole plant stem materials. The only root material (*Valeriana*) and the cone material (*Juniperus*) did not work with any kit, any primer combination or PCR protocol. We think the thick root of *Valeriana* might have been dried with excessive heat degrading DNA to a level not suitable for extraction. In case of the *Juniperus* cone we think high concentrations of terpenoids might interfere with the extraction protocol. We have been working with fresh *Juniperus* materials for many years and generally find that leaf extraction is possible but cone materials do not amplify. Therefore, we base our conclusion in this article only on plant material that we were able to extract and amplify with at least one of the extraction kits.

Correlation Between PCR and Absorbance Ratio

Mean absorbance ratio (A_{260}/A_{280}) measurements were evaluated, and our results indicate that the majority ($n = 101$) of eluted samples are contaminated with high levels of protein, whereas a smaller number of samples ($n = 27$) were found with RNA contaminations (Supplemental Table 1). The ratio of absorbance at A_{260}/A_{280} between 1.7 and 1.9 is generally used as a standard for determining a pure DNA sample. Anything below 1.7 indicates protein, and to a lesser extent latent phenol or carbohydrate, contamination within a sample, and above 1.9 indicates contamination with RNA. Using this standard, Gdmk had the highest number of pure extracts (12 total), followed by Nucl and Nucl2, both of which had eight, Gdmk2 had seven, Qiag and Mobi had six, and Epic and Epic2 which had only one. Correlation between A_{260}/A_{280} ratios and PCR amplifiability is shown in Fig. 1 which illustrates that purity of plant samples measured as absorbance ratio is a poor predictor of whether PCR will work or not if the typical range of 1.7–1.9 is used. However, our data suggest that samples with ratios below about 1.3 and above about 2.3 never amplified and probably do not have to be subjected to PCR testing.

Sequencing and Species Identification

Direct sequencing was performed on the products that amplified using both Nucleospin® plant II extraction methods. One nuclear and one chloroplast marker of interest was used, ITS and trnL-trnF, respectively, and resulting data was characterized taxonomically up to the genus level using BLAST. When we examined the sequence chromatograms, a few plant sample sequences were short and required excessive editing. With ITS, six of the 11 samples did sequence well enough so that we were able to confirm the identity of the reference sample up to the genus level. For trnL-trnF, we were able to confirm eight of the 11 samples. Some samples sequenced better for one marker and some other samples for the alternative marker, overall allowing us to identify all but one sample.

Table 3 Mean DNA concentration (ng/µL) ± SD and PCR results (using ITS region) per plant sample for a given extraction method

Abbreviation	Sample type[a]	Extraction method							
		Qiag	Nucl	Nucl2	Gdmk	Gdmk2	Epic[b]	Epic2[b]	Mobi
GB	Leaf	**55.93 ± 1.10**	**67.05 ± 1.49**	**72.85 ± 0.63**	21.35 ± 5.00	16.57 ± 4.82	93.42 ± 7.83[c]	64.85 ± 5.98[c]	23.84 ± 3.89
CH	Leaf	**40.45 ± 0.50**	**91.76 ± 6.67**	**62.42 ± 1.23**	21.96 ± 0.43	38.68 ± 2.22	18.18 ± 4.37	19.72 ± 0.35	104.31 ± 20.3[c]
TD	Leaf	12.64 ± 1.69	**25.04 ± 2.83**	24.10 ± 3.69	12.80 ± 0.51	15.23 ± 0.10	34.33 ± 2.31	31.88 ± 1.06	10.56 ± 0.42
PD	Leaf	129.02 ± 4.05	**184.41 ± 1.35**	**29.29 ± 1.63**	37.72 ± 1.27	40.12 ± 0.75	41.68 ± 4.64	54.35 ± 1.98	216.72 ± 1.65[c]
DM	Leaf	14.09 ± 1.74	**66.15 ± 2.81**	**33.81 ± 880**	10.61 ± 0.61	16.45 ± 0.45	28.52 ± 4.97	70.07 ± 1.86[c]	13.64 ± 1.90
DDL	Leaf	91.81 ± 2.01	**143.51 ± 0.51**	**114.33 ± 10.4**	94.39 ± 0.37	80.48 ± 4.03	03.29 ± 1.17	03.03 ± 1.41	68.71 ± 5.22[c]
RC	Leaf	**08.41 ± 0.44**	**19.12 ± 0.45**	**11.39 ± 1.53**	14.45 ± 0.37	18.44 ± 1.72	44.37 ± 5.40	36.26 ± 1.62	31.50 ± 3.50
AL	Leaf	**32.54 ± 3.56**	**20.47 ± 0.50**	**44.48 ± 0.60**	13.24 ± 1.55	09.75 ± 0.24	08.16 ± 1.32	03.98 ± 0.48	18.50 ± 0.41
TF	Leaf	75.29 ± 3.01	**123.17 ± 1.72**	**187.45 ± 3.55**	260.22 ± 5.41	**11.25 ± 0.30**	110.9 ± 10.9[c]	185.79 ± 9.78[c]	141.17 ± 9.85[c]
GS	Pill	**21.55 ± 0.77**	**27.05 ± 1.95**	**29.59 ± 3.20**	18.59 ± 1.97	14.53 ± 0.71	39.25 ± 2.97	23.98 ± 2.78	87.01 ± 39.53[c]
HQ	Pill	72.34 ± 1.67	**245.20 ± 31.6**	**48.32 ± 3.62**	**134.49 ± 10.7**	**50.60 ± 0.70**	53.52 ± 13.38	12.78 ± 2.30	66.19 ± 7.64
JB	Cone	13.94 ± 0.33	50.67 ± 31.58	17.49 ± 3.13	14.72 ± 0.32	12.56 ± 2.56	24.26 ± 3.59	44.44 ± 3.80	239.48 ± 43.7[c]
GB	Stem	**58.35 ± 1.39**	**65.06 ± 7.33**	**20.42 ± 0.43**	36.86 ± 0.59	**14.07 ± 0.18**	37.36 ± 1.52	36.60 ± 3.70	33.74 ± 6.27
CH	Stem	47.80 ± 1.21	**41.75 ± 1.81**	**45.16 ± 4.26**	14.94 ± 0.22	**09.61 ± 0.55**	19.34 ± 4.70	13.14 ± 2.79	123.05 ± 44.4[c]
TD	Stem	13.04 ± 4.84	11.87 ± 1.43	07.49 ± 0.95	15.30 ± 0.85	00.77 ± 0.27	25.18 ± 3.57	45.34 ± 2.82	15.28 ± 3.36
PD	Stem	61.55 ± 9.83	73.71 ± 1.16	32.53 ± 2.45	34.95 ± 0.53	19.46 ± 0.33	39.02 ± 4.77	31.64 ± 6.40	349.89 ± 35.3[c]
DM	Stem	22.67 ± 0.07	39.36 ± 15.24	05.20 ± 0.25	10.78 ± 1.22	07.78 ± 0.13	22.05 ± 2.19	43.09 ± 5.56	87.87 ± 2.14[c]
DDL	Stem	31.36 ± 0.27	91.40 ± 5.79	185.13 ± 27.0	61.28 ± 0.52	37.75 ± 0.74	07.20 ± 1.85	35.23 ± 5.52	116.60 ± 28.5[c]
RC	Stem	**16.97 ± 3.41**	**13.96 ± 1.02**	**10.52 ± 0.28**	**21.60 ± 0.69**	**06.52 ± 0.47**	20.40 ± 3.59	25.61 ± 3.62	04.08 ± 0.28
VR	Root	71.62 ± 40.90	18.33 ± 1.18	16.71 ± 6.89	14.27 ± 1.71	72.89 ± 6.53	26.08 ± 7.36	20.66 ± 2.40	28.25 ± 10.57
TD	Bark	03.29 ± 0.52	06.31 ± 0.21	**17.73 ± 8.21**	07.13 ± 0.46	**03.47 ± 0.45**	04.86 ± 0.68	06.99 ± 1.07	13.82 ± 7.26
RC	Bark	**07.56 ± 0.48**	**09.07 ± 2.89**	**12.58 ± 5.44**	**05.01 ± 0.07**	04.17 ± 1.00	05.19 ± 1.30	08.34 ± 1.82	**10.93 ± 0.50**

Bold PCR amplification successful

[a] Extraction done using 25 mg; eluted with 50 µL respective elution buffer

[b] Dilution 1:100

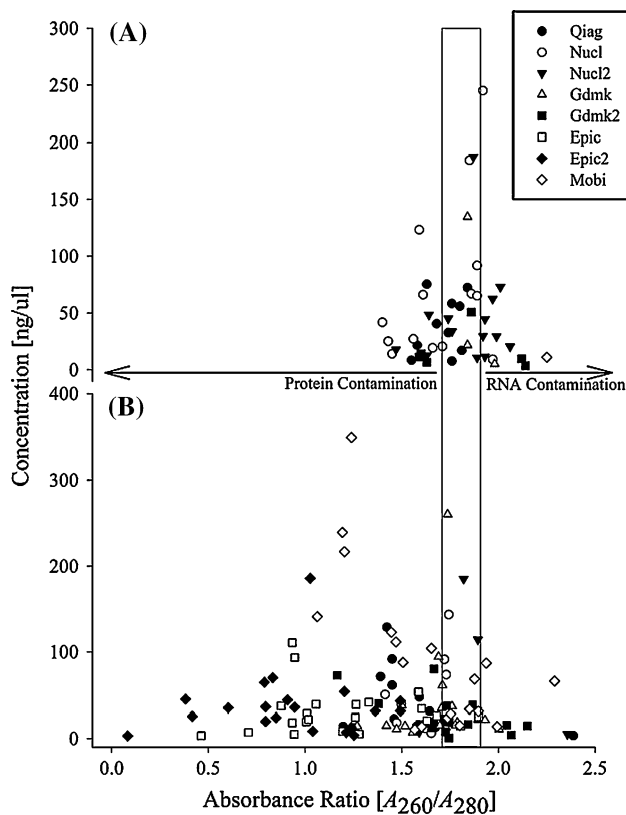[c] High DNA concentration measured probably due to impure elution

Fig. 1 Comparison of plant extracts: mean DNA concentrations (ng/μL) and absorbance ratios (A_{260}/A_{280}) against successful PCR amplification using the ITS region. Those that PCR amplified (**a**) and did not PCR amplify (**b**) are separated for clarity, and the boxed region indicates "pure" elution with an absorbance ratio between 1.7 and 1.9. Samples with absorbance ratios above 2.5 have been omitted due to their inability to amplify and for easier graphical representation. *Symbols* represent the mean value of at least three replicate measurements

Discussion

In order to identify plant supplements accurately and efficiently using DNA barcoding methods a reliable technique for extraction of high quality genomic DNA is needed. Although extensive studies and reviews have been published outlining different methods for secondary compound extraction from medicinal plants [31], DNA extraction methods are not as well characterized and no single method has established itself as commonly applicable to all plant types [12]. As such, commercially available kits are marketed as a fast option that provides a comprehensive and slightly more expensive method of selectively isolating DNA from various sources of materials [32]. In our study, both the Nucleospin® PL1 (Nucl) buffer, based on the widely established CTAB method [33], and the Nucleospin® PL2/PL3 (Nulc2) buffers, which use an SDS-based buffer along with potassium acetate, produced the best quality of DNA for PCR amplification. The underlying

distinction between both methods is the positive charge of CTAB and the negative charge of SDS as the lysis buffer, both of which have been highly successful and widely used in plant DNA extraction. Qiagen's DNeasy plant mini kit has been very successful in the extraction of quality DNA from ancient olive specimens [34], historic herbarium specimens [21, 22], and other herbal dietary supplements [20]. Since the Nucelospin® plant II kit was not used in these studies, it may also be superior in the extraction of DNA from herbarium specimens or other herbal supplements, but this has yet to be confirmed. The MO BIO PowerPlant® DNA isolation kit has also been extensively used for DNA extraction [35–37], though it had not been tested with medicinal plants. As for the other extraction methods, both Epicentre QuickExtract™ solutions and the IBI genomic DNA mini kit, relatively little research has been published at this time and to our knowledge none have been attempted in any other sort of comparative extraction study.

Spectrophotometric quantitation is the simplest way of estimating the concentration of DNA, and A_{260}/A_{280} absorbance ratio is commonly used to assess the purity of nucleic acid samples. The procedure, first described as a way to determine protein purity in the presence of nucleic acid contaminants [38], is now more frequently used for exactly the opposite, as a measure of nucleic acid purity. A_{260}/A_{280} absorbance ratios are determined by calculating the ratio of UV absorbance at 260 nm (for DNA) to the absorbance at 280 nm (for proteins), and are normally considered "pure" when the value lies between 1.7 and 1.9. Higher ratio values than 1.9 typically indicate RNA contamination, and lower ratios indicate the presence of proteins or other substances (such as polysaccharides and other secondary metabolites) not successfully removed during DNA extraction [12, 23]. Previously, it was suggested that the A_{260}/A_{280} ratio may not be an appropriate indicator of nucleic acid purity, as it was first used to detect nucleic acid contamination in protein preparations [39, 40]. Figure 1 shows that many of our extraction samples that are considered "pure" according to the typical ratio do not amplify via PCR. Likewise, many samples that are considered "impure" (either by RNA or protein/other contaminants, at least those with A_{260}/A_{280} ratios between about 1.3 and 2.3) do amplify via PCR. In most studies, protein and RNA contamination normally means that the DNA cannot be amplified [32]. For plant tissues, however, co-purified polysaccharides and other metabolites might interfere with absorbance readings. There is a possibility that PCR inhibitors, such as secondary compounds in woody stem structures or cones, contaminate the extract despite the fact that proteins and RNAs have been cleaned away, resulting in a PCR reaction failure but indicating a "clean" absorbance reading. On the other hand, absorbance

readings outside the "clean" range might be the result of compounds, other than proteins or RNA, which do not inhibit the PCR reaction, and would therefore result in PCR amplifiability. Our results certainly do not refute these two scenarios. As a result, we conclude that absorbance ratios are not a reliable method for global quality assessment of plant DNA. Our results show that if the ratio is between about 1.3 and 2.3 the absorbance measurements could not predict whether the samples would amplify or not. However, the ratio seems to be useful in removing poor samples below a ratio of 1.3 and above 2.3, reducing the PCR testing needs to samples within the range of 1.3–2.3 only. When testing a large number of samples this might considerably reduce the cost of overall testing. Additional data sets need to be evaluated whether this increased range compared to the traditionally used 1.7–1.9 range can always be used as a measure to exclude bad samples.

Using DNA extracted from both Nucleospin® plant II extraction methods, we were able to correctly identify, down to the genus level, eight out of the 11 plant samples that had originally amplified. It seems that, in our medicinal plant samples, the cpDNA was well preserved and therefore led to identification of more of our samples than the nrDNA. Unfortunately, in some cases both trnL-trnF and ITS produced short or messy sequences that gave ambiguous BLAST results. Most of the dirty sequences resembled mixtures or some form of contamination, and were therefore unusable for authentication and identification of those plants.

Conclusion

We were able to corroborate that DNA extraction methods do have a significant influence over the yield and quality of DNA extricable from medicinal plant samples. We tested a fraction of the large number of DNA extraction kits currently marketed, and it is clear that determination of a single kit able to extract DNA from all medicinal plants is far from being entirely resolved. In our study, the Nucleospin® plant II kit performed best for extraction of DNA from degraded samples, closely followed by the Qiagen DNeasy kit, providing amplifiable and sequenceable DNA for molecular identification studies of low quality medicinal plant materials. Furthermore, we concluded that DNA concentration measurements are not good predictors of plant DNA quality, and that PCR amplification, though often costly and tedious, is a more suitable indicator of DNA sequenceability. We also concluded that absorbance ratios in the extended range of 1.3–2.3 are not a good predictor of PCR amplifiability, but outside of this range might be useful to exclude poor samples below 1.3 and above 2.3 from the testing pool. Clearly more detailed studies including other plant groups are needed to confirm this novel finding.

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