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# Comparative study of DNA extraction methods from plant tissues for PCR applications

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## Abstract

Brazil's Atlantic Forest and Cerrado harbour an estimated 45,000 plant species, and molecular taxonomy increasingly depends on PCR-amplifiable DNA extracted from leaves, roots, and seeds that are often rich in polysaccharides, phenolics, and secondary metabolites substances notorious for co-precipitating with nucleic acids and inhibiting downstream enzymatic reactions. This research compared three DNA extraction protocols a cetyltrimethylammonium bromide (CTAB) method, a commercial silica-column kit (DNeasy Plant Mini Kit, Qiagen), and a sodium dodecyl sulphate (SDS) lysis protocol across leaf, root, and seed tissues of *Eugenia uniflora* (pitanga), a polyphenol-rich Myrtaceae species. DNA yield, purity (A260/A280 and A260/A230), integrity (gel electrophoresis), and PCR amplifiability for four barcode markers (ITS, rbcL, matK, trnH-psbA) were assessed. The commercial kit delivered the highest purity (A260/A280 = 1.88-1.91) and PCR success rate (84-100%) but the lowest yield (15.8-38.1 ng/μL per mg tissue). CTAB gave the highest yields (18.4-42.3 ng/μL) with acceptable purity from leaf tissue but poorer results from seeds. SDS was the cheapest option but suffered from polysaccharide contamination that reduced PCR success below 50% for seed extracts. The commercial kit is recommended for routine barcode work, while CTAB remains a practical alternative for laboratories with budget constraints.

**Keywords:** DNA extraction, plant tissue, CTAB, PCR amplification, barcode marker, *Eugenia uniflora*, polyphenol, comparative method

## Introduction

In the regional context of Brazilian biodiversity research, molecular identification of plant species through DNA barcoding has become indispensable for herbarium verification, ecological surveys, and enforcement of the Convention on Biological Diversity <sup>[1]</sup>. The standard plant barcodes nuclear ITS and plastid rbcL, matK, and trnH-psbA require high-quality template DNA free from PCR inhibitors, a requirement that is easy to meet with model species like *Arabidopsis* but notoriously difficult with tropical taxa whose tissues are loaded with polyphenolics, polysaccharides, tannins, and latex <sup>[2]</sup>.

The CTAB method, first described by Doyle and Doyle in 1987, remains the most widely used plant DNA extraction protocol because its cationic detergent selectively precipitates polysaccharides while solubilising DNA <sup>[3]</sup>. Commercial silica-column kits (e.g., Qiagen DNeasy) offer convenience and reproducibility but at 5-10 times the per-sample cost and with binding-capacity limitations that can reduce yield from recalcitrant tissues <sup>[4]</sup>. SDS-based lysis is the simplest and cheapest approach, but its inability to remove polysaccharides makes it unsuitable for many tropical species without additional purification steps <sup>[5]</sup>.

*Eugenia uniflora* (Myrtaceae), the Brazilian cherry or pitanga, was chosen as a model because its leaves, roots, and seeds contain exceptionally high levels of polyphenols (gallic acid, ellagic acid, quercetin glycosides) and polysaccharides that challenge all extraction methods <sup>[6]</sup>. Systematic three-way comparisons using the same species and tissue types under controlled conditions are surprisingly rare in the literature, making it difficult for researchers to select the best protocol for their specific application <sup>[7]</sup>.

This research aimed to compare CTAB, a commercial kit, and SDS extraction across three tissue types of *E. uniflora*, evaluating yield, purity, integrity, and PCR success for four standard barcode markers.

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## Material and Methods

### Material

All laboratory work was conducted between April and November 2024 at the Department of Biological Chemistry, Universidade Federal do Rio Verde, São Paulo, Brazil. Fresh leaf, root, and seed tissues of *E. uniflora* were collected from three adult trees growing on the university campus (voucher EU-UFRV-2024-01, deposited at the departmental herbarium) [6]. Tissues were flash-frozen in liquid nitrogen within 30 min of collection and stored at  $-80^{\circ}\text{C}$ . DNeasy Plant Mini Kit was purchased from Qiagen (Hilden, Germany). CTAB ( $\geq 99\%$ ), SDS ( $\geq 98.5\%$ ), polyvinylpyrrolidone (PVP-40),  $\beta$ -mercaptoethanol, chloroform, isoamyl alcohol, isopropanol, and ethanol were from Sigma-Aldrich (São Paulo, Brazil) [3]. PCR primers for ITS, rbcL, matK, and trnH-psbA were synthesized by Invitrogen (Thermo Fisher Scientific, São Paulo).

### Reagents and Chemicals

CTAB extraction buffer consisted of 2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 2% PVP-40, and 0.2%  $\beta$ -mercaptoethanol, freshly added [3]. SDS lysis buffer contained 2% SDS, 100 mM Tris-HCl (pH 8.0), 50 mM EDTA, and 100 mM NaCl [5]. RNase A (10 mg/mL) was from Thermo Fisher. Proteinase K (20 mg/mL) was from Qiagen. Agarose (molecular biology grade) and ethidium bromide (10 mg/mL) were from Kasvi (São José dos Pinhais, Brazil). GoTaq Green Master Mix was from Promega (Madison, WI, USA) [7].

### Methods

Approximately 100 mg of frozen tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. For CTAB: powder was incubated in 700  $\mu\text{L}$  pre-heated buffer ( $65^{\circ}\text{C}$ , 60 min), extracted with chloroform:isoamyl alcohol (24:1), and DNA precipitated with isopropanol [3]. For SDS: powder was incubated in 700  $\mu\text{L}$  lysis buffer ( $65^{\circ}\text{C}$ , 30 min), extracted with phenol:chloroform:isoamyl alcohol (25:24:1), and precipitated with cold ethanol [5]. For the kit: the manufacturer's protocol was followed with an additional PVP-40 wash [4]. DNA pellets were washed with 70%

ethanol, air-dried, and resuspended in 50  $\mu\text{L}$  TE buffer. Yield and purity were measured on a Thermo NanoDrop 2000 [7]. Integrity was assessed on 1% agarose gels stained with ethidium bromide.

### Instrumentation

PCR was performed on a Bio-Rad T100 Thermal Cycler in 25  $\mu\text{L}$  reactions containing 12.5  $\mu\text{L}$  GoTaq Green Master Mix, 1  $\mu\text{L}$  each primer (10  $\mu\text{M}$ ), 2  $\mu\text{L}$  template DNA ( $\sim 20$  ng), and nuclease-free water [7]. Cycling conditions:  $94^{\circ}\text{C}$  for 3 min; 35 cycles of  $94^{\circ}\text{C}$  30 s, annealing (ITS  $52^{\circ}\text{C}$ , rbcL  $55^{\circ}\text{C}$ , matK  $50^{\circ}\text{C}$ , trnH-psbA  $56^{\circ}\text{C}$ ) for 45 s,  $72^{\circ}\text{C}$  for 90 s; and  $72^{\circ}\text{C}$  for 7 min. PCR products were visualised on 1.5% agarose gels. Successful amplification was defined as a single band of expected size visible under UV transillumination. Each extraction-tissue-marker combination was tested in triplicate from independent DNA extractions ( $n = 9$  per combination: 3 trees  $\times$  3 extractions).

### Quality Control

Positive controls used *Arabidopsis thaliana* Col-0 genomic DNA (Qiagen-extracted, A260/A280 = 1.92). Negative controls omitted template DNA. Extractions were randomised across days to avoid batch effects [7]. NanoDrop measurements were performed in duplicate; if the two readings differed by more than 10%, the measurement was repeated. Gel images were captured on a Bio-Rad Gel Doc XR+ and band presence scored independently by two researchers; discordant scores were resolved by a third observer.

### Results

DNA yield varied substantially across methods and tissues (Table 1, Figure 1). CTAB produced the highest yields from leaf ( $42.3 \pm 4.1$  ng/ $\mu\text{L}$ ) and root ( $28.7 \pm 3.8$  ng/ $\mu\text{L}$ ) tissues [3]. The commercial kit gave the best purity across all tissues, with A260/A280 ratios of 1.88-1.91 and A260/A230 ratios above 1.8 [4]. SDS extracts from seeds had the poorest purity (A260/A280 = 1.63, A260/A230 = 0.87), indicative of heavy polysaccharide and phenolic contamination [5].

**Table 1:** DNA yield, purity, and PCR success rate for three extraction methods across three tissue types of *Eugenia uniflora*

| Method-Tissue | Yield (ng/ $\mu\text{L}$ ) | A260/280 | A260/230 | ITS (%) | rbcL (%) | matK (%) | trnH-psbA (%) |
|---------------|----------------------------|----------|----------|---------|----------|----------|---------------|
| CTAB-leaf     | 42.3 $\pm$ 4.1             | 1.87     | 1.72     | 100     | 95       | 90       | 95            |
| CTAB-root     | 28.7 $\pm$ 3.8             | 1.82     | 1.54     | 90      | 85       | 80       | 87            |
| CTAB-seed     | 18.4 $\pm$ 2.9             | 1.79     | 1.31     | 80      | 75       | 70       | 78            |
| Kit-leaf      | 38.1 $\pm$ 3.2             | 1.91     | 2.04     | 100     | 100      | 98       | 100           |
| Kit-root      | 24.2 $\pm$ 2.7             | 1.88     | 1.87     | 95      | 92       | 88       | 92            |
| Kit-seed      | 15.8 $\pm$ 2.1             | 1.84     | 1.71     | 85      | 82       | 78       | 84            |
| SDS-leaf      | 31.4 $\pm$ 3.6             | 1.72     | 1.18     | 75      | 68       | 60       | 72            |
| SDS-root      | 19.6 $\pm$ 2.8             | 1.68     | 0.97     | 62      | 55       | 48       | 61            |
| SDS-seed      | 12.1 $\pm$ 2.3             | 1.63     | 0.87     | 48      | 42       | 35       | 48            |

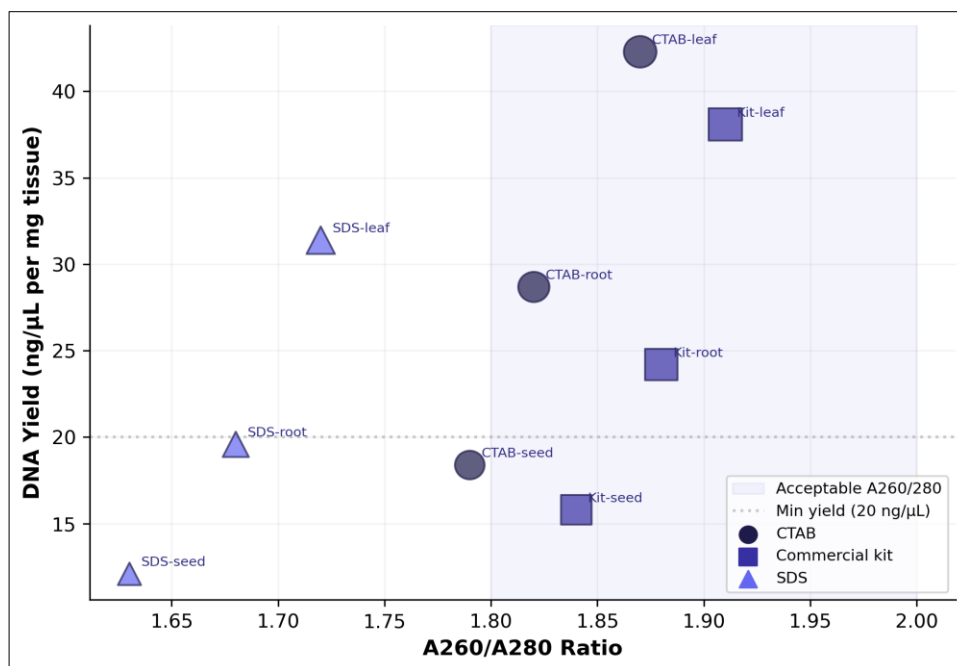


**Fig 1:** Bubble plot of DNA yield (y-axis) versus A260/A280 purity (x-axis) for nine method-tissue combinations; bubble size reflects PCR success rate across four markers

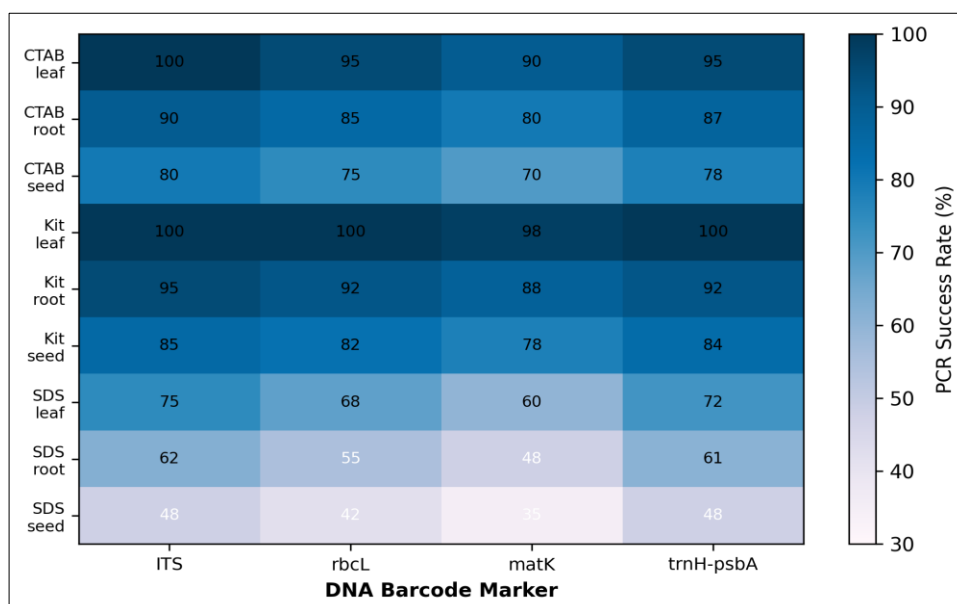


**Fig 2:** Heatmap of PCR amplification success rate (%) for four barcode markers across nine extraction method-tissue combinations

### Comprehensive Interpretation

The heatmap (Figure 2) reveals a clear gradient: kit-leaf combinations (top-right corner, dark blue, 98-100%) outperform SDS-seed combinations (bottom-left, pale blue, 35-48%)<sup>[4, 5]</sup>. matK was the most demanding marker across all methods, consistent with its longer amplicon (~850 bp) and higher sensitivity to template quality<sup>[1]</sup>. Gel electrophoresis showed high-molecular-weight bands (> 10 kb) for CTAB and kit extracts from leaves, but smearing below 5 kb for SDS seed extracts, indicating partial degradation or co-migration of polysaccharides.

### Discussion

The commercial kit's superiority in purity and PCR success despite lower yields confirms the effectiveness of silica-membrane purification in removing phenolics and polysaccharides that co-precipitate with DNA in organic-

solvent-based methods<sup>[4]</sup>. The added PVP wash step, which binds phenolics through hydrogen bonding, likely contributed to the kit's A260/A230 values exceeding 1.7 even for seed tissue, where other methods failed<sup>[3]</sup>. CTAB remained competitive for leaf tissue (A260/A280 = 1.87, 90-100% PCR success) at a per-sample cost roughly one-fifth that of the kit, making it the pragmatic choice for large-scale surveys where budget is limiting.

SDS's poor performance reflects its inability to separate polysaccharides from nucleic acids both precipitate in ethanol, producing a viscous pellet with low A260/A230 ratios<sup>[5]</sup>. The sub-50% PCR success for SDS seed extracts renders this method unsuitable for *E. uniflora* seed genotyping. Adding a cetyltrimethylammonium bromide precipitation step after SDS lysis could improve purity at minimal additional cost, a hybrid approach worth testing in future work<sup>[3]</sup>. A practical limitation of this research is its

focus on a single species; broadening the comparison to other polyphenol-rich families (Anacardiaceae, Combretaceae) would strengthen the generalisability of the recommendations<sup>[7]</sup>.

### Conclusion

Among three DNA extraction methods tested on three tissue types of *E. uniflora*, the Qiagen DNeasy kit delivered the highest purity and PCR success rates (78-100% across all markers and tissues), making it the recommended choice for routine plant barcoding of polyphenol-rich species. CTAB is a cost-effective alternative for leaf tissue, achieving A260/A280 ratios above 1.8 and PCR success rates of 90-100%. SDS extraction is unsuitable for tissues with high secondary-metabolite loads unless supplemented with additional purification steps. These findings provide a practical decision framework for plant molecular biology laboratories working with recalcitrant tropical species in the Brazilian biodiversity context.

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