Methodological article

Optimized protocols for efficient DNA extraction from leaves, stems, flowers, and seeds of *Cannabis sativa* L.

Optimización de protocolos para la extracción eficiente de ADN a partir de hojas, tallos, flores y semillas de *Cannabis sativa* L.

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Abstract

Cannabis *(Cannabis sativa* L.) is becoming an increasingly important crop due to its applications in medicine, industry, and agriculture. Its complex genetics, coupled with varying legal statuses and the demand for high-quality varieties, have made reliable methods essential for the identification, classification, and improvement of cannabis plants. DNA extraction is a key technique enabling such research, which serves as the foundation for various molecular biology applications. The objetive of this study was to evalaute the effciacy of four different DNA extraction methods, using fresh samples of leaves, stems, flowers and seeds of cannabis.DNA extraction was performed in triplicate following four methods: saline precipitation with sodium dodecyl sulfate (SDS), potassium acetate precipitation (KA), DNAzol™ reagent, and the commercial kit DNeasy Mini™ from Qiagen (KC, control). The concentration, purity, and integrity of the extracted DNA were evaluated using spectrophotometry and agarose gel electrophoresis. Furthermore, the efficiency of DNA amplification was assessed through polymerase chain reaction (PCR). The results indicated that the KA extraction method yielded samples with higher DNA concentrations, whereas the SDS method consistently yielded DNA of higher purity across all four tissue types. The use of the KC and SDS methodologies for DNA extraction facilitated the full PCR amplification of all four tissues. In contrast, the DNAzol method achieved 100% amplification solely in the leaf and stem samples. Consequently, the SDS and DNAzol methodologies offer viable alternatives to the KC method, proving to be equally effective while being less labour-intensive and more economical for large-scale DNA extractions.

Keywords: Molecular grade; Nucleic acids; Polymerase chain reaction; Purification methods.

Resumen

Cannabis sativa L. se ha convertido en un cultivo de creciente importancia debido a sus aplicaciones en la medicina, la industria y la agricultura. Dada su compleja estructura genética, las distintas regulaciones legales y la creciente demanda de variedades de alta calidad, resulta esencial disponer de métodos confiables para su identificación, clasificación y mejoramiento. La extracción de ADN constituye una técnica clave que permite desarrollar este tipo de investigaciones, sirviendo como base para múltiples aplicaciones en biología molecular. El objetivo de este estudio fue evaluar la eficacia de cuatro métodos de extracción de ADN en muestras frescas de hojas, tallos, flores y semillas de *Cannabis sativa*. Se utilizaron cuatro tipos de muestras frescas: hojas, tallos, flores y semillas. La extracción de ADN se realizó por triplicado mediante cuatro metodologías: precipitación salina con dodecil sulfato de sodio (SDS), precipitación con acetato de potasio (KA), reactivo DNAzol™ y el kit comercial DNeasy Mini™ de Qiagen (KC, control). La concentración, pureza e integridad del ADN se evaluaron mediante espectrofotometría y electroforesis en gel de agarosa. Además, la eficiencia de amplificación del ADN se analizó mediante reacción en cadena de la polimerasa (PCR). Los resultados indicaron que el método KA produjo muestras con mayores concentraciones de ADN, mientras que el método SDS presentó ADN de mayor pureza en los cuatro tipos de tejidos. El uso de KC y SDS permitió la amplificación completa por PCR en los cuatro tejidos analizados. En contraste, el método DNAzol alcanzó un 100 % de amplificación únicamente en las muestras de hojas y tallos. En consecuencia, los protocolos SDS y DNAzol representan alternativas viables al método KC, demostrando ser igualmente efectivas, menos laboriosas y más económicas para extracciones de ADN a gran escala.

Palabras clave: Ácidos nucleicos; Grado molecular; Métodos de purificación; Reacción en cadena de la polimerasa.

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Introduction

Cannabis *(Cannabis sativa* L.) is an annual, primarily dioecious, and sporadically monoecious herbaceous plant belonging to the Cannabaceae family (Hesami *et al.*, 2020). Cannabis is a highly valuable crop, with its primary applications lying in the medicinal and recreational fields. However, it also has several other potential uses, including in the production of cosmetics, textile fibers, clothing and footwear, biofuels, food, construction materials, paper, fertilizers, automotive parts and bioplastics (Nath, 2022; Palmieri *et al.*, 2019). In this context, the objective of agricultural production of cannabis is to obtain plants, including flowers, leaves, seeds, stems, and roots, with the appropiate characteristics for subsequent uses of the biomass (Amaducci *et al.*, 2008; Krebs *et al.*, 2021; Peng and Shahidi, 2021).

There are hundreds of cultivated cannabis varieties worldwide, differing in aroma, plant size, chemical composition, and cultivation practices, and adapted to diverse agroclimatic conditions (Palmieri *et al.*, 2019). These varieties, in turn, exhibit different yields, applications, and properties. For their identification, gas, liquid, or thin-layer chromatography techniques are typically employed, among other methods. In addition to the most well-known cannabinoids — Δ9-tetrahidrocannabinol (THC) and cannabidiol (CBD) — it is essential to consider other compounds that also exert a significant influence on the characteristics of the various cannabis varieties (Radwan *et al.*, 2017). The advent of molecular techniques has enabled the rapid genotyping of Cannabis varieties, contingent on the availability of high-quality DNA. In this context, polymerase chain reaction (PCR) has been employed for the molecular identification of cannabis materials, with the utilization of various commercial kits being a notable observation. However, the cost associated with these kits has been a limiting factor. Consequently, alternative DNA extraction protocols have been optimized for cannabis seed and resin samples, based on the use of phenol, chloroform and CTAB reagents (Coyle *et al.*, 2003; El Alaoui *et al.*, 2013; Soler *et al.*, 2016). However, these reagents have the potential to be toxic for operators. Furthermore, generally there is no standardized DNA extraction protocol for plant cells, and the method must be adjusted according to the specific characteristics of each case. In particular, the extraction of DNA from plant tissues may yield potential inhibitors for subsequent analysis, including polysaccharides, polyphenols, and other secondary metabolites such as alkaloids and flavonoids, which can impede nucleic acid processing (Paz *et al.*, 2023). Indeed, the unique biochemical profile of cannabis — particularly its high concentrations of cannabinoids and terpenes — can hinder the isolation of pure DNA, thereby complicating subsequent molecular analyses (Sahu *et al.*, 2012). The precipitation of secondary metabolites with nucleic acids can reduce the quality and yield of DNA (Aydin *et al.*, 2018). It is therefore imperative to optimize DNA extraction protocols in order to mitigate these challenges and ensure accurate genetic analysis. For these reasons, it is essential to obtain an adequate quantity and quality of DNA, as well as to ensure the elimination of these inhibitors. Consequently, in the present study, we propose to evaluate alternative DNA extraction protocols in comparison to the commercial kit in different tissues of *C. sativa*. The use of new DNA protocols is essential for advancing cannabis research, particularly in fields like genomics, breeding, biotechnology, pharmacology and medicine.

Materials y methods

Samples. The company CBD AGROCANN S.A., established in Yanda, Santiago del Estero, Argentina, provided samples of cannabis (*Cannabis sativa* L.) cv Pasionaria (Number 21642 of Instituto Nacional de Semillas (INASE) Argentina) tissue, including leaves, stems, flowers, and seeds. For each tissue type (leaves, stems, and flowers), samples consisted of pooled material from ten individual plants, whereas seed samples comprised ten seeds. All tissues were initially placed in sterile tubes containing silica gel to prevent moisture-related degradation during transport to the laboratory. Upon arrival, samples were immediately frozen in liquid nitrogen and stored at −80 °C until DNA extraction. The samples were processed using a grinder (Peabody, PE-MC9100, 220W) for 30 seconds, after which 100 mg of each tissue was weighed (Sartorius M-Power AZ-214). The samples were then analyzed in biological triplicate and technical duplicate.

DNA extraction. All experimental procedures were performed under cold conditions (on ice) to minimize DNA degradation. Furthermore, to ensure RNA removal and allow direct comparison with the commercial kit, all alternative DNA extraction protocols included an RNase A (Genbiotech) treatment performed according to the manufacturer's instructions. The following protocols were employed:

1- Saline precipitation method with sodium dodecyl sulfate (SDS)

Following the methodology outlined by Paz *et al.* (2023) 1 mL of lysis buffer I (10 mM Tris HCl, 25 mM EDTA, 5 mM $MgCl_2$ pH= 7.5) was added to 100 mg of ground sample and gently homogenized. Subsequently, the samples were subjected to centrifugation at 4,600 × *g* for 5 min, after which the supernatant was discarded. The pellet was then resuspended in 450 µL of lysis buffer I, and 20 µL of 10% SDS was added. This mixture was vortexed for 15 s to lyse the cells. Thereafter, 200 µL of 6 M NaCl was added, and the mixture was vortexed for a further 15 s. Centrifugation was performed at 14,000 × *g* for 5 min, after which 500 µL of the supernatant was transferred to another microcentrifuge tube. Isopropanol (500 µL) was added and gently homogenized, after which the mixture was centrifuged at 14,000 × *g* for 5 min. The supernatant was discarded. Two washes were performed with 700 µL of 70% ethanol, after which the mixture was centrifuged at 14,000 × *g* for 5 min. Finally, the DNA was resuspended in 100 µL of sterile water.

2- Potassium acetate method (KA)

In accordance with the methodology delineated by Paz *et al.* (2023), a 100 mg of ground sample was transferred to a tube containing 1000 µL of Buffer Lysis II (50 mM Tris HCl, 10 mM EDTA, 100 mM NaCl, 1% SDS, 10 mM β-mercaptoethanol, pH= 8). The solution was vortexed and incubated for 10 min at 65 °C. Subsequently, 200 µL of Buffer SN3 (comprising 11% glacial acetic acid and 5 M potassium acetate) was added and mixed by inversion. The mixture was then placed on ice for 20 min and subsequently centrifuged for 10 min at 12,000 × *g* at 4 °C. The supernatant was transferred to a new tube and precipitated with an equal volume of isopropanol. This was followed by centrifugation for 15 min at 12,000 × *g* at 4 °C, and two washes with 500 µL of 70% ethanol using a vortex or pipette to dissolve the pellet. Finally, the DNA was resuspended in 100 µL of sterile water.

3- DNAzol™ reagent method

It was performed following the protocol proposed by the manufacturer (Molecular Research Center, Inc.). Briefly, 100 mg of each processed tissue was homogenized with 1 mL of DNAzol reagent. Subsequently, a centrifugation was performed at 10,000 × *g* for 10 min at 4 °C. DNA was precipitated with 500 µL ethanol in a new tube by centrifugation at 5,000 × *g* for 5 min at 4 °C. Then the pellet of DNA was washed twice with 75% ethanol centrifuged at 1,000 × *g* for 2 min at 4 °C and resuspended in 100 µL ultrapure water.

4- Commercial kit DNeasy Mini kit, (QIAGEN)

Extraction was performed according to the manufacturer's instructions. Briefly, 400 µL of AP1 buffer and 4 µL of RNase A solution (100 mg/mL) were added to a tube containing 100 mg of ground sample and mixed vigorously. The mixture was then incubated at 65 °C for 10 min, mixing by inversion 2 or 3 times during the incubation. Then 130 µL of buffer P3 was added to the lysate and incubated in an ice bath for 5 min. The lysate was then centrifuged at 14,000 × *g* for 5 min and transferred to a QIAshredder Mini Spin column, followed by centrifugation at 14,000 × *g* for 2 min. Approximately 300 µL of the liquid obtained in the collection tube was transferred to a new tube and 450 µL of AW1 buffer was added. The liquid was then transferred to a new Mini spin DNeasy column and centrifuged at 7,500 × *g* for 1 min. To perform the wash, 500 µL of buffer AW2 was added and centrifuged for 1 min at 7,500 × *g*, and the column membrane was dried by further centrifugation for 2 min at 14,000 × *g*. Finally, the column was transferred to a new tube, 100 µL of buffer AE was added and after 5 min elution was performed by centrifugation at 10,000 × *g* for 1 min.

Concentration and purity. DNA concentration was determined spectrophotometrically by measuring absorbance at λ = 260 nm, employing the NanoDrop 2000c spectrophotometer (Thermofisher), assuming that an absorbance of 1.0 corresponds to 50 µg/mL of double-stranded DNA. The data on the concentration of DNA in each tissue were subjected to analysis

of variance (ANOVA). The mean values were compared using the least significant difference test (LSD, Fischer) at a 5% significance level, with the use of the InfoStat software (Di Rienzo *et al.*, 2016). Furthermore, the purity of the DNA samples was assessed using the absorbance ratios at $\lambda_{260}/\lambda_{280}$ and $\lambda_{260}/\lambda_{230}$, where A_{260}/A_{280} values ranging 1.8-2.0 and lower values indicates protein contamination; and A_{260}/A_{230} values between 2.0 and 2.2 indicate low levels of organic compounds and salt contamination.

DNA integrity. The quality of the DNA extractions was verified by electrophoresis on 1% agarose gels in TAE 1X buffer, with GelGreen DNA intercalating agent (Biotium), to ensure the integrity and purity of the extracted DNA. To this end, 5 µL of DNA purified using the previously described method from each sample was subjected to electrophoresis with sample buffer (TAE 1X, glycerol, and bromophenol blue). The electrophoretic separation was conducted for 30 min at a constant voltage of 100 V (Cleaver Scientific). DNA bands were observed under blue light on the BluePad transilluminator (Bio-Helix), using a 1 kb or 100 bp DNA ladder (Genbiotech) as a molecular weight marker.

DNA amplification. The reactions were conducted in a 10 µL reaction volume comprising 100 ng of template DNA, 0.5 µM of each primer (F: 5′ TCCTTATGTTCATTTGTAGGTCTTTCA3′ and R: 5′ GTGGTTTCTAATTTGTTATGTTTCTCGTT 3′) designed previously by Weck *et al.* (2021), 5 µL of iTaq Supermix (BioRad) and ultra-pure water. Primers target the hemp-specific spacer DNA sequence between the trnL 3′exon and the trnF gene in *Cannabis sativa* chloroplasts and the amplicon length is 122 base pairs (bp). The following program was employed: an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, hybridization at 58 °C for 30 s and extension at 60 °C for 2 min, conducted using the Veriti thermal cycler (Applied Biosystems). Subsequently, an extension was conducted at 60 °C for 7 min. The results were visualized on agarose gels using a 100 bp DNA ladder (Genbiotech) as the molecular weight marker, following the previously described methodology. The amplification rate was estimated based on the number of samples yielding positive amplification relative to the total number of samples analyzed.

Table 1. Comparison of DNA yield, purity, and amplification efficiency from different cannabis tissues using various extraction methods.

Tissue	Extraction Protocol	Concentration (ng/µL)	Purity		Amplification rate (%)
			$\lambda_{260}/\lambda_{280}$	$\lambda_{260}/\lambda_{230}$	
Seeds	KC	162 ± 13 a,b	1.8	1.8	100
	SDS	78 ± 64 a	2.0	2.2	100
	KA	2433 ±163 c	2.1	1.9	0
	DZ	448 ± 20 b	3.0	3.7	0
Leaves	KC	26 ± 10 a	1.2	1.4	100
	SDS	82 ± 45 a	2.0	1.6	100
	KA	791 ± 207 b	2.0	1.2	0
	DZ	134 ± 97 a	1.8	0.2	100
Stems	KC	18 ± 3 a	1.7	2.1	100
	SDS	13 ± 9 a	2.0	1.2	100
	KA	285 ± 172 a	1.8	1.3	50
	DZ	220 ± 261 a	2.0	0.3	100
Flowers	KC	60 ± 126 a,b	1.4	0.7	100
	SDS	2 ± 20 a	2.1	0.4	100
	KA	179 ± 52 c	1.5	0.7	0
	DZ	90 ± 19 b	1.6	0.1	0

KC: Commercial kit DNeasy Mini kit; SDS: Saline precipitation method with sodium dodecylsulfate; KA: Potassium acetate method and DZ: DNAzol method. Different letters indicate significant differences among DNA protocols in the same tissue using the Least Significant Difference (LSD) test at a p-value of 0.05.

Results

Mean DNA concentration values (ng/µL) obtained from cannabis tissues are shown in Table 1. The concentration of the methods ranged from 2 to 2433 ng/µL of DNA. Compared with the commercial kit, used as the control method (KC), the potassium acetate (KA) protocol yielded significantly higher DNA concentrations in seeds, leaves, and flowers ($p < 0.05$). In contrast, the SDS and DZ methods produced DNA concentrations comparable to or lower than those obtained with KC, depending on the tissue analyzed. Across all extraction protocols, seed samples exhibited higher DNA concentrations than vegetative tissues. Notably, the KA method produced DNA extraction with a significantly higher concentration than the other methods in seeds, leaves, and flowers.

Using the commercial kit as the control and considering acceptable purity values of 1.8–2.0 for the A_{260}/A_{280} ratio and 2.0–2.2 for the A_{260}/A_{230} ratio, the SDS and KA methods showed purity comparable to or slightly better than KC in seeds, particularly for the A_{260}/A_{230} ratio, whereas the DZ method deviated from the optimal range, indicating contamination by proteins or residual reagents. In leaf samples, none of the alternative protocols clearly improved DNA purity relative to KC; SDS and KA showed acceptable A_{260}/A_{280} values but reduced A_{260}/A_{230} ratios, while DZ exhibited marked deviations from the optimal range, suggesting substantial co-purification of organic compounds or salts. In stems, KC and SDS produced A_{260}/A_{280} ratios within the acceptable range and A_{260}/A_{230} values close to optimal. In flower tissues, all protocols, including KC, showed lower A_{260}/A_{230} ratios; however SDS maiange.

Figure 1 shows the electrophoretic profiles of genomic DNA extracted from different cannabis tissues. High–molecular weight DNA was observed as bands retained near the loading wells, indicating the presence of non-degraded genomic DNA in samples. However, the appearance of diffuse or smeared bands migrating toward the direction of electrophoresis suggests partial DNA degradation in specific lanes. Notably, lane 2 exhibited a higher degree of DNA degradation across tissues, as evidenced by smearing. In addition, partial degradation was observed in lane 1 of leaf samples and in lane 3 of seed samples. Despite these differences, intact high–molecular weight DNA was detected in all cases. In some samples, the DNA band was barely visible, which can be attributed to the low DNA concentration rather than to degradation, as supported by the spectrophotometric measurements.

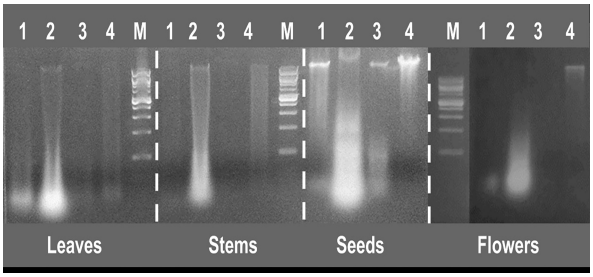

Figure 1. Evaluation of the integrity of DNA samples obtained using different extraction methods from various cannabis tissues. Agarose (1%) gel electrophoresis was conducted on DNA samples extracted from cannabis leaves, stems, seeds and flowers. The samples were prepared using 4 protocols: DNAzol (lanes 1), potassium acetate (lanes 2), precipitation with SDS (lanes 3) and a commercial kit (lanes 4). The 1 kb molecular weight marker (Genbiotech) is shown in lane M.

Figure 2 illustrates the outcomes of PCR product amplification. Extractions conducted using the KC method yielded amplified products in all four evaluated tissues (lanes 7 and 8). Similarly,

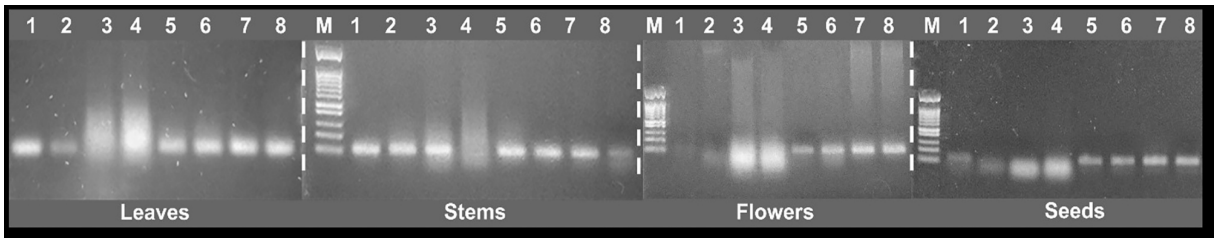

Figure 2. Assessment of amplification efficiency of DNA extracted from different cannabis tissues using multiple extraction methods. Agarose (1%) gel electrophoresis was conducted on PCR product from cannabis leaves, stems, flowers, and seeds using the four distinct protocols: DNAzol (lanes 1 and 2), potassium acetate (lanes 3 and 4), precipitation with SDS (lanes 5 and 6), and a commercial kit (lanes 7 and 8). Primers target the hemp-specific spacer DNA sequence between the trnL 3´exon and the trnF gene in *Cannabis sativa* chloroplasts and the amplicon length is 122 base pairs (bp). The 100 bp molecular weight marker (Genbiotech) was also included (lanes M).

the SDS method facilitated the amplification of all samples (lanes 5 and 6), while the DNAZol method amplified 100% of the cannabis leaf and stem samples (lanes 1 and 2). In PCR assays, specific amplification is characterized by the presence of a well-defined band of the expected size; therefore, diffuse bands observed in some tissues following PCR analysis, were not considered indicative of successful amplification, because they could result from degraded template DNA, nonspecific amplification, primer–dimer formation, or residual inhibitors co-purified during DNA extraction. In this sense, the KA extraction method only amplified 50% of the stem samples, with no amplification observed in the remaining tissues.

Table 2 summarizes the main differences among the DNA extraction protocols evaluated in terms of reagents, processing time, and cost per sample. All protocols shared similar homogenization steps; however, they differed in lysis composition, protein and lipid removal strategies, and DNA precipitation and washing procedures, which may account for the differences observed in DNA yield, purity, and amplification performance. The commercial kit involved fewer manual steps and the shortest processing time (32 min), but showed the highest cost per sample (USD 19.56). In contrast, the alternative protocols (SDS, KA and DZ) relied on commonly available laboratory reagents and significantly reduced costs, ranging from USD 2.35 to 3.61 per sample. Among these methods, the SDS protocol required a moderate processing time (45 min), whereas the potassium acetate method was the most time-consuming (80 min) due to additional incubation and centrifugation steps. The DNAzol protocol showed a processing time comparable to the commercial kit (35 min), although it involved the use of chaotropic agents.

Discussion

The findings of this study offer a comprehensive comparison of four DNA extraction methods applied to diverse cannabis tissues, including leaf, stem, seed, and flower. Each method demonstrated distinctive performance characteristics contingent on the tissue type, thereby elucidating pivotal insights into their efficiency. Notably, the effectiveness of the methods exhibited variability across tissues, underscoring the necessity of selecting an optimized extraction protocol tailored to specific cannabis tissues. Previously, methods for the extraction of DNA from samples of cannabis, including leaves, seeds, and resin, have been reported. These methods have typically employed the use of commercial kits or cetyltrimethylammonium bromide (CTAB) (Coyle *et al.*, 2003; El Alaoui *et al.*, 2013; Soler *et al.*, 2016, 2013). The principal benefit of utilizing commercial kits is that their protocols are straightforward, rapid and uncomplicated, rendering them eminently suitable for routine DNA extraction from cannabis tissues. However, commercial kits have the disadvantage of generating lower yields and a high cost per sample evaluated (Table 2). In this regard, Soler *et al.* (2013) and El Alaoui *et al.* (2013) demonstrated that CTAB-based protocols yielded higher results than commercial kits in seeds and resins. It should be noted that this methodology is more labour-intensive and time-consuming and represents a risk for operators since it uses reagents that can be toxic (Coyle *et al.*, 2003).

The present study evaluated several DNA extraction methods across four distinct cannabis tissue types, highlighting important trade-offs among DNA yield, purity, integrity, amplification efficiency, processing time, and cost. Leaves and stems represent particularly valuable tissues for molecular analyses, as they are available throughout most of the plant's phenological cycle, in contrast to flowers and seeds, which are restricted to specific developmental stages. Despite their relevance, most previous studies have focused on commercial kits or CTAB-based protocols, leaving alternative methodologies and tissue-specific performance largely unexplored (Soler *et al.*, 2016; Toth *et al.*, 2020; Weck *et al.*, 2021). To our knowledge, this study constitutes the first systematic evaluation of non-commercial DNA extraction protocols applied to multiple cannabis tissues.

Although the potassium acetate (KA) method consistently produced the highest DNA yields across tissues, this advantage was offset by reduced DNA purity, compromised integrity, and low amplification efficiency. Suboptimal A_{260}/A_{280} and A_{260}/A_{230} ratios indicate the co-purification of proteins and organic contaminants, which likely interfered with PCR performance. This suggests that DNA yield alone is not a reliable indicator of extract quality, particularly for downstream

Table 2. Comparison of reagents, time and costs of the different protocols studied.

Protocol	Commercial Kit	SDS Precipitation	Potassium Acetate	DNAzol
	QIAGEN	Chen *et al.* (2010)	Dellaporta *et al.* (1983)	Molecular Research Center
Homogenization	Grinding	Grinding	Grinding	Grinding
Cell lysis	400 µL AP1 Buffer Tris-HCl 10 mM EDTA 1 mM NaCl 100 mM PVP 1X DTT 10 mM 4 µL Rnasa A pH= 8 10 min at 65 °C	1mL Lysis I Buffer Tris-HCl 10 mM EDTA 25 mM $MgCl_2$ 5 mM 4 µL Rnasa A pH= 7.5 Centrifugation 4,600 × *g* 450 µL Lysis I Buffer	1 mL Lysis II Buffer Tris-HCl 50 mM EDTA 10 mM NaCl 100 mM SDS 1% β-mercaptoethanol 10 Mm 4 µL Rnasa A pH= 8 10 min at 65 °C	1 mL DNAzol reagent Guanidine thiocyanate Detergents 4 µL Rnasa A pH= 8.7
Protein and lipid separation	P3 Buffer Potassium Acetate 3 M Acetic Acid 5 min in ice bath Centrifugation 14,000 × *g* Mini Spin column (lilac) Centrifugation 14,000 × *g*	20 µL SDS 10% 200 µL NaCl 6 M Centrifug. 14,000 × *g*	SN3 Buffer Potassium Acetate 3 Acetic Acid 11% 20 min in ice bath Centrifug.12,000 × *g*	Centrifug. 10,000 × *g*
DNA precipitation	AW1 Buffer (Ethanol) Purification column Centrifugation 14,000 × *g*	Isopropanol Centrifug. 14,000 × *g*	Isopropanol Centrifug. 14,000 × *g*	500 µL ethanol Centrifugation 5,000 × *g*
Washings	Buffer AW2 (Ethanol 70%) Centrifugation 7,600 × *g*	2 with Ethanol 70% Centrifug. 14,000 × *g*	2 with Ethanol 70% Centrifug. 14,000 × *g*	2 with Ethanol 75% Centrifugation 1,000 × *g*
Redissolution	25 µL AE Buffer	25 µL sterile water	25 µL sterile water	25 µL sterile water
Time (min)	32	45	80	35
Cost per sample	19.56	2.35	3.61	2.50

*Cost estimates were calculated based on the reagents and disposable materials required for each protocol, using price quotations obtained on the same date from Argentine suppliers. This approach ensured a consistent and comparable cost assessment across all DNA extraction methods evaluated

downstream applications such as amplification-based assays. The diffuse DNA patterns observed in agarose gels further support the presence of partial degradation or shearing, which may result from prolonged processing time and harsh lysis conditions associated with the KA protocol.

In contrast, the SDS precipitation method showed a more balanced performance, yielding moderate

DNA concentrations while maintaining acceptable purity and consistently high amplification efficiency across tissues. These results indicate a clear association between DNA purity, structural integrity, and successful PCR amplification, reinforcing the importance of minimizing inhibitor carryover rather than maximizing yield. The DNAzol method, while rapid and cost-effective, showed variable performance depending on tissue type, with evidence of residual chaotropic agents reflected in low A_{260}/A_{230} ratios, particularly in flower samples, which likely contributed to PCR inhibition despite the presence of detectable genomic DNA.

Across all extraction protocols, flower tissues exhibited persistently low A_{260}/A_{230} ratios, indicating a higher susceptibility to contamination by secondary metabolites, polysaccharides, or residual solvents. This is consistent with the complex biochemical composition of cannabis inflorescences and suggests that additional purification steps, such as extended ethanol washes, reduced reagent volumes, or post-extraction clean-up columns, may be required regardless of the extraction method employed (Friar, 2005; Sahu *et al.*, 2012).

Several strategies could be implemented to improve DNA quality in the KA protocol, the one that yielded higher concentration, including reducing incubation times, performing all steps under strict cold conditions, incorporating polyvinylpyrrolidone (PVP) or additional antioxidant agents to limit phenolic oxidation, and adding an extra chloroform-based or silica-column purification step before DNA precipitation (Sahu *et al.*, 2012). Furthermore, amplification efficiency from KA-derived DNA could potentially be enhanced by template dilution, the use of PCR facilitators such as bovine serum albumin (BSA), or additional ethanol-based purification to remove inhibitory compounds (Farell and Alexandre, 2012; Samarakoon *et al.*, 2013).

Overall, the results demonstrate a clear association between DNA purity and integrity and PCR amplification efficiency, whereas high DNA yield without adequate purity compromises downstream performance. While commercial kits remain the fastest and most reliable option, their high cost limits routine application. Among the non-commercial alternatives evaluated, the SDS precipitation method emerged as the most cost-effective compromise, combining acceptable processing time, low cost, adequate DNA purity, and robust amplification efficiency, making it particularly suitable for large-scale or resource-limited genetic studies in cannabis.

The evaluation of alternative DNA extraction methods provides researchers with cost-effective and efficient protocols that can be widely adopted in laboratories, particularly those with limited budgets. The ability to extract amplifiable DNA from commonly available tissues such as leaves and stems throughout the biological cycle of cannabis ensures a consistent and reliable source of genetic material for various applications. This can lead to a better understanding of the genetic diversity within cannabis species and improve breeding programs aimed at developing varieties with desirable traits, such as higher cannabinoid content, disease resistance or improved growth characteristics.

Conclusions

Considering DNA yield, purity, amplification efficiency, processing time, and cost, the SDS-based saline precipitation method emerged as the most reliable and cost-effective protocol across all *Cannabis sativa* tissues evaluated, providing consistently amplifiable DNA from leaves, stems, flowers, and seeds. For leaf and stem samples, SDS offered the best balance between DNA quality and resource efficiency, while DNAzol represented a faster but less consistent alternative. In flower tissues, SDS was the only method that consistently supported PCR amplification despite moderate DNA yields, likely due to reduced co-extraction of inhibitory compounds. In seed samples, SDS again provided the most favorable compromise between yield, purity, and amplification success, whereas protocols yielding higher DNA concentrations did not translate into improved downstream performance. Given that leaves and stems are available throughout most of the cannabis life cycle, they constitute a consistent and accessible source of genetic material for molecular analyses. Overall, the results highlight the importance of selecting tissue- and method-specific extraction strategies to balance DNA quality, amplification efficiency, and resource efficiency.

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