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Original article

Influence of Extraction Method and Leaf Age on Genomic DNA Quality in Rosemary (*Rosmarinus officinalis L*)

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ABSTRACT

Rosmarinus officinalis L. *Salvia rosmarinus* L. (syn. *Rosmarinus officinalis* L.) is well known for its varied pharmacological qualities and rich phytochemical content. The secondary metabolites such as phenolic acids, flavonoids, terpenoids, and essential oils are largely responsible for its biological use. Furthermore, studies of genetic variety are essential for plant improvement programs, which lead to an increase its pharmaceutical value. However, the high concentration of polysaccharides, polyphenols, and other secondary metabolites in rosemary makes DNA extraction a difficult task. In order to separate genomic DNA from young and mature rosemary leaves, this study compared the effectiveness of CTAB and SDS extraction techniques. Spectrophotometric ratios (A260/A280 and A260/A230) were used to assess the quality and purity of DNA. The results of this study showed that in terms of DNA yield and purity, the CTAB approach performed noticeably better than the SDS method. High-quality DNA was extracted using CTAB from young leaves; A260/A280 values ranged from 1.84 to 1.95 (average = 1.91), indicating excellent purity. On the other hand, the purity of DNA isolated from old leaves using CTAB method clearly lower but still adequate (1.71–1.81). In both leaf types, the SDS method produced lower-quality DNA with lower purity ratios and overall efficiency. Finally, the CTAB method provides an efficient way to acquire high-quality genomic DNA appropriate for subsequent molecular applications, especially when used to young rosemary leaves.

Introduction

Salvia rosmarinus L. (synonym: Rosmarinus officinalis L.), a perennial evergreen aromatic shrub belonging to the family Lamiaceae, has considered one of the most interested studied species due to its long history of traditional use and its wide range of proved pharmacological properties [1–3]. The plant is well known as a medicinal plant due to its medicinal importance. *R. officinalis* has been acknowledged since ancient times as an important medicinal herb due to its exceptional therapeutic benefits. These biological effects are primarily linked to its varied range of bioactive components, such as phenolic compounds, flavonoids, and essential oils, which have shown considerable promise in preventing and treating numerous diseases. Whereas, its biological effects strongly support its traditional use in the heal and treatment of neurological, gastrointestinal, cardiovascular, inflammatory, and metabolic disorders [2,3]. These properties make rosemary extracts and its essential oils commonly used as natural antioxidants in food preservation, as well as active ingredients in pharmaceutical and cosmetic products [1,4].

On the other hand, Molecular studies are currently widely used to improve the production of medicinal plants to yield more active ingredients, given their increasing use in alternative medicine. However, for subsequent molecular studies and applications, such as PCR amplification, molecular marker analysis, sequencing, and genetic fingerprinting, DNA purity and concentration are consequently crucial prerequisites [5–6]. The reliability of molecular tests may be limited by compromised DNA quality, which can lead to PCR inhibition, poor replicability, and incorrect genetic interpretations [7,6]. Diversity analysis represents an academic approach to understanding the characteristics of various plants. In medicinal plants, such analyses are essential and have resulted in improved growth performance, enhanced stress tolerance, and higher levels of valuable autonomy [8,5]. Particularly, compounds such as rosmarinic acid, carnosic acid, carnosol, caffeic acid, unsolid acid, and components of essential oils like camphor are some of the most common bioactive substances isolated from rosemary [1,3,5].

Additionally, biotic stresses might lead to extensive yield losses and reduced active compound which, used in pharmaceutical sector [9]. These factors highly contribute to apply more genetic studies for investigating genetic variability in rosemary populations in order to improve rosemary cultivation and bioactive compound productivity. In fact, genetic engineering offers a practical way to enhance the growth, development, and ideal growing conditions of wild medicinal plants through genetic research and comprehension of their genetic composition, given the substantial influence of harsh environmental conditions on their growth, which contributes to their disrupted and unstable growth and productivity. The extraction of genomic DNA from rosemary is still technically difficult, despite its significance. High concentrations of polysaccharides, polyphenols, and other secondary compounds that obstruct DNA isolation processes are present in rosemary tissues, as they are in many aromatic and therapeutic plants [5,10,11]. The most common contaminants in plant DNA extracts, according to research, are polysaccharides, which often reduce DNA quantity and quality and make DNA pellets sticky and difficult to handle [12]. Moreover, inhibitor compounds such as polyphenolic compounds are easily oxidized during extraction and irreversibly binds

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to DNA, leading to degradation and reduced purity [13,11]. As a result, conventional DNA extraction protocols often fail to yield DNA with satiable quality when applied to rosemary tissues [5,11]. Typical plant DNA isolation methods require effective breakdown of the cell wall, which is normally obtained using detergents such as CTAB or SDS [12]. Particularly there are, several methods are used and tested for purpose of obtaining high quality plant DNA. Furthermore, most of those methods modified and improved to obtain high yield and high quality DNA whereas; among those methods, CTAB cetyltrimethylammonium bromide based methods are commonly used as the as the most effective for deterging and removing inhibitor compound such as polysaccharides and polyphenols [12]. Consequently, systematic comparative evaluation and optimization of CTAB- and SDS-based extraction methods are essential for establishing reliable DNA isolation protocols suitable for rosemary molecular studies [14,12].

Additionally, in order to successfully removing secondary metabolites and contaminants that have been shown to impair DNA quality, this work attempts to optimize important extraction parameters, such as incubation time, chemical concentrations, and detergi ng processes [5,14,15,12]. In this context, several previous studies investigated the performance of CTAB and SDS DNA extraction methods in various plant tissues, aiming to identify the most efficient protocol for obtaining high-quality genomic DNA suitable for genomic polymorphism analysis. Number of studies have evaluated DNA extraction methods (CTAB and SDS) in medicinal plants rich in secondary metabolites such as phenols and terpenes, with particular focus on DNA purity and yield molecular techniques conducted a comparative study between CTAB and SDS protocols reported that the CTAB-based method produced DNA with higher purity and fewer polysaccharide contaminants compared to SDS. Obtained plant DNA using CTAB methods produce acceptable purity levels suitable for following molecular applications, whereas SDS-based extracts showed reduced purity in some cases [12].

In addition to that, DNA extracted using CTAB method from both fresh and dried medicinal plant leaves, and found that adding PVP and a high concentration of NaCl to the extraction buffer resulted in high DNA yield and purity. On the other hand, other studies indicated that SDS was effective in cell lysis, but it was less efficient in removing polysaccharides from plant tissue [1 6]. Collectively, these previous studies indicate that CTAB-based extraction protocols are generally superior to SDS-based methods in terms of DNA purity and consistency, particularly when applied to leaf tissues rich in polysaccharides and secondary metabo lites. However, the efficiency of each method may vary depending on plant species and tissue type. These findings provide a strong scientific basis for the present study, which aims to evaluate and optimize CTAB and SDS protocols for genomic DNA extraction from rosemary tissues for genetic polymorphism analysis.

Materials and Methods

Location of Experimentation

This study was carried out at the Libyan Biotechnology Research Center, Tripoli (Al-Fernaj), Libya. The experimental work focused on the extraction of high-quality genomic DNA from rosemary (*R. officinalis*) leaves at different maturity stages (fresh and old), employing various DNA extraction methods.

Plant Material

Leaves of rosemary (*R. officinalis*) were collected for genomic DNA extraction and analysis. Fresh, healthy leaf samples were selected to ensure the integrity and quality of the extracted DNA. Sampling included both fresh (young) and old (mature) leaves in order to evaluate the efficiency of the applied DNA extraction methods across different physiological conditions. Rosemary leaves are known to contain high levels of secondary metabolites, including polyphenols and essential oils, which may interfere with DNA isolation and do wnstream molecular applications. Therefore, the inclusion of leaves at different maturity stages was intended to assess the ability of the extraction methods to yield high-quality and pure DNA despite the presence of such inhibitory compounds. All collected samples were handled carefully to minimize degradation and contamination prior DNA extraction. This step was primarily conducted to determine the most suitable DNA extraction protocol for obtaining high-quality genomic DNA from R. officinalis leaf tissues. Leaves of rosemary (*R. officinalis*) were used in this study for plant DNA extraction. Particularly Two types of leaves collected from the same plant were analyze d, including fresh (young) leaves and old leaves, in order to evaluate the efficiency of DNA extraction from different physiological stages of the plant tissue.

Chemicals

CTAB, SDS, sodium chloride (NaCl), Tris-HCl, EDTA, sodium hydroxide (NaOH), hydrochloric acid (HCl), β-mercaptoethanol, Proteinase K, chloroform: isoamyl alcohol (24:1), ice-cold absolute ethanol, ice-cold 70% ethanol, isopropanol, TE buffer, RNase, ethidi um bromide, glycerol, bromophenol blue, and sterile distilled water.

Equipment and Instruments

All laboratory equipment and instruments used in this study were selected to ensure accurate sample preparation, efficient DN A extraction, and reliable analysis of DNA quality and quantity. The equipment included an analytical balance, spatula, mortar and pestle, and liquid nitrogen for sample preparation and homogenization. Eppendorf tubes were used for sample handling, while a centrif uge and vortex mixer facilitated sample processing and mixing. A thermoshaker and water baths (set at 37 °C and 55 °C) were utilized to maintain controlled incubation conditions during DNA extraction procedures. Micropipettes with appropriate pipette tips were used for precise liquid handling, and graduated cylinders and volumetric flasks were employed for solution preparation. DNA analysis was perfo rmed using an electrophoresis apparatus with a gel casting tray, while a microwave oven was used for agarose gel preparation. The concentration and purity of extracted DNA were measured using a NanoDrop spectrophotometer. Analytical balance, spatula, mortar and pestle, liquid nitrogen, Eppendorf tubes, centrifuge, vortex mixer, thermoshaker, water bath (37°C and 55°C), micropipett es and pipette tips, graduated cylinders, volumetric flasks, electrophoresis apparatus, gel casting tray, microwave oven, and NanoDrop spectrophotometer. Preparation of Stock Solutions (100 mL).

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DNA Isolation Buffers

DNA extraction typically involves several well-organized steps, including the preparation of extraction buffers. Each step in the process must be performed with care and attention to avoid degradation or loss of DNA. The DNA extraction buffers used in this experiment include detergents such as CTAB (cetyl trimethyl ammonium bromide) and SDS (sodium dodecyl sulfate), which help disrupt cell membranes. β-mercaptoethanol is used to denature proteins by breaking disulfide bonds and removing polyphenols. EDTA is added to chelate magnesium ions, which are necessary for DNA-degrading enzymes. Tris-HCl at pH 8.0 maintains buffer stability, while salts such as sodium chloride neutralize the negative charges on DNA. The DNA extraction process began with the preparation of stock solutions. Three main stock solutions were prepared first:

NaCl (5 M)
5 M NaCl was prepared by dissolving 29.2 g of NaCl in 100 ml of double-distilled water. (For 1 L of 5 M NaCl, 292.2 g would be required; however, the volume was reduced to 100 ml).

Tris (1 M, pH 8.0)
Dissolve 121 g of Tris in 1000 mL of double-distilled water, using 49 mL of concentrated HCl to adjust the pH to 8.0 (to maintain the acidity level suitable for genetic material). Since the resulting volume exceeds requirements, the volume is reduced to 50 mL (i.e., 121 g ÷ 20 = 6.05 g; 49 mL ÷ 20 = 2.45 ml).

EDTA (0.5 M)
Dissolve 186.1 g of EDTA in 1000 mL of double-distilled water, adding 20 g of NaOH and adjusting the pH to 8.0. Since the resulting volume is large, it is scaled down to 50 mL (i.e., 186.1 g / 20 = 9.305 g of EDTA and 20 g / 20 = 1 g of NaOH).

2% CTAB Extraction Buffer
5 M NaCl Solution
29.22 g of NaCl were dissolved in distilled water, and the final volume was adjusted to 100 mL.

1 M Tris-HCl Solution (pH 8.0)
1 g of Tris base were dissolved in distilled water. The pH was adjusted to 8.0 using HCl, and the volume was completed to 100 ml.

0.5 M EDTA Solution (pH 8.0)
61 g of EDTA were dissolved in distilled water. The pH was adjusted to 8.0 using NaOH, and the volume was completed to 100 ml.

Preparation of Extraction Buffers

CTAB Extraction Buffer (2%, 50 ml)

Reagent	Amount	Final Concentration
CTAB	1 g	2%
5 M NaCl	14 ml	1.4 M
0.5 M EDTA	2 ml	20 mM
1 M Tris-HCl	5 ml	100 mM
Distilled water	Up to 50 mL	

β-mercaptoethanol: 12 µl per 6 mL buffer
Proteinase K: 60 µl per 6 mL buffer

SDS Extraction Buffer (2%, 50 ml)

Reagent	Amount	Final Concentration
SDS	1 g	2%
5 M NaCl	14 ml	1.4 M
0.5 M EDTA	2 ml	20 mM
1 M Tris-HCl	5 ml	100 mM
Distilled water	Up to 50 mL	

β-mercaptoethanol: 12 µl per 6 mL buffer
Proteinase K: 60 µl per 6 mL buffer

Preparation of Other Buffers

TE Buffer (100 mL)

Reagent	Amount	Final Concentration
1 M Tris	1 ml	10 mM
0.5 M EDTA	0.2 ml	1 mM
Distilled water	Up to 100 mL	.

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10X TAE Buffer (1000 ml)

Reagent	Amount
Tris base	48.4 g
Glacial acetic acid	11.42 ml
0.5 M EDTA	20 ml
Distilled water	Up to 1000 mL

DNA Extraction Procedure

Approximately 70–100 mg of rosemary leaves were weighed for each sample. Liquid nitrogen was added, and the plant material was ground thoroughly using a mortar and pestle until a fine powder was obtained. The powdered tissue was transferred into Eppendorf tubes, and 600 µl of extraction buffer (CTAB or SDS) was added. Samples were mixed using a vortex mixer and incubated in a thermoshaker at 55°C for 60 minutes. After incubation, an equal volume of chloroform: isoamyl alcohol (24:1) was added. Samples were gently inverted and centrifuged at 13,000 rpm for 5 minutes at 24°C. The aqueous phase was transferred to a new tube. DNA was precipitated using ice-cold ethanol or isopropanol, washed with 70% ethanol, air-dried, and resuspended in 20–50 µl of TE buffer. RNase was added, and samples were incubated at 37°C for 30–60 minutes.

Estimation of DNA Purity and Integrity

Preparation of 0.7% Agarose Gel

0.49 g agarose was dissolved in 70 mL of 1X TAE buffer. The solution was heated until completely dissolved and allowed to cool. Ethidium bromide was added to obtain a final concentration of 0.5 µg/ml.

NanoDrop Spectrophotometer Analysis

DNA concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Scientific). The instrument was calibrated using 5 µl of TE buffer. Then 5 µl of each DNA sample was placed on the measurement pedestal. DNA purity was evaluated using the absorbance ratios A260/A280 and A260/A230.

General Notes

All samples were processed under identical experimental conditions. DNA extraction was performed using both CTAB and SDS methods for comparative evaluation.

Statistical Analysis

Statistical analysis was performed using R software. Data were expressed as mean ± standard deviation (SD). Differences between DNA extraction methods and leaf ages were analyzed using appropriate statistical tests, and p value $p < 0.05$ was considered statistically significant.

Results and Discussion

Efficient DNA extraction is crucial for studying plant genes and enhancing breeding practices, with methods varying by species and growth stage. Extracting DNA from leaves high in polysaccharides and polyphenols poses challenges. Pure DNA is essential for molecular applications like PCR and genetic analysis, as highlighted by (17). The CTAB method is especially effective for difficult plants, underscoring the need for careful selection of leaves and growth stages to ensure optimal DNA purity. This study assessed two DNA extraction methods using different plant leaves to identify the best approach for high-quality DNA.

Nanodrop Spectrophotometer analysis for DNA Purity Assessment Using A260/A280 Ratio

This study evaluated the purity of genomic DNA extracted from rosemary (*R. officinalis*) using absorbance ratios at 260/280 nm. A ratio close to 1.8 indicates DNA purity, while deviations suggest potential contamination by proteins or phenols. Most of the extracted DNA samples fell within the optimal purity range, demonstrating their good quality for testing purposes. However, some samples showed ratios outside this range, indicating the potential presence of impurities or extraction problems. The results of this study (Figure 1,2) deeply demonstrated the effect of plant leaves age and DNA extraction method on DNA purity. The results of this study for ratio of 260/280 proved that DNA extracted from young leaves yields high quality DNA compared with old leaves; this may due to low inhibitor compound content in young leaves. Moreover, the results proved also that the purity of plant DNA extracted from CTAB method was in higher levels of purity compared with DNA extracted using the SDS method, which proved the efficiency of CTAB method for obtaining high quality DNA.

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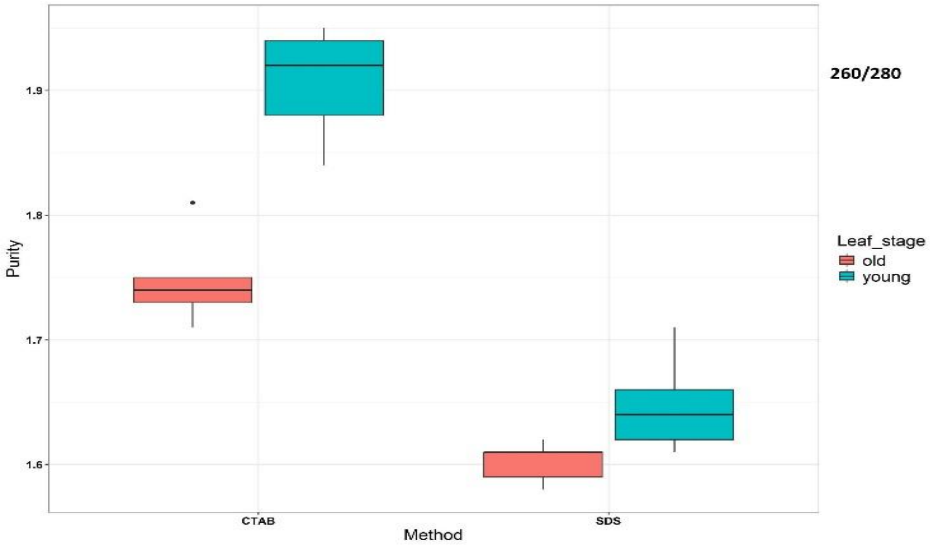

Figure 1. Comparison of DNA Purity (260/280 Ratio) using CTAB and SDS Methods across Different Leaf Stages

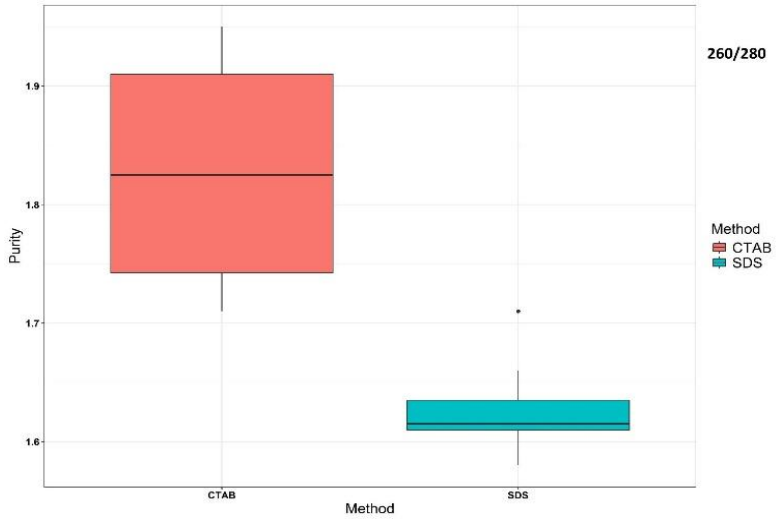

Figure 2. Comparison of DNA Purity (260/280 Ratio) between CTAB and SDS Extraction Methods

This study was conducted to determine the efficiency of the extraction method in obtaining pure genetic material by its ability to remove contaminants such as polysaccharides. The results of this study were analyzed using the CTAB and SDS methods, with each method applied to both young and older leaves. The A260/A280 absorbance ratio is widely used to evaluate DNA purity, particularly for detecting protein contamination. Pure double-stranded DNA typically exhibits values between 1.8 and 2.0, while lower values indicate the presence of proteins, phenolic compounds, or other contaminants (19–20).

CTAB Method

This study evaluates the effectiveness of genomic DNA extraction from rosemary (*R. officinalis*) leaves using cetyltrimethylammonium bromide (CTAB) at different maturity stages, focusing on young and mature leaves. The CTAB method is effective in removing inhibitors, such as polysaccharides and phenolic compounds, that affect DNA purity. Given that rosemary contains high levels of essential oils and phenolic compounds that vary with leaf age, this research aims to assess the effect of leaf maturity on the quantity and purity of extracted DNA and its suitability for molecular applications. Comparing young and mature leaves seeks to identify the optimal plant material for DNA extraction.

CTAB Method Using Young Leaves

CTAB methods largely used for plant DNA extraction with high quality, and many studies reported that CTAB DNA extraction methods are able to extract high quality DNA from plants, even those contain high amount of inhibitor compounds such as polysaccharides and polyphenols. All values fall within the optimal purity range, indicating high-quality DNA with minimal contamination. These findings are consistent with previous studies showing that younger plant tissues yield higher-quality DNA due to their lower content of polysaccharides and phenolic compounds (21,22). The A260/A280 purity ratios obtained from this study using CTAB method with young fresh rosemary (*R. officinalis*) leaves were 1.94, 1.84, 1.88, 1.92, and 1.95 (figure 1,2), with average of 1.91. The results, with respect to the 260/280 ratio, were very favorable, indicating that the CTAB method with young rosemary leaves was highly effective and that the purity of the extracted DNA was excellent. This proves the effectiveness of this method in obtaining pure DNA, which is attributed to

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CTAB's ability to remove and eliminate inhibitor compound and other substances. Furthermore, the accumulation of these inhibitors in the young leaves was minimal compared to the older leaves. The study affirms the effectiveness of the CTAB extraction method for obtaining high quality plant genomic DNA, particularly from young rosemary leaves. This success is attributed to a lower concentration of inhibitor compounds in younger leaves, which enhances DNA purity and extraction performance. The CTAB method surpassed all other tested extraction techniques, producing reduced DNA yield and quality from mature leaves and SDS-based methods. The A260/A280 ratio values indicated potential protein contamination or interfering substances, which can adversely affect DNA purity and its applications. Overall, the findings confirm CTAB's superiority in extracting DNA from plants, especially those rich in inhibitor compounds like polysaccharides and polyphenols. This obtained results were in full agreement with earlier studies, which reported that younger plant tissues normally yield DNA of higher quality due to reduced accumulation of polysaccharides (21,22). Our obtained results were in full agree with (12), who found that purity of DNA extracted from faba bean plant leaves at an age of 7 days was significantly higher than the other two treatments, which are 14 and 30 days. Furthermore, (23) found also that the optimized CTAB method able to extract high quality DNA from 100mg fresh leaf tissue of Yam, banana, and tomato leaves. In the same line, (24) reported the use of the CTAB method for rapid extraction of genomic DNA from small amount of plant leaves materials for the purpose of PCR.

CTAB Method using Old Leaves

Although a slight deviation from the ideal range suggests minimal protein contamination, these results demonstrate highly satisfactory DNA purity. This can be explained by the increased accumulation of structural compounds and secondary metabolites in mature leaves, which may hinder the efficiency of DNA extraction (25). The results demonstrate that the CTAB method performs efficiently, particularly when applied to young leaves, highlighting the importance of tissue selection in DNA extraction protocols. In contrast, DNA extracted from old (mature) leaves showed relatively acceptable purity values; however, some readings exhibited slight deviations below the optimal range. This suggests the presence of minor protein contamination or residual impurities. The reduced purity in mature leaves may be associated with the higher accumulation of secondary metabolites, such as polyphenols and structural compounds, which are known to interfere with DNA extraction efficiency (25). Overall, these findings indicate that the CTAB method is effective for genomic DNA extraction from rosemary leaves, with significantly better performance observed in young tissues compared to old leaves, highlighting the importance of tissue selection in DNA extraction protocols.

The results of the present study indicate that the CTAB extraction method applied to mature rosemary leaves was capable of isolating genomic DNA; however, with lower efficiency compared to its application on young leaves. This reduced performance may be attributed to the higher accumulation of complex polysaccharides and secondary metabolites in older tissues, which can interfere with DNA extraction and purity. Despite this limitation, the CTAB method with mature leaves still produced better results than all SDS-based extraction protocols evaluated in this study. The obtained A260/A280 ratios ranged from 1.71 to 1.81, indicating relatively acceptable DNA purity and suggesting effective removal of protein contaminants and other impurities. Nevertheless, the slightly lower and more variable ratios compared to those obtained from young leaves reflect reduced DNA quality. Overall, these findings demonstrate that while CTAB remains a reliable method for DNA extraction from mature rosemary leaves, its efficiency and DNA quality are inferior to those achieved with young plant material. The CTAB method demonstrated high efficiency in DNA extraction, yielding superior DNA quality and concertation, which aligns with findings of (26).

SDS Method

The sodium dodecyl sulfate (SDS) method, while effective for general DNA extraction due to its detergent properties, faces limitations in plant species abundant in secondary metabolites like rosemary. This study evaluates the SDS method's performance in extracting genomic DNA from rosemary leaves at different maturity stages, young and old, to assess its efficiency, purity, and yield. Rosemary's high content of essential oils and polyphenols, which can vary with leaf age, poses challenges for SDS extraction, as it is less effective than CTAB methods in removing polysaccharides and phenolic compounds that interfere with DNA purity and downstream applications. The research aims to determine how leaf maturity impacts the SDS method's effectiveness in metabolite-rich tissues, providing insights into its limitations and the influence of tissue characteristics on DNA extraction outcomes.

SDS Method Using Young Leaves

Although slightly improved compared to old leaves, the values remain below the optimal range, indicating persistent contamination. The relatively poor performance of the SDS method may be explained by its limited ability to remove the inhibitor compounds, which highly existed in plant cell, particularly in species rich in secondary metabolites (25,27).

The results of this study (Figure1,2) proved that SDS method was not able to produce high quality DNA, as reflected by the A260/A280 ratios. The recorded values for SDS with young rosemary leaves ranged from 1.61 to 1.71, with a mean of approximately 1.65, indicating suboptimal protein removal and lower DNA purity. In comparison to the CTAB method, SDS yielded poorer DNA quality. However, SDS method able to isolate plant DNA but with low purity levels, which performed relatively better with young leaves than with mature leaves, indicating that tissue age influences extraction efficiency even when using SDS.

SDS Method Using Old Leaves

These values are significantly below the acceptable threshold, indicating considerable protein contamination. This suggests that SDS method is less efficient in removing proteins during extraction, especially from mature plant tissues. The SDS extraction method with mature rosemary leaves showed low DNA purity based on the A260/A280 ratios. The recorded values ranged from 1.58 to 1.62, with a mean of approximately 1.60, indicating inadequate removal of protein contaminants. These results confirm that SDS is less effective in extracting high-quality DNA, particularly from mature leaves. Furthermore, the lower mean value compared to SDS with young leaves reflects a decline in extraction efficiency with increasing leaf age. Our obtained results were agreeing with (23), which proved that the use of SDS buffer was not able to isolate high quality plant DNA. The results of this study proved that the SDS method is less efficient in

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removing proteins during extraction, especially from mature plant tissues. Mature rosemary leaves' SDS-extracted DNA yielded A260/A280 ratios ranging from 1.58 to 1.62, with a mean value of roughly 1.60. These readings indicate substantial protein contamination and poor DNA quality, as they fall well below the ideal purity range of approximately 1.8. This decreased purity is a reflection of the SDS method's limited ability to effectively remove proteinaceous contaminants, especially in mature tissues. The accumulation of higher concentrations of secondary metabolites, phenolic compounds, and complex structural elements in older leaves can impede cell lysis and subsequent purification processes. Protein residues and other impurities are therefore more likely to remain in the finished extract. The CTAB method has proven to be superior to the SDS method, particularly when dealing with recalcitrant tissue, providing high-quality DNA suitable for other studies such as PCR applications (28). In the same vein, [18], who found that CTAB-based methods are extremely successful in extracting high-quality DNA from plants containing high levels of secondary metabolites. SDS method is considerably less effective than the CTAB method at handling this level of biochemical complexity, as evidenced by the latter's superior performance in comparable samples. Additionally, the decreased A260/A280 mean value in comparison to DNA extracted by using SDS extraction from young leaves suggests that leaf maturity has a detrimental effect on extraction efficiency, indicating the importance of both the extraction technique and the stage of plant growth. All things considered, these results indicate that SDS approach is not the best for separating high-quality DNA from mature rosemary.

Nanodrop NanoDrop Spectrophotometer analysis for DNA Purity Assessment Using A260/A230 Ratio

The A260/A230 ratio is widely employed as a marker of contamination caused by leftover extraction chemicals and organic inhibitor substances such phenolic compounds. Values between 2.0 and 2.2 are ideal for pure DNA. The existence of co-extracted impurities that could impede downstream molecular applications was typically indicated by discrepancies from this range. The A260/A230 ratio is an important indicator of contamination by organic compounds such as polysaccharides, phenolics, and residual extraction reagents. (29,30,31). The results of this study, as showed in figure (3,4) represented that the effect of CTAB and SDS on both young and old leaves, and results discussed as follows.

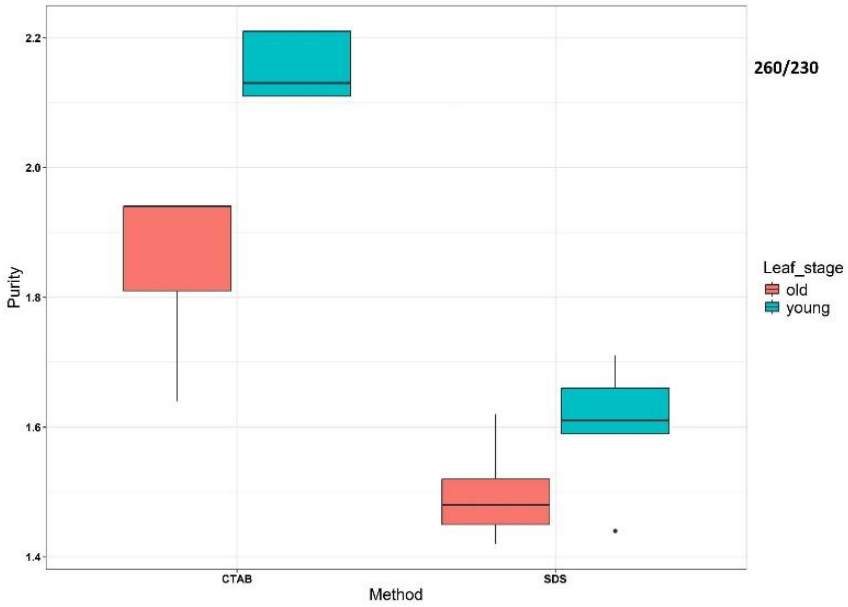

Figure 3. Comparison of DNA Purity (260/280 Ratio) using CTAB and SDS methods across different leaf stages

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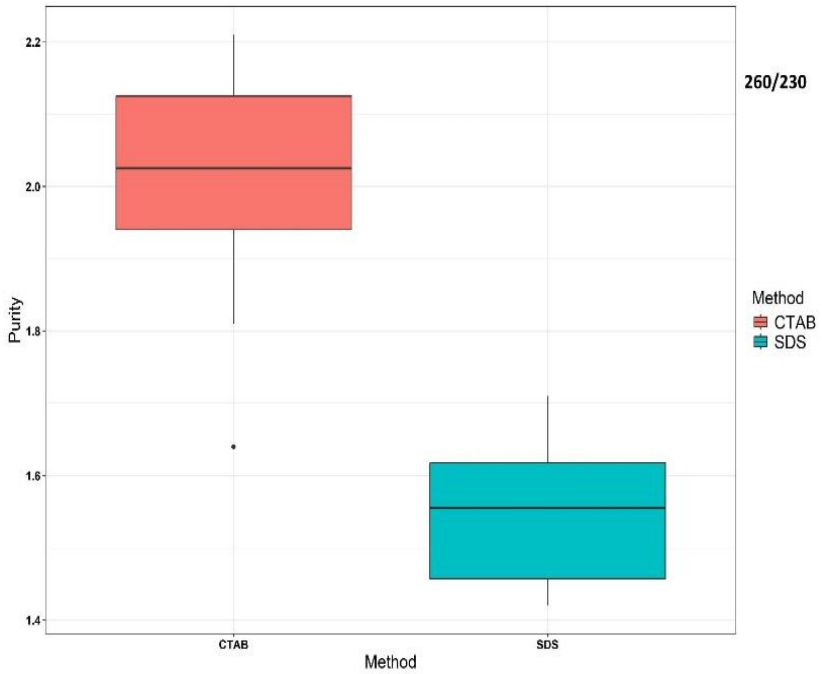

Figure 4. Comparison of DNA Purity (260/230 Ratio) between CTAB and SDS Extraction Methods

CTAB Methods

CTAB with Young Leaves

All values fall within the optimal range, demonstrating effective removal of polysaccharides and phenolic compounds. Similar results have been reported in studies highlighting the efficiency of CTAB in purifying DNA from plant tissues rich in secondary metabolites (32). These results (Figures 3 and 4) proved that the CTAB method yielded plant DNA with high purity under ration of 260/230 which, mean that CTAB was able to remove inhibitor compound perfectly. Whereas, the recorded results were CTAB with Young Leaves: (2.21, 2.21, 2.11, 2.13, 2.11) with an average of 2.15. However, Values between 2.0 and 2.2 are ideal for pure DNA. Our recorded results strongly prove that DNA purity isolated by the CTAB method from young leaves is high, regarding the 260/230 ratio. The high purity of the obtained DNA is not just due to the efficiency of CTAB but also because young leaves contain low levels of inhibitor compound. These findings confirm that CTAB method is highly effective in removing organic contaminants, particularly when combined with young leaf tissue.

CTAB with Old Leaves.

Old leaves contain high levels of polysaccharides, which normally exhibit the extraction of plant DNA. The results of this study confirmed what several previous studies had demonstrated. Furthermore, the results at the 260/230 average were identical to those at the 260/280 average, proving the accuracy of the work performed during the experiment. The results as represented in (Figure 3,4) also showed that using the extraction method with older leaves yielded lower purity for the extracted DNA compared to that obtained with newer leaves, a finding corroborated by previous results in this study. CTAB results were as follows: 1.64, 1.81, 1.94, 1.94, 1.94, with average of 1.85. These findings demonstrate that CTAB method for DNA extraction exhibits varying efficiency based on plant tissue age. Mature rosemary leaves showed moderate contamination (A260/A230 average = 1.85) due to the accumulation of secondary metabolites such as phenolics and polysaccharides. In contrast, DNA from young leaves achieved optimal purity (A260/A230 mean ≈ 2.15), attributed to fewer interfering compounds. The results underscore CTAB's effectiveness, particularly for species rich in secondary metabolites, and highlight the importance of sample selection in achieving higher DNA purity during extraction. These results proved the benefit of CTAB in the extraction of plant DNA, especially for plants that are high in secondary metabolites. Improved DNA purity is greatly aided by CTAB's detergent qualities and ability to remove polysaccharides from DNA. Furthermore, the better results shown with young leaves emphasize how crucial sample selection is to the outcome of extraction. Since young leaves have fewer interfering chemicals that would otherwise prevent the CTAB approach from isolating pure DNA, this observation supports the efficient removal of organic contaminants and reflects the reduced metabolic complexity of younger tissues. These results support the proven benefit of CTAB in the extraction of plant DNA, especially for species that are high in secondary metabolites. Improved DNA purity is greatly aided by CTAB's detergent qualities and capacity to remove polysaccharides from nucleic acids. Furthermore, the better results with young leaves emphasize how crucial sample selection is to the success of extraction.

SDS Methods

SDS with Young Leaves

In this study, SDS method was tested. It was used to compare it with CTAB method and determine its ability to extract pure DNA. However, SDS method is generally less efficient than CTAB method. The recorded results (figures 3 and 4) were: 1.42, 1.48, 1.45, 1.52, 1.62, with average of 1.5. Although slightly improved compared to old leaves, the values remain below acceptable levels. SDS method appears less effective in removing organic contaminants, which may negatively impact DNA quality and downstream applications (27).

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SDS with Old Leaves

These low values indicate substantial contamination by organic compounds, suggesting inefficient purification. In comparison, the SDS method exhibited consistently lower A260/A230 ratios for both young and mature leaves, indicating substantial contamination by organic compounds. The mean value for mature leaves (≈ 1.50) reflects poor removal of polysaccharides and phenolic compounds, likely due to the limited capacity of SDS to effectively separate these contaminants from DNA. Although a slight improvement was observed in young leaves (mean ≈ 1.60), the values remained below the acceptable threshold for pure DNA. This suggests that while reduced levels of secondary metabolites in younger tissues may partially enhance extraction efficiency, the inherent limitations of the SDS method still result in suboptimal purification. SDS Method Performance: Across both young and mature leaves, the SDS method consistently yielded lower A260/A230 ratios, signaling significant contamination by organic compounds. The mean ratio for mature leaves (≈ 1.50) reflects the limited capacity of SDS to effectively partition polysaccharides and polyphenols from the genomic DNA. While younger tissues—which typically harbor lower levels of secondary metabolites—showed a slight improvement (mean = 1.60), these values remained below the optimal threshold for DNA purity. These results suggest that the inherent limitations of the SDS approach preclude high-purity extraction, regardless of the tissue's developmental stage.

The deficiency of the SDS method compared to CTAB can be attributed to differences in extraction mechanisms. Unlike CTAB, SDS lacks strong selectivity in removing polysaccharides and phenolic compounds, which leads to their co-extraction with DNA. As a result, DNA obtained using SDS may be less suitable for sensitive downstream applications such as PCR and other molecular analyses. Differences in extraction methods may explain why SDS performs worse than CTAB. Unlike CTAB, SDS lacks the selectivity to avoid co-extracting polysaccharides and phenolic compounds along with DNA. Consequently, DNA obtained using SDS may be less suitable for sensitive downstream applications such as PCR, sequencing, or molecular marker analysis. However, the results demonstrate the clear superiority of the CTAB method over the SDS approach in mitigating organic contamination and yielding high-purity DNA, particularly from young rosemary leaves. Furthermore, the impact of tissue age across both protocols highlights the critical role of plant physiological characteristics in determining extraction success. These findings underscore that achieving optimal DNA quality requires a strategic selection of both the extraction chemistry and the developmental stage of the starting material. Overall, the results clearly demonstrate that both the extraction method and the type of plant tissue significantly influence DNA purity. The CTAB method showed superior performance compared to the SDS method in both A260/A280 and A260/A230 ratios. This can be attributed to the ability of CTAB to bind polysaccharides and facilitate their removal during the extraction process, as well as its efficiency in separating nucleic acids from proteins and other contaminants (5,23). In contrast, the SDS method lacks specificity in removing plant-derived contaminants, particularly in tissues rich in phenolic compounds and polysaccharides, leading to lower DNA purity. Moreover, young leaves consistently yielded higher purity DNA than old leaves. This can be explained by their lower levels of lignification and reduced accumulation of secondary metabolites, which simplifies cell lysis and improves DNA extraction efficiency (25).

Conclusion

In conclusion, it can be said that the results of this study have strongly demonstrated that using the CTAB method for extracting DNA from young leaves of Rosemary (*R. officinalis*) is the optimal solution for obtaining highly purified plant DNA and can be considered a reliable method for obtaining pure genetic material that can be successfully used in future studies such as PCR; this method is strongly recommended. The results also proved that the SDS method is less effective compared with the CTAB method, even with young leaves, which indicated that the SDS method is not recommended for obtaining high-quality DNA from Rosemary.

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