Journal of Forestry Research and Management. Vol. 16(2).64-71; 2019, ISSN 0189-8418

www.jfrm.org.ng

Comparative analysis of two methods of isolation of the genetic materials in four *Terminalia species*

Adekunle E. A

Biotechnology Section, Bioscience Department, Forestry Research Institute of Nigeria.
P.M.B 5054, Ibadan, Nigeria.
adekunleea@gmail.com, adekunle.ea@frin.gov.ng +2347062295251

ABSTRACT

Terminalia species are one of the most important forest trees whose use transverse pharmaceutical, chemical, and therapeutic industries. For proper molecular characterization and classification of these species, the first and the most frequent step requires the isolation of the genetic material, DeoxyRiboseNucleic Acid (DNA). This step is crucial as extraction of high quality DNA from these species will enhance good results from further analysis techniques and molecular biology experiments. Comparative investigation was carried out to determine which of the two methods, contemporary and kit DNA extraction will give the best quality of DNA from four *Terminalia species* (*T. ivorensis, T. superba T. catappa* and *T. mantaly*). Highest concentration of 852.4 ng/µl DNA was obtained from *T. ivorensis* using the contemporary DNA extraction method, while the highest purity 1.93 (260/280 ratio) was obtained from *T. superba* using kit extraction method. There was significant difference (P = 0.05) in the two extractions methods used on the concentration and purity of the nucleic acid material extracted from the tree species used for this study. The contemporary DNA extraction is a low-cost method with high yield of DNA from plant tissue. The current study provides insight to the use of simple and reliable method for the isolation of genomic DNA from fresh leaves of some forest trees.

Keywords: Concentration, Deoxyribosenucleic acid, Forest trees, Genomic, purity

Introduction

Terminalia is genus with approximately 200 species which is widely distributed across different types of vegetation worldwide (de Morais *et al*., 2012). Some of these species are deciduous shrubs or trees with large trunk and very tall height while others are evergreen used to provide shade as ornamental plant. The evergreen umbrella tree, *Terminalia mantaly*, grows with straight stem up to 20 m and layered branches with ability to resist drought (Orwa *et al*., 2009). Several reports have shown the trees in this family to contain antioxidants, tannins, flavonoids phenolic compounds, organic acids and its derivatives (Garcez *et al*., 2003; Latha and Daisy 2011; Pellati *et al*., 2013).

Due to their rich chemical compositions, *Terminalia spp* are known to be potent against diseases such as hypertension, inflammation, hyperlipidemia, gastric ulcers and wound healing (Li *et al.*, 2011; Latha and Daisy 2011; Nair *et al*., 2012; Mishra *et al*., 2013; Tom *et al*., 2014).

Extracts and compounds from various *Terminalia spp* have been reported to possess antimicrobial, antiviral, antiparasitic, anti-diabetic, hepato protective, nephro-protective and cardioprotective activities (Cheng *et al.*, 2002; Masoko and Eloff 2005; Singh *et al.*, 2008; Senthilkumar 2008; Kuete *et al.*, 2010;

Adetunji and Salawu 2010, Eesha *et al*., 2011; Fahmy *et al*., 2015).

Although, Terminalia species are multipurpose forest trees with innumerable importance, their taxonomy remains polemic due to inconsistent morphology and genetic diversity they exhibit across different regions (Deshmukh *et al.,* 2009). Understanding the molecular basis for the vast genetic diversity and morphological differences of these species could be a key to proper identification, genetic characterization, genetic improvement and conservation. To achieve this, the basic genetic information of this species must be accessed and processed using bioinformatic tools.

This study compared two methods of isolation of the genetic material in four *Terminalia* species and determined which of these methods is more reliable to obtain good qualities of the genetic material.

Materials and Methods

All four *Terminalia* species used for the study were obtained from the Forestry Research Institute of Nigeria, Ibadan, Nigeria on longitude 07^{o}23'18'' N to 07^{o}23'43''N and latitude 03^{o}51'20''E to 03^{o}23'43''E. Fresh leaves of *T. ivorensis*, *T. superb* and *T catappa* were obtained from the Central Nursery while *T. mantaly* were obtained from the Ornamental Nursery. These samples were collected with separately into pre-labelled tubes and transferred on ice to the Biotechnology section were this study was carried out.

Sample preparation

For each species, young leaves were randomly collected from three pots and labelled as replicates A, B and C. For each of the replicates, approximately 60 mg was weighed into a mortar with a spatula full of sterilized glass bead and ground with the pestle. The prepared sample was divided into two equal halves and each half was placed in two Eppendorf tubes. The two samples from each replicated were subjected to two different DNA extraction methods (Contemporary and kit extraction).

Contemporary DNA extraction from *Terminalia* species

Plant DNA isolation was carried out using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1990). The ground leave sample prepared above was re- suspended in 567 µl Tris-EDTA buffer (TE). 3 µl proteinase K was added to the mixture followed by the addition of 30 µl 10% SDS. This was incubated for 1 h at 37^{O}C on the block heater. 100 µl of 5 M NaCl was added and mixed well this was followed by the addition of 80 µl of CTAB/NaCl mixture. The content of the tube was mixed well and incubated at 65^{O}C for 10 min. 750 µl chloroform was added to the aliquot, mixed well and centrifuged for 5 min. The top layer was removed by pipetting into a new tube while trying to avoid interface. 450 µl isopropanol was added to the collected supernatant and shaken back and forth until a clump of DNA is visible. The tube was centrifuged at 10,000 rpm for 1 min to pellet the DNA. The liquid was carefully removed and the pelleted DNA was washed with 1000 µl 70% ethanol. The tube was centrifuged for 10 sec to re-pellet the DNA and the liquid was carefully removed. The tube was uncapped to air dry for about 10 min. the extracted DNA was re-suspended in 500 µl TE buffer.

Kit of DNA extraction from *Terminalia* species

The kit extraction of the four *Terminalia* species was done for Quick-DNA^{TM} Miniprep Plus Kit, Zymo Research. 95 µl of water was

added to the sample previously prepared by grinding. This was followed by addition of 95 µl Solid Tissue Buffer and 10 µl of Proteinase K. the content of the tube were mixed thoroughly by voltex mixer for 15 sec and incubated on the block heater at 55 ^{o}C for 3 h. After incubation, samples were mixed thoroughly and centrifuged at 10,000 rpm for 1 min. The supernatants were collected into fresh tubes and 400 µl of Genomic Binding Buffer was added to the samples and mixed thoroughly. The mixture was transferred into Zymo-Spin IIC-XL Column in a collection tube and centrifuged at 10,000 rpm for 1 min. The flow though was discarded and 400 µl of DNA Pre-Wash Buffer was added to the spin column and centrifuged at 10,000 rpm. 700 µl g-DNA Wash Buffer was added to the spin column and centrifuged at 10,000 rpm and this step was repeated with 200 µl of g-DNA Wash Buffer. The spin column was transferred into a clean micro centrifuge tube and 50 µl ofn DNA elution Buffer was added and incubated at room temperature for 5 min. Thereafter, the DNA was eluted by centrifuging the tube at 10,000 rpm for 1 min.

Quantification of the extracted DNA from *Terminalia species*

Quantification of DNA concentration and purity of the samples were measured using NanoDrop® 2000 spectrophotometer. The ratio of 260/280 absorbance was used to assess the purity of DNA with ratios ˜ 1.8 being accepted as pure.

DNA Electrophoresis of the extracted DNA from *Terminalia species*

The extracted DNA was subjected to gel electrophoresis to determine the quality and integrity of the DNA. 1.0% agarose gel was prepared by weighing 1 g of agarose into 100 ml TAE buffer solution. The solution was placed in the microwave for heating and proper melting. The hot gel was allowed to cool down to ambient temperature before the addition and thorough mixing of 5 ul of Ethidium bromide (EB). The mixture was poured into the gel-cast with the combs that forms the wells. Gel was allowed to solidify and the DNA marker and the genomic DNA were loaded as follows. 5µl of loading buffer and 5 µl of the extracted DNA were mixed gently and loaded into the wells. The electrophoresis was programmed at 80V, 40A, 8 W and 60 min. The gel was visualized under UV light and the photograph of the result was taken.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using GenStat package (Edition 4) while significantly different means were separated using Duncan Multiple Range Test (DMRT).

Results and Discussion

Basically, the four major steps involved in DNA extraction are cell lysis, precipitation, washing and re-suspension. The amount of DNA obtained from a particular experiment depends on a number of factors such as type of tissue involved and the particular isolation method used (Holden *et al.*, 2009). The concentration and purity of the Nucleic acid extracted from *Terminalia* spp are presented in Table 1. These values show the quality and the quantity of DNA extracted in the samples. The statistical analysis of these results show that there was significant difference (P = 0.05) in the two extraction methods used on the concentration and purity of the nucleic acid material extracted from the tree species used for this work (Table 1, Fig. 1). Generally, the quantity and quality of DNA extracted in plant samples are affected by small changes in the pH, presence of protein, phenol, lignin or other contaminants that absorb strongly at or

near 280 nm (Kotchoni *et al.*, 2011). Overall, results show that the contemporary DNA extraction method was more efficient in obtaining high quantity of DNA with low purity. On the other hand, the use of kit for DNA extraction produced DNA with high purity but low quantity. One of the reasons for the low concentration obtained from kit extraction could be due to loss of genetic materials during the many purification steps as specified by the manufacturers. The reason for the variation within each group, where same method of extraction was used, could be: handling, variation in lignin and other chemical content of individual species (Sangwan *et al.*, 1998; Deshmukh *et al.*, 2009). The modified CTAB method employed for DNA extraction from *Terminalia* species was effective and gave good yield of the genetic material with low purity. Akada *et al.*, (2000) had earlier suggested that the purity of samples is of less importance for some rapid PCR-based applications. However, the presence of impurity may be of hindrance to further utilization sensitive molecular procedures such as digestion with restriction enzymes and cloning (Ahmad *et al.*, 2016). However, removal of the impurities could be done to obtain a pure DNA extract for further sensitive molecular biology techniques.

Table 1. Interactive effect of extraction methods and species on the concentration and purity of extracted DNA from Terminalia species

Factor		Concentration (ng/µl)	Purity
Extraction method	Species		
Contemporary	*T. ivorensis*	852.4 ± 120.9^{a}	1.12 ± 0.31^{e}
	T. superba	644.1 ± 63.5^{b}	1.18 ± 0.08^{e}
	T catappa	804.8 ± 20.9^{a}	1.49 ± 0.21^{f}
	T. mantaly	453.3 ± 107.2^{c}	1.04 ± 0.06^{e}
Kit	*T. ivorensis*	134.3 ± 28.8^{d}	1.85 ± 0.15^{g}
	T. superba	163.5 ± 9.9^{d}	1.93 ± 0.09^{h}
	T catappa	232.2 ± 22.4^{d}	1.76 ± 0.06^{g}
	T. mantaly	119.3 ± 9.5^{d}	1.87 ± 0.11^{g}
L.S.D @ p = 0.05			
Extraction method		54.8	0.14
Species		77.6	ns
Extraction method x Species		109.7	0.27

On the other hand, the species had significant effect (P = 0.05) on the concentration but not on the purity of the genomic DNA obtained from these forest trees (Table 1, Fig 2).The concentration of the Extracted genomic DNA from *T. catappa*. was significantly higher than recorded in other species. The order of concentration of extracted DNA obtained from the different species used for this experiments was *T. catappa* > *T. ivorensis* > *T. superba* > *T. mantaly*.

The results of the gel electrophoresis are shown in figure 3. Similar results obtained from this work had been previously reported from the isolation of genomic DNA from fresh leaves of *Terminalia arjuna* (Deshmukh *et al.*, 2007). The images show that the genomic DNA from these species has similar molecular weight. The clarity of the DNA

samples extracted by the kit methods may be due to absence of impurity. Although the amount of the DNA extracted by the kit method is not the highest in this study, its image on the gel showed better. This suggests that the quality and purity of extracted DNA play significant role in further molecular application (Khosravinia *et al.*, 2007).

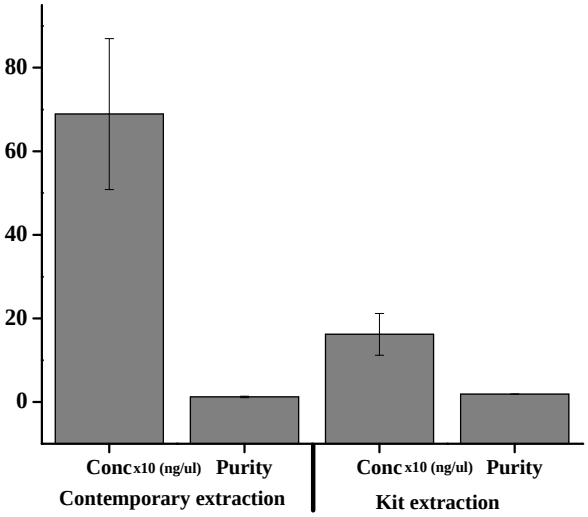


Figure 1. Effect of extraction method on the concentration and purity on of extracted DNA from *Terminalia* species

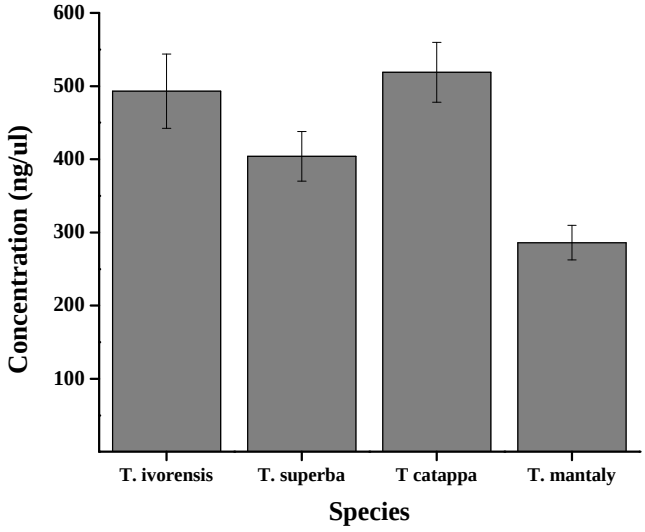
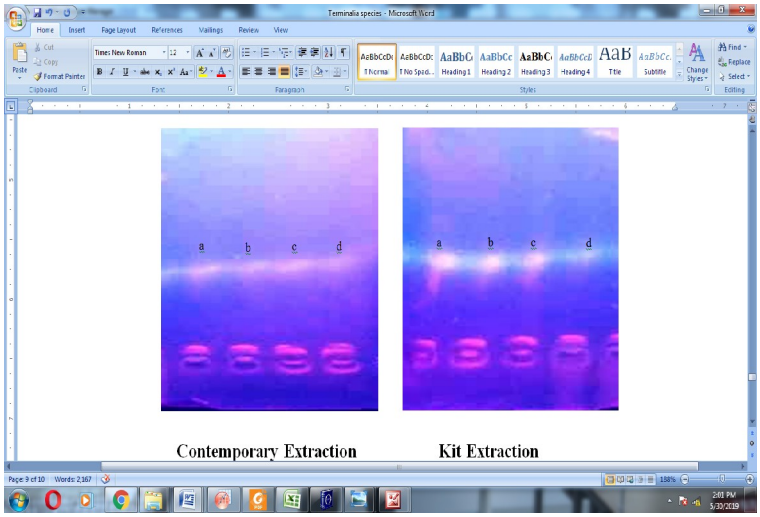


Figure 2. Effect of different species on the concentration of extracted DNA from *Terminalia* species



Contemporary Extraction **Kit Extraction**

Figure 3. Gel electrophoresis pictures (**a:** *T. ivorensis*, **b**: *T. superb*, **c:** *T. catappa*, **d**: *T. mantaly*)

Conclusion

The genetic information in organism is carried in the DNA molecules. Developing a suitable method for plant species DNA extraction and storage is of great importance to their use for molecular works such as polymerase chain reaction, characterization, sequencing, cloning, phylogeny tree construction, Southern blotting, genetic manipulation, generation of barcode and genetic germplasm conservation. One of the great impediments to molecular biology work is the expensive reagents and long procedures which sometimes does not guarantee result. This study provides a direction for researchers to be able to choose a desired method for obtaining nucleic acids for molecular work.

Reference

Adetunji V, Salawu O (2010). Effcacy of ethanolic leaf extracts of Carica papaya and Terminalia catappa as molluscicides against the snail intermediate hosts of schistosomiasis. Journal of Medicinal Plants Research 4: 2348-2352.

Ahmad J, Ibrahim M.M, Baif A, Qureshi M. I (2016). Standardization of DNA extraction from invasive alien weed *Parthenium hysterophorus* 15(22):1035-1040. DOI: 0.5897/AJB2016.15232

Cheng HY, Lin CC, Lin TC (2002). Antiherpes simplex virus type 2 activity of casuarinin from the bark of Terminalia arjuna Linn. Antiviral Res 55: 447-455.

De Morais Lima GR, de Sales IR, Caldas Filho MR, de Jesus NZ, de Sousa Falcão H, Barbosa-Filho JM, Cabral AG, Souto AL, Tavares JF, Batista LM (2012) Bioactivities of the genus Combretum (Combretaceae): a review. Molecules 17: 9142-9206.

Deshmukh V P, Thakare P V, Chaudhari U S, Gawande P A, Undal V S (2009). Assessment of Genetic Diversity among Terminalia Species Using RAPD Markers Global Journal of Biotechnology & Biochemistry 4 (2): 70-74

Deshmukh V P, Thakare P V, Chaudhari U S, Gawande P A. (2007). A simple method for isolation of genomic DNA from fresh and dry leaves of *Terminalia arjuna* (Roxb.)

Wight and Argot. Electronic Journal of Biotechnology. 10:3 http://www.ejbiotechnology.info/content/vol10/issue3/full/5/

Doyle, J.J. and Doyle J.L (1990). Isolation of plant DNA from fresh tissue. Focus, v.12, p.13-15,

Eesha BR, Mohanbabu AV, Meena KK, Babu S, Vijay M, Lalit M, Rajput R. (2011). Hepatoprotective activity of Terminalia paniculata against paracetamol induced hepatocellular damage in Wistar albino rats. Asian Pac J Trop Biomed 4: 466-469.

Fahmy NM, Al-Sayed E, Abdel-Daim MM, Karonen M, Singab A (2015). Protective effect of Terminalia muelleri against carbon tetrachloride-induced hepato– nephro toxicity in mice and characterization of its bioactive constituents. Pharm Biol 1-11.

Garcez FR, Garcez WS, Miguel DL, Serea AA, Prado FC (2003). Chemical constituents from Terminalia glabrescens. J Braz Chem Soc 14: 461-465

Holden M J, Haynes R. J, Rabb S A, Satija N, Yang K,. Blasic J R (2009). Factors Affecting Quantification of Total DNA by UV Spectroscopy and PicoGreen Fluorescence. J. Agric. Food Chem., 57, 7221–7226. DOI:10.1021/jf901165h

Khosravinia H, Narasimha H N, Parasad D, Pirany N (2007). Optimizing factors influencing DNA extraction from fresh whole avian blood African Journal of Biotechnology Vol. 6 (4), pp. 481-486

Kotchoni SO, Gachomo EW, Jimenez-Lopez JC (2011). A plant cocktail amenable for PCR-based genetic analysis in *Arabidopsis thaliana*. Mol. Biol. Rep. 38(8):5281-5284. Akada R, Murakane T, Nishizawa Y. 2000. DNA extraction method for screening yeast clones by PCR. Biotechniques 28:668–674.

Kuete V, Tabopda TK, Ngameni B, Nana F, Tshikalange TE, Nana F, Tshikalange TE, Ngadjui BT. (2010). Antimycobacterial, antibacterial and antifungal activities of Terminalia superb (Combretaceae). S Afr J Bot 76: 125-131.

Latha R, Daisy P (2011) Insulin-secretagogue, antihyperlipidemic and other protective effects of gallic acid isolated from Terminalia bellerica Roxb. In streptozotocin-induced diabetic rats. Chemico-Biological Interactions 189: 112-118.

Li K, Diao Y, Zhang H, Wang S, Zhang Z, Yu B, Huang S, Yang H (2011). Tannin extracts from immature fruits of Terminalia chebula Fructus Retz. promote cutaneous wound healing in rats. BMC Complement Altern Med 11: 86.

Masoko P., Eloff J.N. (2005).The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography. African Journal of Biotechnology Vol. 4 (12), pp. 1425-1431.

Mishra V, Agrawal M, Onasanwo SA, Madhur G, Rastogi P, Pandey HP, Palit G, Narender T (2013). Antisecretory and cyto-protective effects of chebulinic acid isolated from the fruits of Terminalia chebula on gastric ulcers. Phytomedicine 20: 506-511

Nair JJ, Aremu AO, Van Staden J (2012). Anti-inflammatory effects of Terminalia phanerophlebia (Combretaceae) and identifcation of the active constituent principles. S Afr J Bot 81: 79-80.

Orwa C, Mutua A , Kindt R , Jamnadass R, Simons A. (2009). Agroforestree Database:a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/)

Pellati F, Bruni R, Righi D, Grandini A, Tognolini M, Pio Prencipe F, Poli

F, Benvenuti S, Del Rio D, Rossi D (2013). Metabolite profling of polyphenols in a Terminalia chebula Retzius ayurvedic decoction and evaluation of its chemopreventive activity. J Ethnopharmacol 147: 277-285

Sangwan NS, Sangwan, RS, Kumar S (1998). Isolation of genomic DNA from the antimalarial plant *Artemisia annua*. Plant Mol. Biol.Rep. 16(4):365-365

Senthilkumar GP (2008). Biochemical studies on the effect of Terminalia chebula on the levels of glycoproteins in streptozotocin-induced experimental diabetes in rats. J Agric Food Chem 6: 105-115.

Singh G, Singh AT, Abraham A, Bhat B, Mukherjee A, Verma R, Agarwal SK, Jha S, Mukherjee R, Burman AC (2008) Protective effects of Terminalia arjuna against Doxorubicin-induced cardiotoxicity. J Ethnopharmacol 117: 123-129.

Tom ENL, Thernier CG, Martin H, Dimo T, Alvergnas M, Nappey M, Berthelot A, Demougeot C (2014). Treatment with an extract of Terminalia superba Engler & Diels decreases blood pressure and improves endothelial function in spontaneously hypertensive rats. J Ethnopharmacol 151: 372-379.