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Conservation of tree genetic resources of North-Eastern Lagos Nigeria

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This study investigates the rate of concurrent depletion on the remnant flora growing in the North-eastern part of Lagos, which lies in the South-western part of Nigeria. Tree species growing in this area are not spared from advancing civilization, which has resulted in inevitable loss of genetic resources. Hence, molecular technique is adopted in an effort to conserve the genetic resources of the tree species. Samples were collected at random from various sites in north eastern part of Lagos and identified. A total of 66 tree species was recorded. Genomic DNA was extracted from fresh leaves samples following modified cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol. The DNA when viewed on 1% agarose revealed bands of high molecular weight. Also, spectrophotometric check on the genomic DNA showed a good quality DNA samples with absorbance ratio of 1.7 to 1.8. The purified DNA was dissolved in buffer and stored at -80°C in the established DNA Bank at the University of Lagos, Akoka, Lagos, Nigeria. This can be used for further investigations including understanding genetic and evolutionary relationships between taxa, functional analysis of genes, comparative genomics, DNA barcoding and plant breeding amongst others.

Key words: Bio-conservation, cetyltrimethyl ammonium bromide (CTAB), Lagos, trees, genetic resources.

INTRODUCTION

Globally, the removal or destruction of significant areas of forest cover is moving apace, where every year an integral part of the nation's forest is destroyed through industrialization, urbanization, road construction, commercial agriculture amongst others (Okafor et al., 2009). These cumulative anthropogenic activities have resulted in a degraded environment with reduced biodiversity. The effects of these impacts are mostly evident in the developing countries, with highest rate of notoriety in Nigeria, where almost all the ancestral forest is lost with an alarming rate of disappearance of the remnant vegetation (Batta et al., 2013; Pelemo et al., 2011; Ladipo, 2010; FAO, 2010; Kabiru, 2008). This massive incessant deforestation is shaping climate and geography of several plants species.

Of all the species of plants exploited, the trees are mostly targeted (Elsiddig, 2003; Alamu and Agbeja, 2011) owing to their vast values ranging from economic, social to spiritual paraphernalia amongst others (Seth, 2002). In fact, several authors, including Okafor et al.

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(2009), Ihenyen (2009), Keay (1989) and Redhead (1971) lamented that out of about 565 species of trees existing in Nigeria, over 60 species are faced with extinction and various forms of risk. Despite studying trees for centuries and chronicling their vital importance to humans, there still exists lack of reliable information on where and when the indigenous trees are disappearing. In fact, the tree species growing in the study area, situated in the commercial and most urbanized state in Nigeria and which accommodates about 10% of the entire population of the country (Pelemo et al., 2011), are not spared from the above aforementioned threats. The influx of human population in search of white collar jobs in the study area has necessitated the development of several infrastructural facilities so as to provide comfort to the populace and this has led to the destruction of almost all the ancestral and proximate vegetation in the study. This is a socio-economic problem which seems to be too difficult to be controlled (Pelemo et al., 2011). As a result of massive loss of valuable plant species and adverse impact on environmental and socio-economic values, policies have been formulated for proper conservation and management of the genetic diversity through establishment of several nature reserves and botanical gardens amongst others in ensuring *in situ* conservation strategy. Despite these, it is very evident that *in situ* conservation is no longer effective given the global socio-economic problems aforementioned. The need to adopt molecular technique is appreciable given its advantage of providing a less laborious means for assigning known and unknown plant taxa. Molecular techniques such as DNA barcoding, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP) have recently been used for plant diversity studies and are referred to as easy way of conserving biodiversity (Arif et al., 2010; Pagnotta, 2009).

According to Arif et al. (2010), appropriate identification and characterization of plant materials are essential for the successful conservation of plant resources and to ensure their sustainable use. Hence, in other to conserve the trees species growing in the study area for posterity, attempts have been made by several researchers (Adekanmbi and Ogundipe, 2009; Shonubi and Okusanya, 2007; Orebamjo and Njoku, 1970) to list and highlight the existing species in the study area. Since effective conservation of plant genetic resources requires a complementary approach which makes use of both *ex situ* and *in situ* conservation methods to maximize the genetic diversity available for use, this study aims at conserving the tree genetic resources in northeastern Lagos using DNA banking techniques.

Description of study area

The study was conducted in the University of Lagos campus at Akoka, Yaba, Lagos, Nigeria. The area which is largely surrounded by the scenic view of the Lagos lagoon comprising a total of 802 acres (3.25 km^2) of land. It is located on longitude 3° 24' E and latitude 6° 30' N and on elevation of 40-90 m, which makes flooding difficult. The vegetation in this area is half cleared and developed and the remainder is represented by mangrove vegetation and most of the species recorded by Orebamjo and Njoku (1970) have diminished in number and density. It has an undulating terrain, half of which represents buildings, with various fresh water channels and creeks passing across at different location of this area. A large area of mangrove swamps, roughly 50%, dominates the vegetation. In the north and south east lies the brackish water lagoon which supports a typical terrestrial habitat, and experiences less human disturbance while in the south and south west lies the fresh water, where the soil is highly rich and supports a rich flora which is highly favored by the climate type much disturbed by human activities (Figure 1).

MATERIALS AND METHODS

Sample collection and identification

This study is based on extensive field surveys conducted in the North-eastern Lagos, Akoka Yaba, Lagos. A Global Position System (GPS) was used during the sampling period. For sample collection, the study area was divided into four sampling plots. Trees were enumerated in 50 x 20 m plots, whereas 0.5 x 2 m quadrat was used to study herbs and grasses. Samples were collected at random within each plot and identified. The assessment of native versus introduced status of the trees was done following Keay (1989) Keay et al. (1964) Hutchinson and Dalziel (1954) and Dalziel (1937). Voucher specimens of all plants have been collected and deposited at the University of Lagos Herbarium, Lagos, Nigeria. For DNA analysis, fresh young leaves, fruit, seed and flower samples were collected and silica-gel was added to the each sample in a zip lock bag and preserved in freezer for molecular analysis.

DNA extraction and purification

Genomic DNA was extracted from fresh leaf samples using the modified cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1989). The phenolic compounds were removed by passing the extracted DNA through the vacuum cleaner.

Gel electrophoresis

This involved quality check of the DNA samples on 1% agarose gel. The gel was run on 0.5x tris Borate EDTA (TBE) buffer at 75 V for 1 h 30 min. The gel was visualized by staining with 10 mg/ml ethidium bromide under ultra violet (UV) light and photographed with the gel documentation system (UVitec).

Quantification of DNA samples

This involved the determination of the concentration and

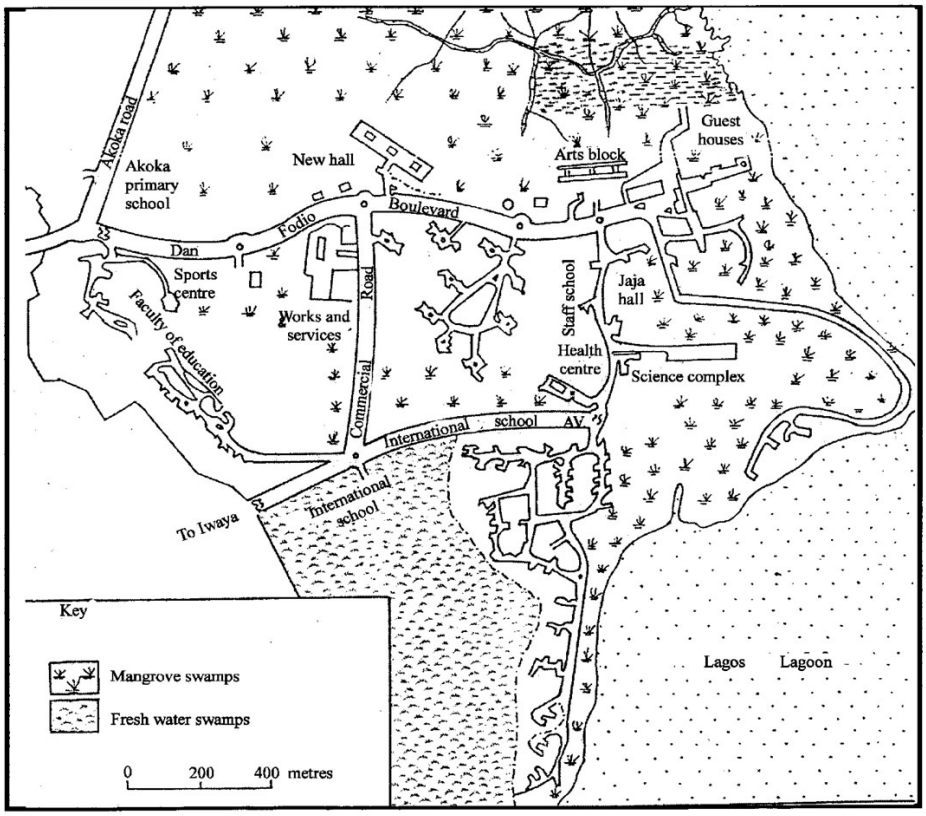

Figure 1. Map showing study area (fresh and mangrove swamp of University of Lagos). Source: Curled from Shonubi and Okusanya (2007).

relative absorbance of each DNA samples using an Eppendorf biophotometer. It was achieved by mixing 55 µL of sterile water with 2 µL of the DNA sample in a cuvette. The cuvette was then placed in an Eppendorf Biophotometer Plus, and readings were documented at 260 and 280 nm, respectively.

RESULTS

Our sampling showed a total of 66 woody tree species capable of attaining a maximum height of 12 m and girth of 60 cm (Table 1). They are made up of 58 genera which can be grouped into 27 families. Of these, only 42 species are indigenous to the environment (Plate 1) while 24 species are categorized as introduced (Plate 2). Most of the species encountered belong to the legume family Fabaceae (Plate 3) representing 20.89% of total number of species recorded. The vegetation of the study area is undulating, as some species were found existing in higher elevated areas, whereas some were found to exist in low areas.

All the samples yielded good quality DNA with high level of purity. The absorbance ratio of the extracted DNA samples as recorded from the spectrophotometric analysis ranging from 1.74 to 1.84 (Figure 2). Four of the samples however had absorbance ratio of > 1.9 *Casuarina equisetifolia, Callophyllum innophylum, Spondias mombin* and *Tabebuia rosea*) showing that impurities might be present in the samples. The concentration of the DNA samples obtained ranges from123 to 1670 ng/µl (Figure 3). All the samples have been deposited in the DNA bank at the University of Lagos, Akoka, Lagos, Nigeria for conservation purposes.

DISCUSSION

The tree species encountered in this study have a maximum height of 12 m and girth of 60 cm and this conforms to the definition of trees as stated by Redhead (1971). However, continuous existence of these species is doubtful owing to the fact that nearly all the ancestral vegetation in the study area has been degraded mainly as a result of clearing secondary vegetation and Mangrove forest to build public houses or infrastructures. Also, the drainage patterns are drastically changed as streams are straightened, redirected and made into concretized canals and ditches. When compared with the report given by Orebamjo and Njoku (1970), many species were found to be missing (especially species such as *Anogeissus leiocarpus, Triplochyton scleroxylon,*

Table 1. List of trees in North-Eastern Lagos, their location and conservation status.

Species	Family	Conservation status	Location	Elevation (m)
Adansonia digitata A.L	Bombacaceae	LC *	N06°31.139° E003°24.033°	69
Albizzia lebbeck Benth	Fabaceae	LC	N06°30.666° E003°23.799°	54
Albizzia zygia (DC.) Macbor.	Fabaceae	LC	N06°31.032° E003°23.976°	32
Alstonia boonei De Wild.	Apocynaceae	LC	N06°30.803° E003°23.642°	54
Anacardium occidentale De Wild.	Anacardiaceae	LC	N06°31.116° E003°24.043°	27
Annona muricata L	Annonaceae	LC *	N06°31.078° E003°24.052°	59
Anthocleista djalonensis A Chev	Loganiaceae	LC	N06°30.987° E003°23.925°	32
Anthocleista vogelii Planch	Loganiaceae	LC	N06°30.610° E003°23.708°	43
Artocarpus communis J.R Forst. & G. Forst	Moraceae	LC	N06°31.292° E003°23.933°	28
Avicennia germinans (L.) L	Avicennaceae	LC	N06°31.292° E003°23.933°	28
Azadirachta indica A Juss	Meliaceae	LC	N06°30.608° E003°23.743°	57
Bauhinia monandra Kurz	Fabaceae	LC	N06°30.064° E003°23.078°	33
Blighia sapida K. Koenig	Sapindaceae	LC	N06°31.066° E003°24.043°	55
Bombax buonopozense P Beauv	Bombacaceae	LC	N06°31.086° E003°24.052°	99
Bridelia micrantha (Hochst) Baill	Euphorbiaceae	LC	N06°31.097° E003°24.048°	26
Calophyllum inophyllum L	Calophyllaceae	LR *	N06°31.088° E003°24.050°	43
Carica papaya L.	Caricaceae	LC	N06°30.380° E003°23.800°	55
Cassia siamea (Lamarck) Irwin et. Barneby	Fabaceae	LC	N06°31.201° E003°23.823°	40
Casuarina equisetifolia L	Casuarinaceae	LC *	N06°30.461° E003°23.812°	60
Ceiba pentandra (L.) Gaertn	Bombacaceae	LC *	N06°31.082° E003°24.055°	83
Chrysophyllum albidum G. Don	Sapotaceae	LC *	N06°31.116° E003°24.047°	27
Citrus sinensis Osbeck	Rubiaceae	LC	N06°30.506° E003°23.823°	47
Cocos nucifera G. Don	Arecaceae	LC	N06°30.455° E003°23.809°	73
Cola gigantea L	Sterculiaceae	LC	N06°31.042° E003°24.047°	21

Table 1. Contd.

Cola nitida et. Endl. Schot	Sterculiaceae	LC	N06°31.070° E003°24.052°	83
Cordia abyssinica Lam	Boraginaceae	LC *	N06°31.112° E003°24.043°	62
Delonix regia (Hook)Raf	Fabaceae	VU	N06°30.445° E003°23.779°	45
Dialium guineensis Willd	Fabaceae	LC	N06°30.476° E003°23.795°	25
Elaeis guineensis Jacq	Arecaceae	LC	N06°30.605° E003°23.763°	58
Erythrina senegalensis DC	Fabaceae	LC	N06°30.596° E003°23.743°	44
Eugenia malaccensis L.	Myrtaceae	LC *	N06°30.613° E003°23.743°	45
Ficus congoensis	Moraceae	LC	N06°30.334° E003°23.788°	46
Ficus exasperata L.	Moraceae	LC	N06°31.037° E003°23.903°	32
Ficus sycomorus L	Moraceae	LC	N06°31.064° E003°24.111°	32
Ficus vallis-chaudae L	Moraceae	LC	N06°30.529° E003°23.825°	66
Gliricidia sepium (Jacq) Kunth	Fabaceae	LC	N06°30.348° E003°23.783°	71
Gmelina arborea Roxb	Lamiaceae	LC	N06°31.066° E003°24.058°	86
Holarrhena floribunda (G. Don) T. Durand& Schinz	Apocynaceae	LC	N06°30.599° E003°23,765°	40
Hildegardia barteri Roxb	Malvaceae	LC	N06°31.225° E003°23.961°	94
Hura crepitans L	Euphorbiaceae	LC *	N06°31.088° E003°24.063°	29
Jacaranda mimosifolia G. Don	Bignonaceae	VU	N06°30.870° E003°23.875°	34
Khaya grandifoliola C.DC	Meliaceae	VU	N06°31.063° E003°24.036°	61
Lagerstroemia speciosa (L.) Pers	Lythraceae	LC	N06°31.113° E003°23.925°	38
Mangifera indica L	Anacardiaceae	LC	N06°30.296° E003°23.785°	45
Milicia excelsa (Welw.) C.Berg	Moraceae	EN*	N06°31.080° E003°24.052°	52
Millettia thonningii (Schum. &Thonn.)Baker	Fabaceae	LC	N06°30.424° E003°23.821°	42
Morinda lucida Benth	Rubiaceae	LC	N06°30.367° E003°23.783°	54
Newbouldia laevis (P.Beauv.) Seeman ex Heyne	Bignonaceae	LC	N06°30.330° E003°23.793°	51
Peltophorum pterocarpum (DC.) Baker ex Heyne	Fabaceae	LC *	N06°30.870° E003°23.852°	34

Table 1. Contd.

Persea americana Mill	Lauraceae	LC	N06°30.511° E003°23.828°	34
Phoenix reclinata Jacq	Arecaceae	LC	N06°30.464° E003°23.784°	46
Pithecelobium dulce (Roxb.)Benth	Fabaceae	LC *	N06°31.070° E003°24.114°	60
Psidium guajava L	Myrtaceae	LC	N06°30.296° E003°23.814°	48
Raphia hookeri Marm Wendland	Arecaceae	LC	N06°30.429° E003°23.828°	54
Rauvolfia vomitora Afzel	Apocynaceae	LC	N06°31.054° E003°24.036°	75
Roystonea oleraceae O.F. Cook	Arecaceae	LC	N06°31.114° E003°23.927°	33
Senna alata	Fabaceae	LC	N06°31.201° E003°23.823°	40
Senna fistula	Fabaceae	LC	N06°31.201° E003°23.823°	40
Spondias mombin L	Anacardiaceae	LC*	N06°31.088° E003°24.037°	61
Sterculia tragacantha Lindl	Malvaceae	LC	N06°31.054° E003°24.034°	57
Tabebuia rosea (Bertol.)DC.	Bignonaceae	LC	N06°31.112° E003°23.894°	75
Tectona grandis L	Verbenaceae	LC *	N06°30.467° E003°23.784°	51
Terminalia catappa L	Combretaceae	LC	N06°30.466° E003°23.819°	69
Terminalia randii Baker. f	Combretaceae	LC	N06°31.336° E003°24.406°	71
Terminalia superba Engl.et Diels	Combretaceae	LC *	N06°31.089° E003°24.054°	58
Treculia africana Decne	Moraceae	LC *	N06°31.083° E003°24.052°	56

LC - Least concerned, LR - local risk, VU - vulnerable, EN - endangered, *protected by cites.

Daniellia ogea, Celtis spp. *and Entandrophragma* spp., *Daiella olivieri, Pterocarpous* spp*., Diosporos* spp. and *Pychanthus* spp*. Lovoa* spp*. and Vitex doniana,* among others) suggesting that these trees species are under the threat of anthropogenic human activities, as most of the trees species were probably replaced by buildings after deforestation.

Although DNA extraction is the first step in every molecular studies research (Qi-Xing et al., 2013), the selection of suitable protocol for DNA extraction from a specific plant species has always been problematic, given that some plants are rich in cellulose, polysaccharides, polyphenols, proteins and lipids, which are responsible for the complication of the nucleic acid separation and purification, and this is mostly associated with tropical plants (Li et al., 2011; Mohammad et al., 2008; Tan and Yiap, 2009; Sharma et al., 2008; Wang et al., 2008; de la Cruz et al., 1997; Porebski et al., 1997; John, 1992). As a result, wide variety of DNA extraction techniques has been developed (Mohammad et al., 2008) however, this study adopted the CTAB protocol methods of Doyle and Doyle (1987) for the extraction of genomic DNA from the tree species, given that the samples studied are of tropical origin (Qi-Xing et al., 2013; Sahu et al., 2012; Oboh et al., 2009; Ogunkanmi et al., 2008). The study also highlights how rapid and reliable the CTAB protocol is specifically for extracting DNA from plants which are rich in polysaccharides and

Plate 1. Some indigenous species encountered. a) *Adansonia digitata*; b) *Athrocarpus sp;* c) *Bridelia micrantha;* d) *Cola nitida* e) *Khaya grandifofolia*; f) *Hildegardia barteri*; g) *Holarrhena floribunda;* h) *Raphia hookeri*; i) *Treculia africana.*

secondary metabolites, and the protocol also excludes the use of expensive liquid nitrogen and toxic phenols. Purity of extracted DNA was excellent as evident in the absorbance ratio recorded (1.74 to 1.84). This suggests that the preparations were sufficiently free of proteins and polyphenolics/polysaccharide compounds, with minimal contamination (Clark and Christopher, 2000). Hence, the purified DNA was dissolved in buffer and stored at -80°C in the established DNA Bank at the University of Lagos, Akoka, Lagos, Nigeria. The pure DNA extracted is a prerequisite to reliable molecular biology research (Maltas et al., 2011; Pagnotta, 2009; Savolainen et al., 2007; Mace, 2003) including molecular marker study such as AFLP, RAPD or any other PCR, based analysis or research. It could also be used in forensic research, understanding genetic and evolutionary relationships between taxa, functional analysis of genes, comparative genomics research, DNA barcoding and plant breeding. Furthermore, it can be used for studying DNA structure and chemistry, examining DNA-protein interactions, carrying out DNA hybridizations, and for cloning and sequencing; which provide additional option for conservation of biodiversity.

Conclusion

This study is probably the first attempt at using molecular techniques in conserving the flora of northeastern Lagos. Hence, this study has contributed to the genomic conservation of the tree species in Nigeria and the geno-

Plate 2. Some Introduced species encountered. a) *Casuarina equisetiifolia;* b) *Cocos nucifera;* c) *Delonix regia;* d) *Eugenia malaccensis;* e-f) *Lagerstroemia speciosa*.

Plate 3. Some members of the family Fabaceae encountered. a) *Albizzia lebbeck;* b) *Albizzia zygia;* c) *Senna siamea;* d) *Millettia thonningii;* e) *Bauhinia monandra;* f) *Peltophorum pterocarpum.*

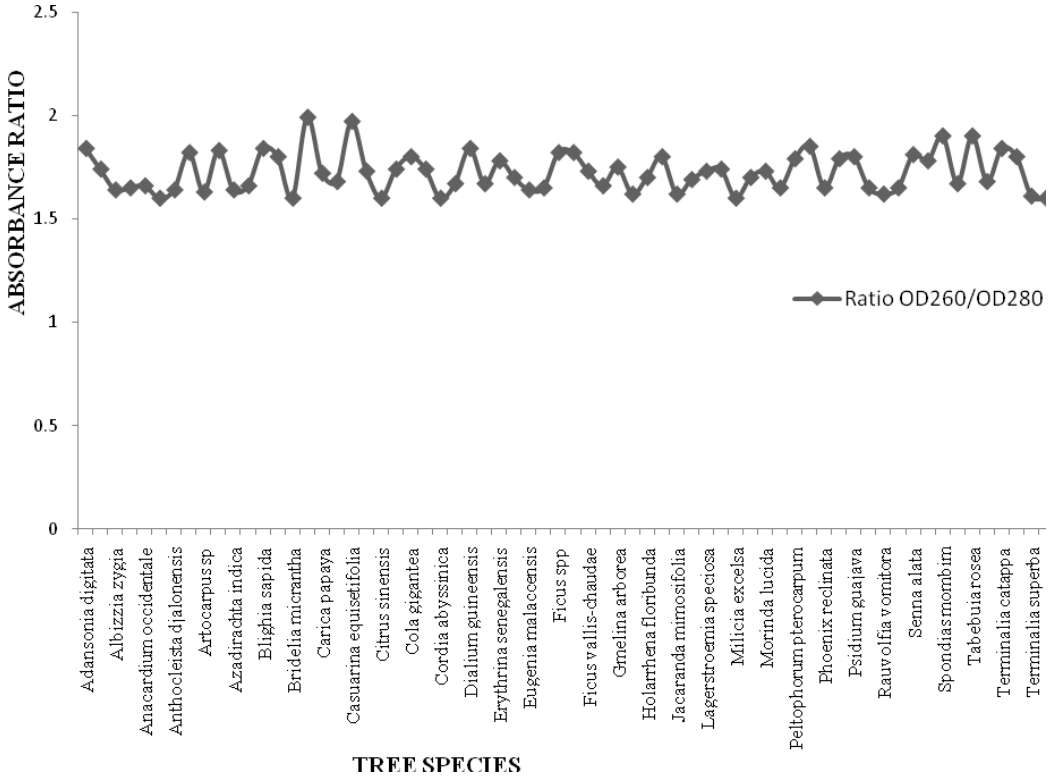

Figure 2. Absorbance ratio of DNA samples of the tree species.

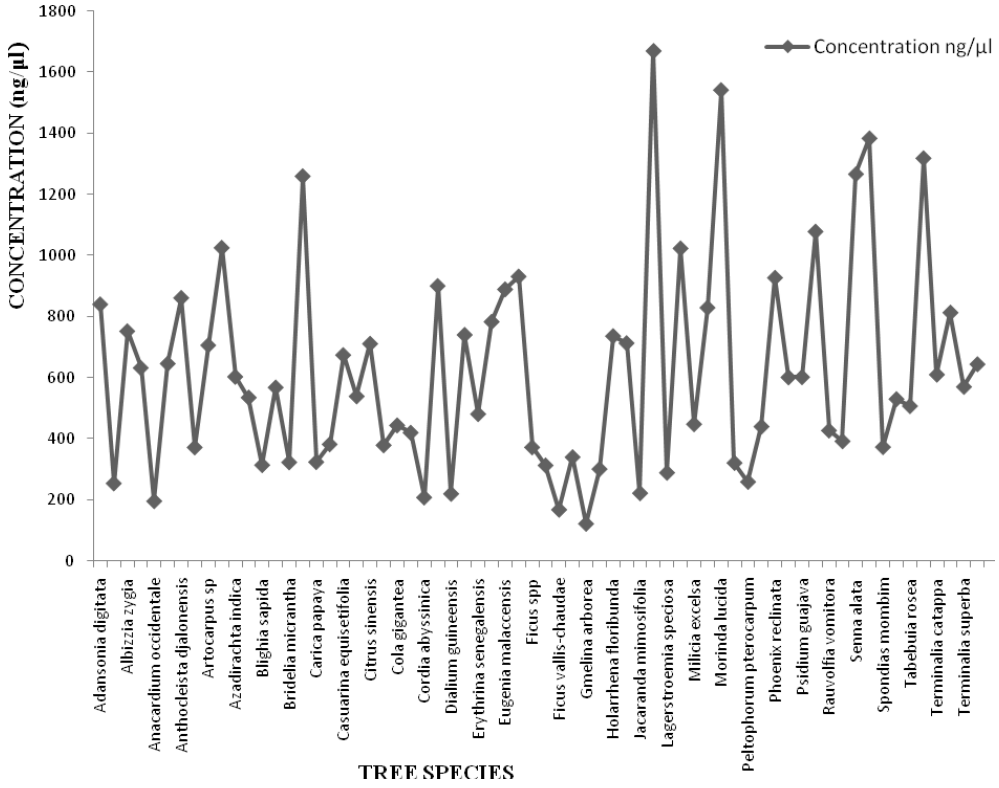

Figure 3. Concentration of DNA samples of the tree species in ng/µl.

mic DNA extracted would serve as a bench mark for further researches.

Conflict of Interests

The author(s) have declared that there is no conflict of interests.

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