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Research Article

DNA fingerprinting of five *Tabebuia* species with reference to their anti-trypanosomal activity

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ABSTRACT

Tabebuia is the largest genus of the Bignoniaceae family, with great importance due to its beautiful decorative flowering trees, as well as, its remarkable biological activities. The exact identification of *Tabebuia* species is important, not only, for cultivation purposes but also for exploration of their phytochemical and biological potential. DNA fingerprinting technology is now considered an easily accessible, quick, and accurate method of species identification. The current study investigated the genetic diversity among five *Tabebuia* species, using start codon targeted (SCoT), and inter simple sequence repeats (ISSR) markers. Results indicated the efficiency of both markers for genetic fingerprinting of the five tested *Tabebuia* species with ISSR analysis being more polymorphic than SCoT analysis. The dendrogram generated from the combination of ISSR and SCoT analyses classified the tested species into two main clusters. Cluster I included *T. guayacan*, while Cluster II was separated into sub-cluster I comprising *T. rosea* and sub-cluster II that was further subdivided into sub-cluster IIa (*T. pulcherrima*) and sub-cluster IIb (*T. argentea* and *T. pallida*). Furthermore, the antitrypanosomal activity of the alcohol extracts of stems and leaves of the five tested *Tabebuia* was evaluated. Results revealed a variation in activity between extracts from different species. The highest antitrypanosomal activity was recorded for the stem and leaf extracts from *T. pulcherrima*, with IC_{50} (6.4-7.2 µg/mL) and (8.3 and 8.9 µg/mL) after 48 and 72 h respectively, followed by *T. pallida* leaf then *T. rosea* stem extracts.

Keywords:
Tabebuia, DNA fingerprinting, ISSR marker, SCoT marker, Antitrypanosomal activity

1. INTRODUCTION

Genus *Tabebuia* is the largest genus of Bignoniaceae family[1-2], with more than 100 species in tropical and subtropical areas[3]. Members of this genus are famous as a therapeutic alternative by rural or remote populations and widely applied in traditional medicine for cutaneous infections, stomach disorders, inflammation, depression, diabetes, allergies, and even for controlling cancer[2,4-7]. The biological activities reported, were proved to be associated with the presence of several classes of secondary metabolites such as; naphthoquinones, phenolic compounds, iridoids, lignans, coumarins, and flavonoids[3,8-10].

DNA fingerprinting studies of *Tabebuia* species are somehow limited till now, however, such information is valuable for breeding programs, differentiation between plant species and cultivars, identification of plants in very small amounts, or identification of plants containing a gene of interest to produce genetically engineered plants [11]. Specially, traditional botanical identification methods by taxonomists, are often difficult, time-consuming, and unavailable during field survey[12]. DNA barcoding technology has become reliable bioinformatics and the most stable tool that does not vary seasonally or with the age of the plant[13]. The most common examples of DNA markers are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single-nucleotide polymorphism

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(SNP) and diversity arrays technology (DArT) markers[14]. However recently, promising marker techniques have been developed such as Inter simple sequence repeat marker (ISSR) and Start Codon Targeted (SCoT) marker. Inter simple sequence repeat marker (ISSR) is a PCR-based marker that is highly polymorphic and useful for genetic diversity, genome mapping, and evolutionary biology[15]. It also overcomes many defects of other marker methods, such high cost of amplified fragment length (AFLP) and the low reproducibility of random amplified polymorphic DNA (RAPD)[16]. Also, the start codon targeted marker (SCoT) is simple and popular for its high polymorphism and better marker resolvability[17]. ISSR and SCoT markers were reported to be useful in genetic diversity studies because they are highly reproducible and have great power for polymorphism detection[18]. Thus, further analysis of the genetic diversity and variation among *Tabebuia* species cultivated in Egypt gives a better understanding of the distribution of genetic diversity among this genus.

African trypanosomes are medically important fly-borne neuro-inflammatory parasites that cause sleeping sickness in humans and are fatal if left untreated[19-20]. During the last 6 years, only one drug has been approved for African sleeping sickness. In this regard, natural products are still considered as a good source for drugs with optimized biological activity and minimized-sided effects, or at least they can provide chemical structures as prototypes for new drugs[21]. The current study seeks to explore the antitrypanosomal potential of the genus *Tabebuia*.

This study aimed to assess the genetic diversity and similarity among five *Tabebuia* species using two effective PCR-based markers (ISSR and SCoT markers); as well as to evaluate the antitrypanosomal activity of alcoholic extracts from different organs of the five tested species.

2. MATERIALS AND METHODS

2.1. Plant material

The leaves and stems of five *Tabebuia* species were collected during March 2018 from two different locations; (*T. argentea* Britton *and T. guayacan* (Seem.) Hemsl.), were collected from Al-Zohriya garden, Zamalek, Cairo Governorate, Egypt; (*T. pulcherrima, T. pallida* L. *and T. rosea* (Bertol.) DC.) were collected from El-Nabatate island garden, Aswan, Egypt. All plant samples were identified and authenticated by Prof. Dr. Abdel-Halim Mohammed (Professor of Agriculture, Flora department, Agricultural museum, Dokki, Giza, Egypt) and collected according to plant collections guidelines of Alberta Native Plant Council 2006. Voucher specimens were kept in the botanical garden in Aswan, Aswan, Egypt. The leaves were deposited with the number (Ta 1-Ta 5) corresponding to *T. guayacan, T. pallida, T. argentea, T. rosea,* and *T. pulcherrima.* respectively. while the stems were deposited with the numbers (Ta 6-Ta 10) corresponding to the stems of *T. guayacan, T. pallida, T. argentea, T. rosea,* and *T. pulcherrima.* respectively. Plant materials were washed separately with fresh water. A part of fresh leaves collected, were stored at -5°C for DNA study. While, for the anti-trypanosomal assay, the leaves and stems collected (Ta 1-10) were dried in the shade for several days. The dried materials were ground into a coarse powder using a grinding machine and the materials were stored at room temperature.

2.2. DNA analysis

2.2.1. DNA isolation procedure

Approximately 100 mg of young and healthy leaf tissue of each plant sample was collected and ground by liquid nitrogen to a fine powder which is either immediately used for DNA extraction, or stored at -30°C before DNA isolation. The DNA extraction was done using DNeasy plant Mini Kit (QIAGEN) on Agriculture Research Centre of Cairo University, Giza, according to method[22-23]. 400 µL of buffer AP1 and 4 µL of RNase a stock solution (100 mg/mL) were vigorously vortexed with a maximum of 100 mg of ground plant tissue and the mixture was then incubated for 10 min at 65°C and mixed 2-3 times during incubation by inverting tube. 130 µL of buffer AP2 was added to the lysate, mixed, and incubated for 5 min on ice. Lysate was applied to the QIA shredder spin column sitting in a 2 mL collection tube, centrifuged for 2 min at maximum speed (10,000 rpm) and the supernatant was transferred to a new tube without disturbing the cell-debris pellet. 450 µL of lysate was recovered and 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting. Afterward, 650 µL of the mixture was applied through a DNeasy Mini spin column setting in a 2 mL collection tube, centrifuged for 1 min at 8,000 rpm and flow-through was then discarded. DNeasy column was placed in a new 2 mL collection tube and 500 µL buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8,000 rpm and 500 µL buffer AW was added to the DNeasy column and centrifuged for 2 min at maximum speed (10,000 rpm) to dry the column membrane. DNeasy column was transferred to a 1.5 mL microfuge tube and 100 µL of preheated buffer AE (65°C) was pipetted directly onto the DNeasy column membrane, incubated for 5 min at room temperature, and centrifuged for 1 min at 8,000 rpm to elute. Elution was repeated once as described. A new microfuge can be used for the first eluting. Alternatively, the microfuge tube can be reused for the second elution step to combine elutes.

2.2.2. Polymerase chain reaction (PCR) condition for both ISSR and SCoT markers

The PCR amplification of ISSR and SCoT markers were carried out using 20 µL of reaction mixture containning 10X PCR Buffer, 200 µM each of dGTP, dATP, dCTP, and dTTP; 2.0 mM $MgCl_2$; 100 pM Primer; 30 ng genomic DNA and 1 U Taq polymerase. The PCR schedule followed was 94°C for 4 min followed by 45 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 2 min, and a final incubation at 72°C for 10 min. The PCR product was resolved on a 2.0% agarose gel in TBE buffer (89 mM Tris-HCl, 89 mM Borate, 2 mM EDTA) at a constant current of 200 mA for approximately 4 h, and visualized with ethidium bromide (0.5 µg/mL) under UV light primer selection[24].

2.2.3. Agarose gel electrophoresis

1.50 g agarose was mixed with 100 mL TBE buffer and boiled in the microwave, after the temperature reached 55°C Ethidium bromide (5 µL) was added, and then melted gel was poured into the tray of mini-gel apparatus and covered by the electrophoretic buffer (1xTBE). DNA amplified product (15 µL) was loaded in each well and DNA ladder (1Kbp) mix was used as standard DNA with molecular weights of 3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp. The run was performed for about 30 min at 80 V in mini-submarine gel BioRad.

2.2.4. Data analysis

Every band generated by ISSR and SCoT amplification with every primer was counted. The clear constant repetitive amplified bands were transformed into a binary matrix (presence "1" and absence "0") (Table S1 and S2 in supplementary data). The data were used for estimating the genetic similarity coefficients, following the methods of Nei and Li[25], which is stated as 2Nij/Ni+Nj, where Nij is the number of common bands in i and j varieties and Ni and Nj are the number of bands for accessions i and j, respectively. Cluster analysis was carried out with dissimilarity matrices using UPGMA (Unweighted Pair Group Method using Arithmetic average), a clustering program that compresses the patterns of variation into branch diagrams (dendrogram) using the PHYLIP 3.5c software program. Robustness of the dendrogram was tested by estimating the cophenetic correlation values for each dendrogram and comparing them with the original genetic dissimilarity matrix using Mantel's matrix correspondence test[26-27]. Estimates of the differences between the dendrograms based on SCoT and ISSR markers were also assessed by computing the cophenetic values and constructing the cophenetic matrices for each primer set and for the combination of both markers (Table S3-S5 in supplementary data).

In order to test the genetic variability further, multidimensional scaling of the data was done using the SPSS windows (Version 10) program. DICE computer package was used for the pairwise difference matrix calculation and plotting the phenogram among cultivars[28-29].

2.3. Anti-trypanosomal assay

300 g of powdered material of each plant sample (Ta1-10) was macerated separately in 500 mL of 70% ethanol in sealed amber-colored extraction bottles. Kept for 3 days with occasional shaking and stirring. Filtered through a fresh cotton plug and the filtrates were concentrated using a rotary evaporator under reduced pressure at 40°C. The anti-trypanosomal bioassay was done following Huber and Koella technique[30-31]. Briefly, 10^4 trypanosomes per mL of *Trypanosoma brucei brucei* strain TC 221 were cultivated in Complete Baltz Medium. Trypanosomes were tested in 96-well plate chambers against different concentrations of test extracts at 0.1-100 µg/mL in 1% DMSO to a final volume of 200 µL. For controls, 1% DMSO as well as parasites without any test extracts were used simultaneously in each plate to show no effect of 1% DMSO. The plates were then incubated at 37°C in an atmosphere of 5% CO_2 for 24 h. After the addition of 20 µL of Alamar Blue, the activity was measured after 48 and 72 h by light absorption using an MR 700 Microplate Reader (Dynatech Engineering Ltd., Willenhall, UK) at a wavelength of 550 nm with a reference wavelength of 650 nm. The quantification of the IC_{50} values of the examined extracts was processed by interpolation in triplicate.

Table 1. List of the primers names and their nucleotide sequences used in ISSR and SCoT techniques.

ISSR		SCoT	
Name	**Sequence 5'.....3'**	**Name**	**Sequence 5'.....3'**
14A	CTC TCT CTC TCT CTC TTG	**SCoT 3**	ACG ACA TGG CGA CCC ACA
44B	CTC TCT CTC TCT CTC TGC	**SCoT 4**	ACC ATG GCT ACC ACC GCA
HB-8	GAG AGA GAG AGA GG	**SCoT 6**	CAA TGG CTA CCA CTA CAG
HB-12	CAC CAC CAC GC	**SCoT 8**	ACA ATG GCT ACC ACT ACC
HB-15	GTG GTG GTG GC	**SCoT 9**	ACA ATG GCT ACC ACT GCC

Table 2. Total bands scored by ISSR markers.

Primer name	Total band	1	2	3	4	5
14A	10	7	7	9	5	5
44B	8	5	3	4	2	5
HB-8	4	4	3	3	4	4
HB-12	10	6	5	7	7	9
HB-15	7	4	3	4	5	3
Total	**39**	**26**	**21**	**27**	**23**	**26**

1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

Table 3. The distribution of monomorphic and polymorphic bands generated by ISSR marker.

Primer Name	Size of Bands (bp)	Number of bands	Monomorphic bands	Unique bands	Polymorphic Bands	% Polymorphism
14A	1425-260	10	0	6	10	100%
44B	860-280	8	1	4	7	87.5%
HB-8	635-345	4	3	0	1	25%
HB-12	1270-300	10	5	1	5	50%
HB-15	1070-360	7	0	1	7	100%
Total		39	9	12	30	
Mean		7.8	1.8	2.4	6	
Percentage			**23.07%**	**30.76%**	**76.92%**	

3. RESULTS

3.1. DNA analysis

Two different PCR-based molecular markers (ISSR and SCoT) were used. The two molecular markers were able to distinguish and identify the five species from each other. Data matrices of ISSR and SCoT marker profiles were generated by scoring (1) for presence and (0) for the absence of individual allele (Table S1 and Table S2 in supplementary data). Salient features of the fingerprint database obtained using the different markers are given in (Table 2-5) and (Figure 1-5).

3.1.1. ISSR analysis

Out of fifteen primers used, five primers produced scorable bands and were selected for species identification and genetic relationship analysis (Figure 1). ISSR primers generated a total of 39 bands ranging from 4 (HB-8) to 10 (14A) and ranging in size from 260 bp to 1425 bp. Among the scored bands, 30 were polymorphic, yielding a polymorphism rate of 76.92% indicating that the examined species exhibited a high level of polymorphism, and 9 were monomorphic, yielding a monomorphism rate of 23.07%. The percentage of polymorphism revealed by the different primers ranged from 25% for primer (HB-8) to 100% for primers (14A and HB-15) (Table 2-3). ISSR dendrogram classified the five species into two main clusters (Figure 2). Cluster I included only *T. guayacan* and cluster II is differentiated into two subclusters IIa (*T. pulcherrima* and *T. rosea*) and IIb (*T. argentea* and *T. pallida*).

3.1.2. SCoT analysis

Similarly, fifteen SCoT primers were tested, among them the best five were selected for species identification and relationship analysis of the five *Tabebuia* species (Figure 3). As shown in (Tables 4-5); a total of 27 bands were detected ranging from 2 (SCoT-8) to 9 (SCoT-3). Thirteen were polymorphic yielding a polymorphism rate of 48.14% and fourteen were monomorphic, yielding a monomorphism rate of 51.85%. The percentage of polymorphism revealed by the different primers ranged from 0% for primer (SCoT-8) to 66.66% for primer (SCoT-9). The primer (SCoT-8) was monomorphic with a monomorphism rate of 100%. The dendrogram generated for SCoT marker showed two main clusters (Figure 4). Cluster I included only *T. rosea*) DC., Cluster II is separated into sub-cluster I including (*T. guayacan*) and sub-cluster II that is further divided into sub-cluster IIa (*T. argentea*) and sub-cluster IIb (*T. pulcherrima and T. pallida*).

3.1.3. Similarity Index Combination of SCoT and ISSR Analysis

The reproducible patterns and amplification profiles of both markers were screened for polymorphism among the five species. The number of amplified products was 39 bands for ISSR and 27 bands for SCoT. The molecular size of the produced bands revealed the presence of a wide range of sequences. The total number of polymorphic bands was 43 (30 from ISSR analysis and 13 from SCoT analysis) and the total number of monomorphic bands was 23 (9 from ISSR analysis and 14 from SCoT analysis) as dictated in (Tables 2-5).

Unique markers are defined as bands that are present

Table 4. Total bands scored by SCoT marker.

Primer name	Total band	1	2	3	4	5
SCoT 3	9	8	7	7	6	7
SCoT 4	7	5	7	3	5	7
SCoT 6	6	3	4	4	3	6
SCoT 8	2	2	2	2	2	2
SCoT 9	3	2	2	3	2	3
Total	**27**	**20**	**22**	**19**	**18**	**25**

1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

Table 5. The distribution of monomorphic and polymorphic bands generated by SCoT marker.

Primer Name	Size of bands (bp)	Number of bands	Monomorphic bands	Unique bands	Polymorphic bands	% Polymorphism
SCoT-3	1280-475	9	5	4	4	44.44%
SCoT-4	1370-260	7	3	1	4	57.14%
SCoT-6	1200-340	6	3	2	3	50%
SCoT-8	425-375	2	2	0	0	0%
SCoT-9	600-360	3	1	1	2	66.66%
Total		27	14	8	13	
Mean		5.4	2.8	1.6	2.6	
Percentage			**51.85%**	**29.62%**	**48.14%**	

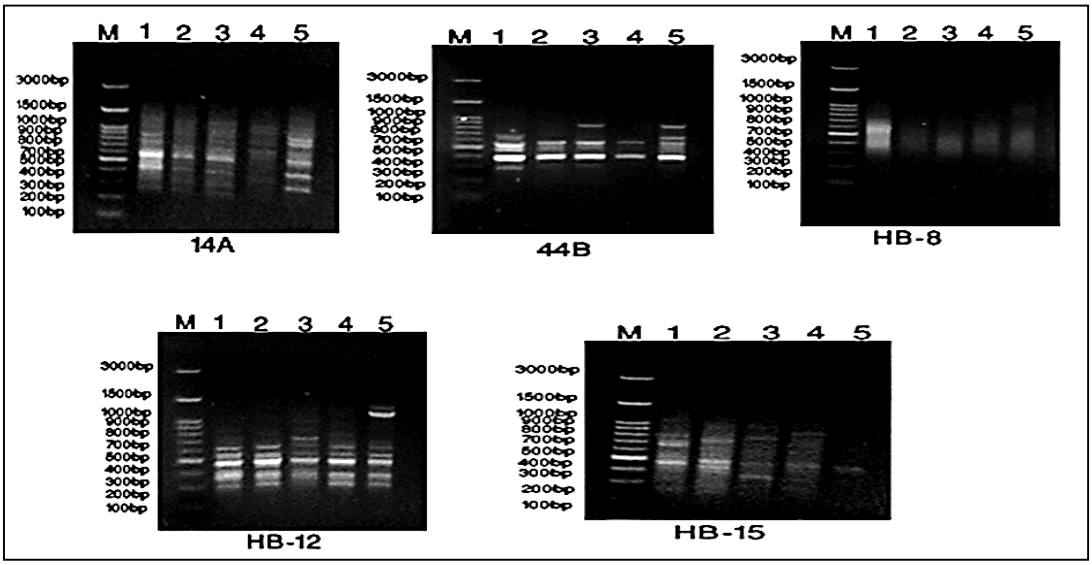

Figure 1. Photograph of DNA electrophoresis. Showed the ISSR bands of the five species using five primers; 1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

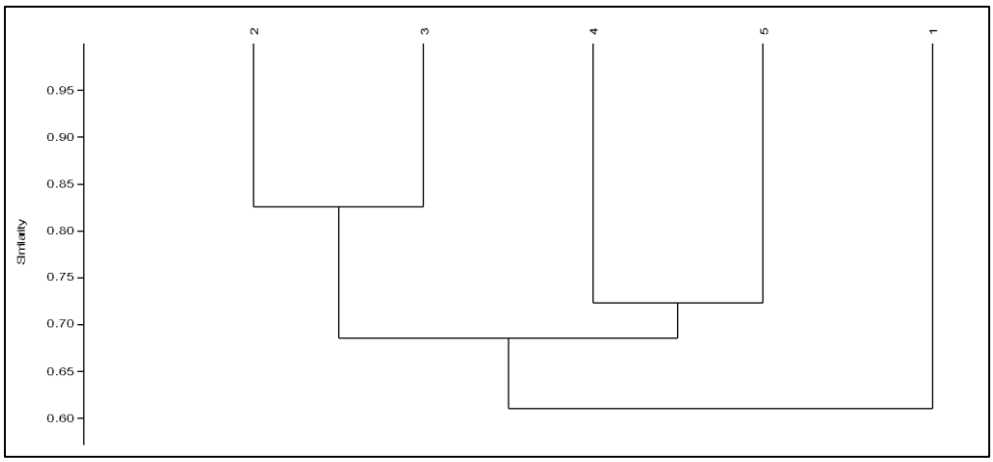

Figure 2. Dendrogram analysis of the five *Tabebuia* species. Using ISSR analysis; 1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

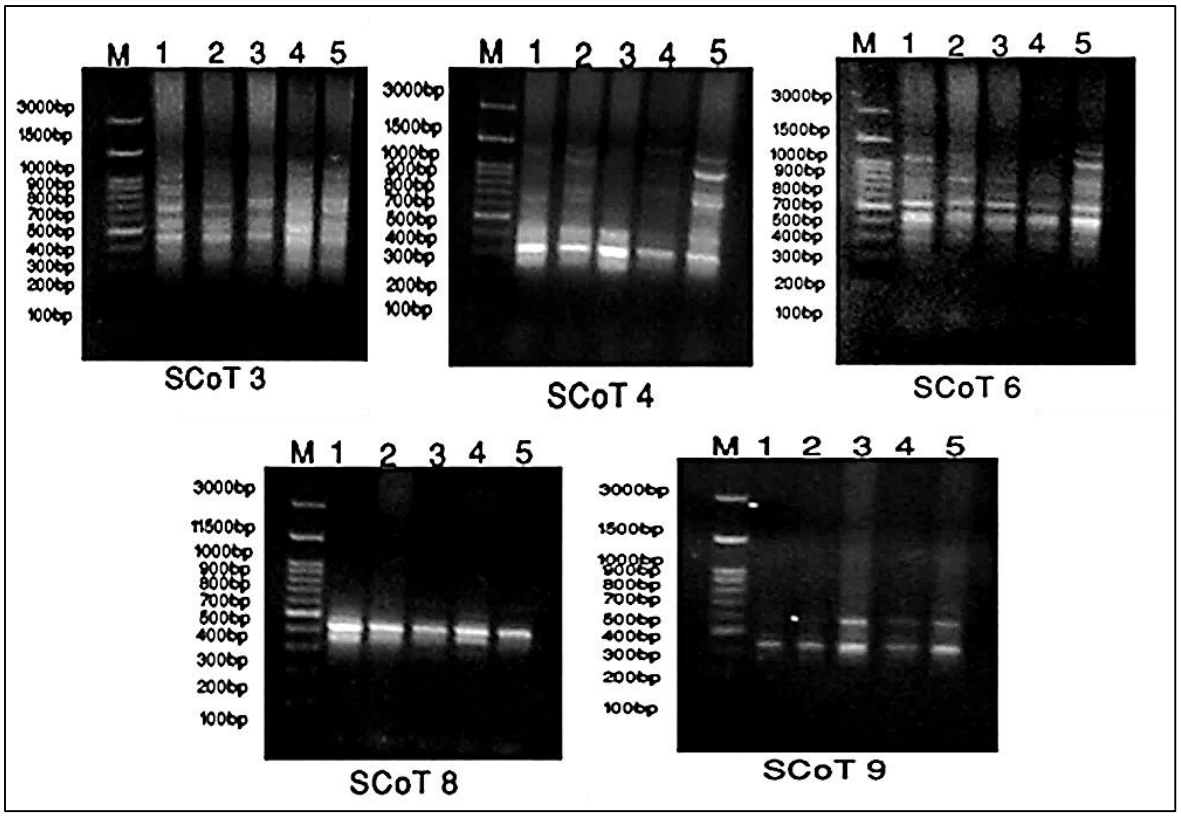

Figure 3. Photograph of DNA electrophoresis. Showed the SCoT bands of the five species using five primers; 1: *T. guayacan*, 2: *T. pallida L.*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

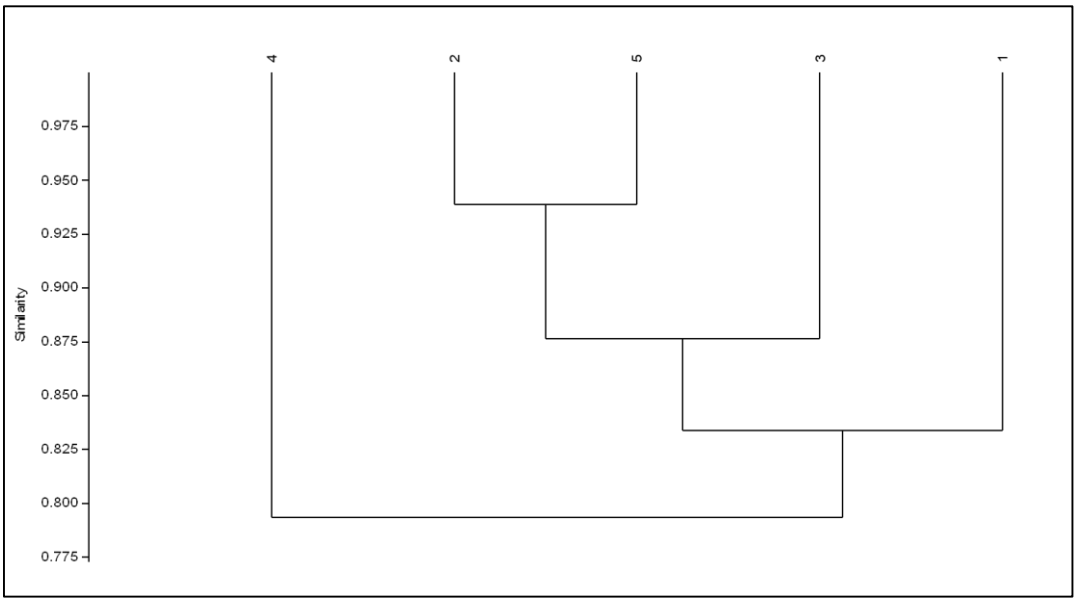

Figure 4. Dendrogram analysis of the five *Tabebuia* species using SCoT analysis; 1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima.*

(Positive Unique Marker (PUM)) or absent (Negative Unique Marker (NPM)) in one species and are specifically useful in identifying species. They facilitate the discrimination between the studied species. The total number of unique bands was 20 bands 12 from ISSR analysis and 8 from SCoT. In ISSR analysis, 14A primer scored the highest number of unique bands (6), while SCoT-3 primer in SCoT analysis scored (4) unique bands. The distribution of unique markers is dictated in (Tables 3 and Table 5). The dendrogram generated from the combination of both ISSR and SCoT analysis is composed of two main clusters (Figure 5), cluster I composed (*T. guayacan*) Cluster II is separated to sub-cluster I including *T. rosea* and sub-cluster II that is further divided into sub-cluster IIa (*T. pulcherrima*) and sub-cluster IIb (*T. argentea and T. pallida).*

3.2. Anti-trypanosomal activity

The anti-trypanosomal activity of alcoholic extracts from leaves and stems of the five *Tabebuia* species was investigated. Among the 10 tested extracts, only four were active against *T. brucei brucei* strain TC 221 with IC_{50} values < 20 µg/mL (Table 6, Figure 6-7). *T. pulcherrima* stem exhibited the most potent inhibition, with IC_{50} values 6.4 and 8.3 µg/mL after 48 h and 72 hr incubation, respectively. Leaves extract from, *T. pallida* and *T. pulcherrima*, showed very close IC_{50} values; 7.2 and 7.8 µg/mL after only 48 hr. Also, *T. rosa* stem extract displayed activity evaluated as IC_{50} 13.4 and 16.2 µg/mL after 48 and 72 hr, respectively. On the other hand; the remaining six extracts displayed IC_{50} >100 µg/mL, after 48 h and 72 hr incubation.

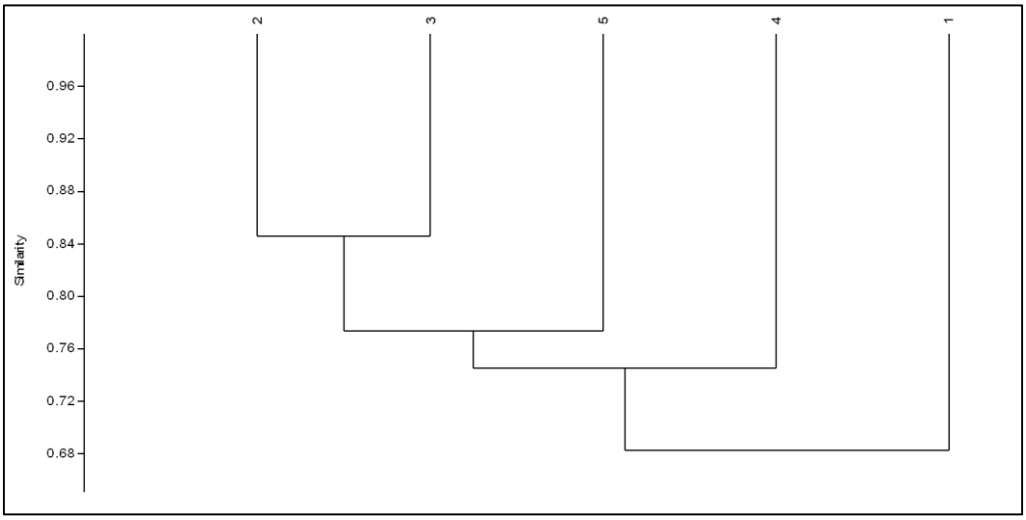

Figure 5. Dendrogram analysis of the five *Tabebuia* species. Using a combination of ISSR and SCoT analysis; 1: *T. guayacan*, 2: *T. pallida*, 3: *T. argentea*, 4: *T. rosea*, 5: *T. pulcherrima*.

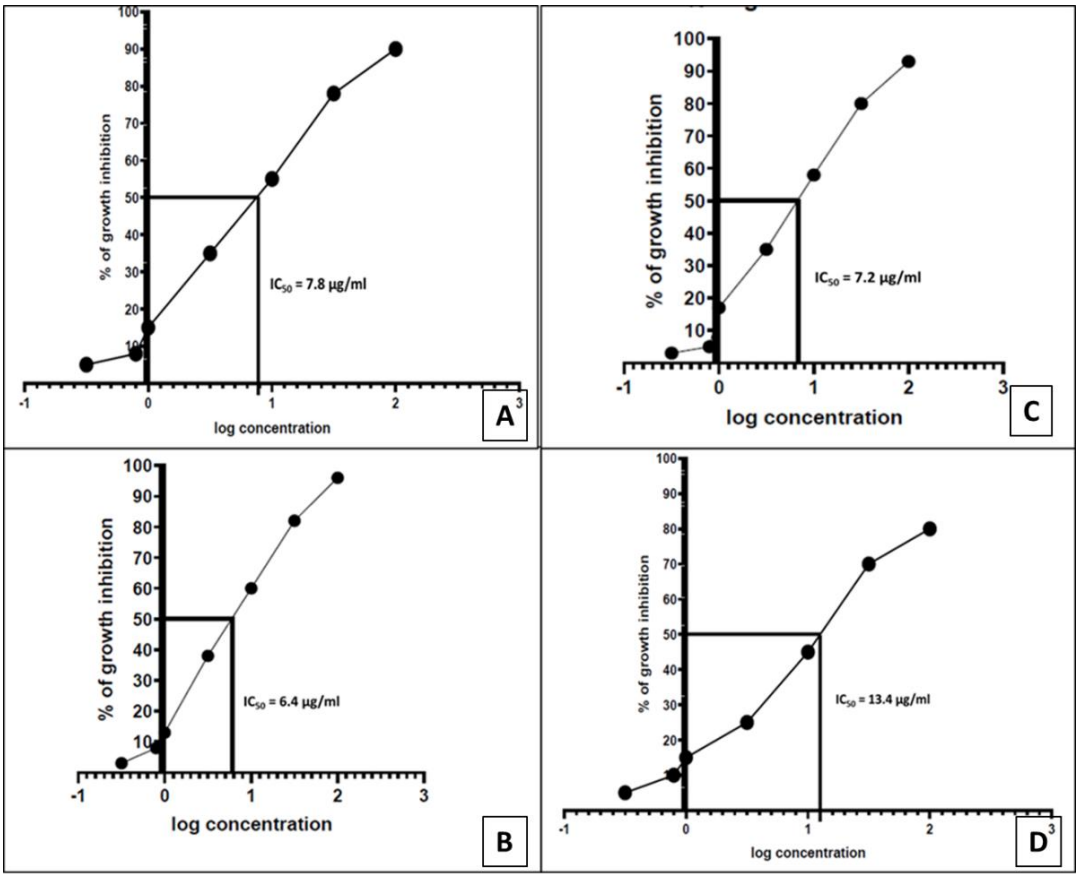

Figure 6. Dose-response curves of the tested extracts show the concentration required to inhibit 50% growth of *Trypanosoma brucei* TC 221, following incubation for 48 h. A= The dose-response curve of *T. pulcherrima* leaf, B= *T. pulcherrima* stem, C= *T. pallida* leaf, and D= *T. rosea* stem.

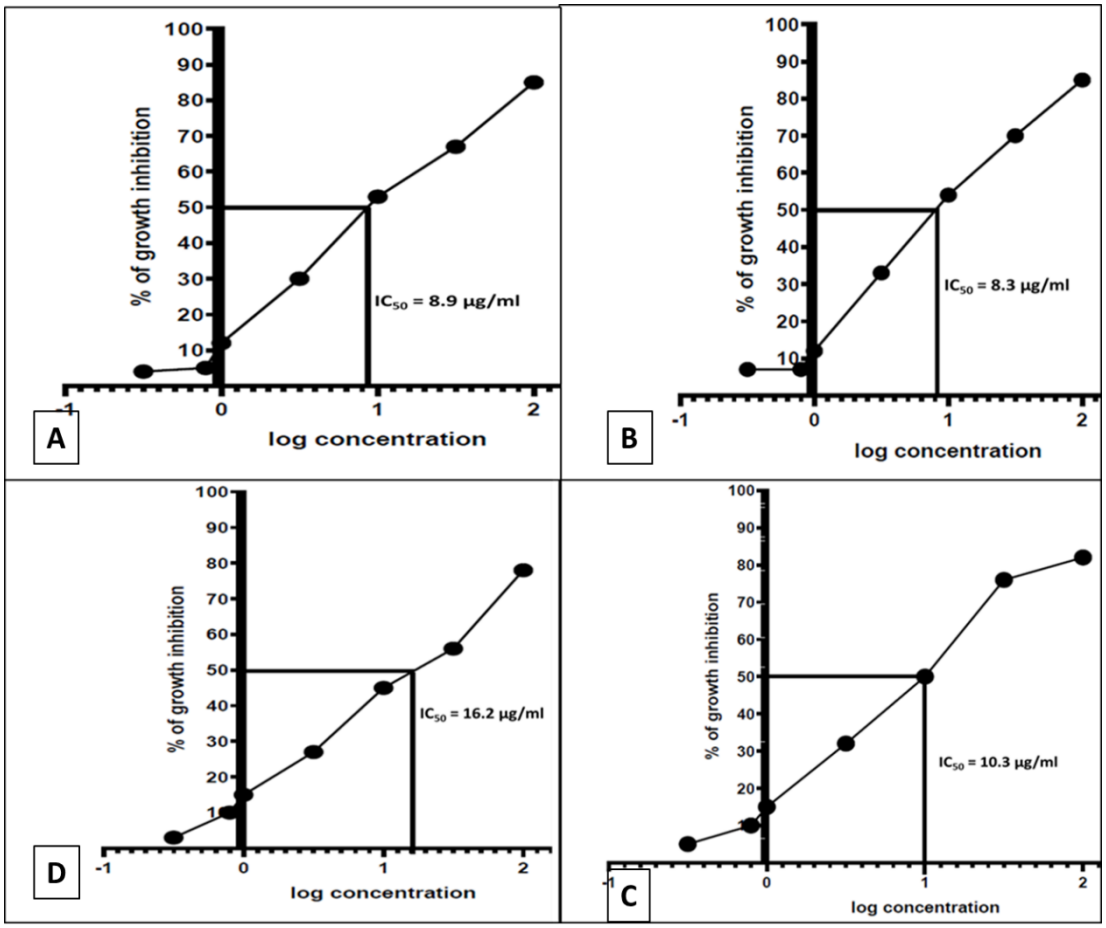

Figure 7. Dose-response curves of the tested extracts show the concentration required to inhibit 50% growth of *Trypanosoma brucei* TC 221, following incubation for 72 h. A= The dose-response curve of *T. pulcherrima* leaf, B= *T. pulcherrima* stem, C= *T. pallida* leaf, and D= *T. rosea* stem.

Table 6. The anti-trypanosomal activity of plants extract.

Plant samples	*Trypanosoma brucei* TC 221, IC_{50} (µg/ml, 48 hr)	SD±	*Trypanosoma brucei* TC 221, IC_{50} (µg/ml, 72 hr)	SD±
T. guayacane leaf	>100	---	>100	---
T. guayacane stem	>100	---	>100	---
T. pulcherrima leaf	7.8	0.023	8.9	0.074
T. pulcherrima stem	6.4	0.008	8.3	0.012
T. rosea leaf	>100	---	>100	---
T. rosea stem	13.4	0.007	16.2	0.023
T. pallida leaf	7.2	0.011	10.3	0.034
T. pallida stem	>100	---	>100	---
T. argentea leaf	>100	---	>100	---
T. argentea stem	>100	---	>100	---

4. DISCUSSION

DNA fingerprinting of genus *Tabebuia* was previously reported, using SNPs and INDELs markers that proved to be useful for genetic diversity and phylogeography within the genus[32]. However, this is the first time for SCoT and ISSR markers to be reported for genus *Tabebuia*. Both markers have proven efficacy in several previous studies e.g. for identification and genetic comparison analysis of 23 mango germplasm accession[33] and for fingerprinting of 40 varieties of bread wheat[34]. Archak et al., reported that the efficiency of a molecular marker technique depends upon the amount of polymorphism it can detect[35]. Herein, the two markers represented sufficient information for diagnostic fingerprinting of the five *Tabebuia* species, however depending on the total number of polymorphic and unique bands, obtained from these two markers, ISSR marker (76.92% polymorphism and 12 unique bands) was more effective for identification and assessment of the genetic diversity among the tested *Tabebuia* species.

The antitrypanosomal activity of the leaves and

stems of five *Tabebuia* species was investigated. Results revealed significant differences in activity among tested extracts from different organs. The highest activity was observed for the stem extract of *T. pulcherrima* followed by the leaf extracts from *T. pallida.* and *T. pulcherrima,* then the stem extract of *T. rosea.* Interestingly, were the results, indicating inactivity of *T. pallida* stem and *T. rosea* leaf extracts. This could be attributable to the difference in the metabolic content of the leaf and stem in each of the two species. On the other hand, alcoholic extracts from the stem and leaf organs of the two species, *T. guayacane* and *T. argentea,* displayed no antitryoanosomal activity (Table 6).

The antitrypanosomal activity of the bark of *T. serratifolia* was previously investigated, where, the chloroform extract was found effective against *Trypanosoma cruzi* with inhibition percent greater than 96%, [36-37]. However, no previous records were found regarding the antitrypanosomal activity of any of the five tested species.

According to our previous published work[38], the metabolic profiling of the five *Tabebuia* species was performed using LC-HRMS. Results indicated that iridoids, phenyl ethanoids and furanonaphthoquinons are the major classes of metabolites present in the five tested species. Reviewing the antitrypanosomal activity of these metabolite classes, revealed that the identified furanonaphthoquinones like 2-(1-hydroxyethyl)-4H,9H-naphtho [2,3-b] furano-4,9-dione along with 2-acetyl-naphtho [2,3-b]furan-4,9-dione, had significant antitrypanosomal activity[33]. While, the iridoids derivatives ajugol, aucubin, and catalpol had mild activity against *T. brucei*[39].

It is worth noting that; despite the recorded biological efficacy of leaf and stem of *T. pulcherrima,* whether antitrypanosomal activity (current study) or the cytotoxic activity[38], no phytochemical investigation was reported for *T. pulcherrima.* Accordingly, this species is strongly recommended for more phytochemical and biological future research.

5. CONCLUSION

In the present study, the genetic diversity of five *Tabebuia* species was studied using the two markers; SCoT and ISSR. Both markers were found efficient in genetic fingerprinting, with ISSR marker being more polymorphic. The antitrypanosomal activity was also assessed where *T. pullcherima* was shown to be the most active. The study suggests the species; *T. pulcherrima,* as a promising candidate for more bioassay-guided phytochemical investigation.

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Conflict of interest
The authors declare that they have no conflict of interest.

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