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Intra Specific Relationships and Biochemical Composition of *Laportea Aestuans* (L.) Chew, *Sida Rhombifolia* L. *And Commelina Verginica* L. in Lagos State

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ABSTRACT

Laportea aestuans L. (Chew), *Commelina virginica* (L.) and *Sida rhombifolia* (L.) are common wild plants used in treating several ailments including diarrhea, dysentery, hernia, oedema, ulcers and many more in traditional African medicine especially, Nigeria. The potentials of Random Amplified polymorphic DNA (RAPD) primers in delimiting intra-specific variation in *L. aestuans, C. virginica* and *S. rhombifolia* was assessed using three RAPD primers. Plant and soil samples were collected from 19 local government areas in Lagos State and assessed for genetic and biochemical relationships. A total of 56 bands were produced of which 44 were polymorphic. Maximum number of bands (21) was produced by OPY20 while OPC04 produced minimum number of bands (14). The maximum and minimum percentage polymorphisms were 100% (OPC04) and 50% (OPA12), The dendrogram of genetic diversity had a genetic distance range of 0.57 to 1.00, 0.58 to 1.00 and 0.52 to 1.00 and clustered at 0.57, 0.58, 0.52 implying 57%, 58%, 52% similarity and 43%, 42% and 48% variability for *L. aestuans, C. virginica* and *S. rhombifolia* respectively. Results of phytochemical analysis show that *S. rhombifolia* is particularly high in steroids (I134.19±0.23), flavonoids (118.65±0.18), phenolics (125.25±1.43), alkaloids (63.56±0.11) and triterpenes (72.44±0.12) but low in saponins (23.29±0.05), glycosides (6.79±0.01) and tannins (7.10±0.02).

Keywords; Urticaceae, polymorphism, Dendrogram, Diversity,.

INTRODUCTION

Laportea aestuans (L.) chew is an annual herb of the Urticaceae family, generally referred to as 'white nettle' or 'tropical nettle weed' [1]. *Laportae aestuans* is a popular multi-purpose plant in tropical Africa especially Nigeria. It has different Nigerian local names including fiyafiya/ofuefue (Yoruba), bulsum fage (Hausa), Ile-nkita (Igbo), Oho-ghogho (Benin) and many more. Among the many ethno-botanical uses of *L. aestuans* are; as an abortifacient, antimicrobial, laxative, in eye treatments and pain-killers, in treating pulmonary and stomach troubles, for diarrhea and dysentery, in treatment of hernia, oedema and ulcers [2, 3]. Results

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of epidemiological studies showed that consumption *L. aestuans* inhibits the damaging activities of free radicals in human body [4]). Also essential oil from *L. aestuans* containe methyl salicylate which had significant antioxidant and antimicrobial activities [5]. *Sida rhombifolia* is an annual plant in the family malvaceae. The genus *Sida* has over 200 species distributed throughout the tropics [6]. *Sida rhombifolia* has a wide range of medicinal uses including treatment of stings and bites of scorpion and snakes [7]. Hot aqueous extract, decoctions and infusions of *S. rhombifolia* is used as an abortifacient, to reduce rheumatic pain, in treatment of cough, as an aphrodisiac and in treatment of fever and urinary diseases, for dysentery, in treatment of gonorrhea [8, 9]. Stem of *S. rhombofolia* is chewed for dental hygiene and the infusion for prevention of miscarriage [10]. *Commelina virginica* on the other hand is one of five families, with about 731 known species in 41 genera [11]. It is an annual, monocotyledonous plant occurring worldwide in tropical and sub-tropical countries [12]. The plant has been shown to have potential as a bio-herbicide [13]. *Commelina virginic* has Ethno-botanical applications in blood clotting, relieve of fever among others [14].

Plate 1: (A) *Laportae aestuans* (B)*Commelina virginica* and (C) *Sida rhombifolia*

Genetic diversity has been defined as the quantitative measurement of the variability of a population, which reflects the equilibrium between mutation and the loss of genetic variation [15, 16]. Genetic diversity is an important component of biological diversity [17]. Genetic diversity are influenced mostly by genetic Structure, reproductive characters, population history and habitat fragmentation [18, 19]. Habitat fragmentation affects population by reducing populations to small isolates, which results in increasing genetic drift and inbreeding, as well as reduced gene flow [20]. Inbreeding species have homozygosity and lesser genetic diversity within populations and higher genetic differentiation among populations [21]. Other factors that may also affect genetic diversity are; life-history traits, such as pollination and seed dispersal modes. Medicinal plants are increasingly endangered owing to over exploitation, habitat loss and fragmentation as well as other environmental factors. Adaptability of plants to environmental changes is influenced by the genetic variability of the plant. Hence, Understanding the genetic variation within populations is essential in establishing proper conservation approach. In this study, intra specific variation and biochemical relationships among populations of *L. aestuans, C. virginica* and *S. rhombifolia* were assessed.

MATERIALS AND METHODS

Sample collection

Fresh whole plants of *Laportea aestuans, Commelina virginica* and *Sida rhombifolia* and soil were collected from 19 Local Government areas (Mushin, Shomolu, Mainland, Oshodi/Isolo, Apapa, Kosofe, Ifako-Ijaye, Ibeju-lekki, Eti- osa, Lagos Island, Ikeja, Amuwo-odofin, Epe, Agege, Ojo, Ajeromi-Ifelodun, Surulere, Ikorodu, Alimosho, Badagry) in Lagos State. The samples were collected between March and August 2020. The GPS of sample

locations were used to generate Figure 1. Collected plants were authenticated at the University of Lagos Herbarium with the following Herbarium numbers *Laportea aestuans* (LUH: 8675), *Commelina virginica* (LUH 8673) and *Sida rhombifolia* (LUH 8674).

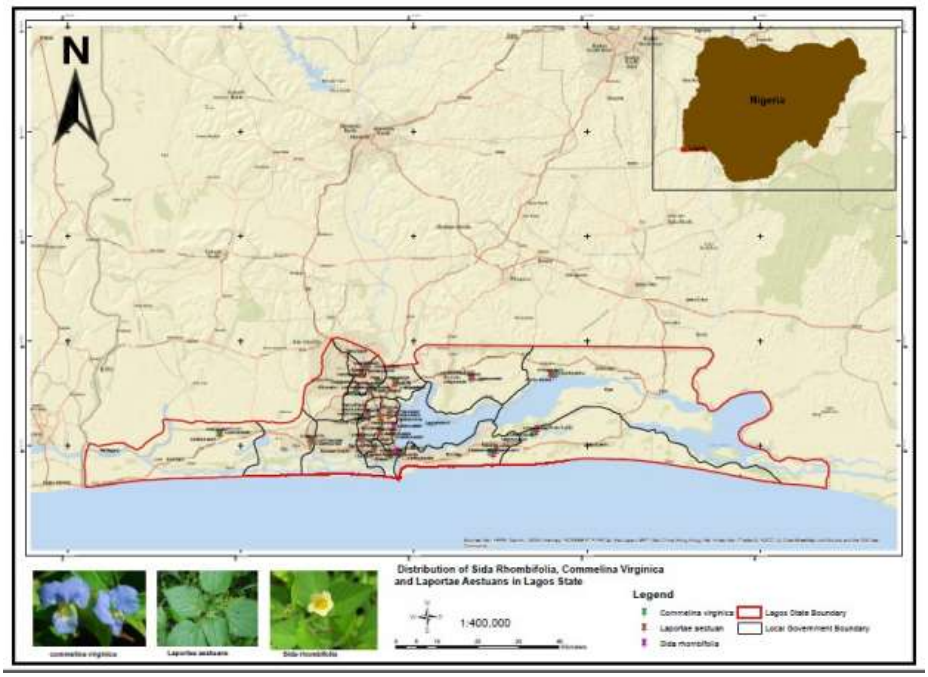

Figure 1: Distribution of *Laportea aestuans, Sida rhombifolia, Commelina virginica* in Lagos State.

GENETIC DIVERSITY STUDY

DNA extraction

The DNA of *L. aestuans, C. virginica* and *S. rhombifolia* were extracted separately using a slight modification of the method of Srilekha and Ravi [22]; Hamzah [23]. Fresh samples (leaves) of *L. aestuans, C. virginica* and *S. rhombifolia* were first homogenized using mortar and pestle. Then, 500µl is transferred into 2ml test tube and 250µl of hexadecyl trimethyl ammonium bromide (CTAB) and 20µl mercapto-ethanol were added. The tube was incubated at 60°C for 45min, and mixed every 5-10min by inverting. 100µl of chloroform: isoamyl alcohol (24:1) was added and mixed for 30mins by inverting. The tube was then centrifuged at 12000rpm for 10 min. The aqueous upper phase (containing the DNA) was transferred to a new 1.5ml tube and the steps repeated, but centrifuged for 5min. The aqueous upper phase was then precipitated with 0.6 volume of ice cold Isopropanol and 0.1vol of 3M Sodium acetate (pH 5.2) and centrifuged at 15,000rpm for 15 min. The pellets obtained were washed with 70% Ethanol and kept to dry at room temperature. The DNA obtained was dissolved in sterile distilled water and stored at -20ºC until used for PCR amplification

PCR amplification

Extracted DNA samples were adjusted to a final concentration of 50 ng/µL. Twelve (12) RAPD primers (Operon Technologies, Alameda, CA, USA) were selected and used for amplification. The RAPD reactions were performed with 50ng genomic DNA, 1X Buffer, 2mM MgCl2, 0.1mM dNTPs, 0.4 µM primer, 1 uni Taq DNA polymerase, and 25µl distilled water. Amplification was programmed in a Corbett Research CG1- 96 PCR thermal cycler: denaturation at 95ºC for 15 min, then 40 cycles at 95ºC for 1 min, annealing at 30ºC for 1 min, extension at 72ºC for 2 min, a final elongation was performed at 72ºC for 10 mins

and finally hold at 4ºC. The PCR products were separated on a 1% Agarose gel. Then the gel was stained with 5mg/ml ethidium bromide solution. Out of 12 decamer primers used, only 3 (OPC-04 CCGCATCTAC), (OPA-12 GAAACGGGTG), (OPY-20 AGCCGTGGAA) consistently produced reproducible bands and were therefor used

Determination of Heavy metal, Nutritional and Phytochemical composition

Samples from 10 most industrialized local government areas were randomly selected and assessed for heavy metal composition using Atomic Absorption Spectrophotometric (Buck Scientific, East Norwalk, CT06855, USA) as described by [24]. Composite samples of each plant (*L. aestuans*, *C. virginica* and *S. rhombifolia*) were analyzed for mineral nutrition and phytochemical composition. Electrical conductivity and PH was determined by the method of [25]. Mineral nutrition was determined by wet digestion procedure. Calcium and Magnesium were determined using the Atomic Absorption Spectrophotometer (Buck Scientific, East Norwalk, CT06855, USA). Sodium (Na) and Potassium (K) were determined by flame photometry (Jenway Ltd, Dunmow, Essex, UK) [26]. Phytochemical composition of aqueous extracts were done using the method of [27]

Data Analysis

Clear and repeatable amplification products were scored as 1 for presence of bands and 0 for absence of bands. Data generated were used to generate dendogram for Similarity metrics using NTSYSpc version 2.10e. Using Hardy-Weinberg equilibrium assumption, the null allele frequency (q) was calculated according to [28]

$$Null'\ allele\ frequency(\mathrm{q}) = \left(\frac{\mathrm{N}}{\mathrm{n}}\right)\frac{1}{2}$$

Where: N= number of negative bands

n= sample size

The similarity index values (SI) of individuals on the same gel were also calculated using a modified method of [28]

$$Similarity\ index\ (\mathrm{SI}) = \left(\frac{2\mathrm{NABC}}{(\mathrm{NA+NB+NC})}\right)$$

Where: N_{ABC} = No of RAPD bands shared by individual A, B and C

$N_A + N_B + N_C$ = No of fragments scored for each individual respectively.

Data for heavy metal were analyzed statistically using IBM SPSS statistics (version 24.0) at ($p<0.05$).

RESULTS AND DISCUSSION

Results for genetic diversity study of *L. aestuans*, *C. virginica* and *S. rhombifolia* are presented in plate 1, Table 1 and Figures 1 and 2. Plate 1 is the gel electropherogram (A) OPA12, (B) OPY20 and (C) OPC04. A total of 56 markers were produced of which 44 were polymorphic. Size of amplified products ranged from 500 to1500bp. Maximum number of bands (21) was produced by OPY20 while OPC04 produced minimum number of bands (14). Maximum and minimum percentage polymorphism were 100% (OPC04) and 50% (OPA12) with an average of 80.5%. Similarity index values were 1.13 (OPA12) and 1.00 each for (OPY20 and OPC04) while Null frequency values ranges from 0.05 to 0.34. Several authors have assessed the genetic diversity/relatedness of plant species with RAPD primers [29, 30, 26, 22, 31]. In the work of Srilekha and Ravi, [22], 100 percent polymorphism was observed in OPE-4 and OPA-9 primers similar to the finding of this research. Relatively high level of polymorphism were obtained in this study indicating moderate diversity between the genotypes similar to [32]. Dendrogram of similarity (Figure 1) constructed using Unweighted Pair Group of Method for arithmetic (UPGMA) with SM coefficient showed a genetic distance range of 0.57 to 1.00, 0.58 to 1.00 and 0.52 to 1.00 and clustered at 0.57, 0.58, 0.52 implying 57%, 58%, 52% similarity and 43%, 42% and 48% variability for *C. virginica*, *L.*

aestuans and *S. rhombifolia* respectively. The cluster tree analysis of *C. virginica* (Figure 1A) show that the genotype is divided into 5 main clusters. The 5 clusters were further divided into subgroups according to their similarity. Cluster one were the most closely related genotype. Samples from LA, BA (Lagos Island, Badagry) and OJ, AJ (Ojo, Ajeromi-Ifelodun) had 100% similarity while sample from Amuwo-odofin (AM) did not fall into any of the clusters, it is an out group separating from others at a similarity coefficient of approximately 0.60. Dendrogram of *L. aestuans* (Figure 1B) show 4 main genotypic groupings with several subgroupings. Samples from ET and LA (Eti-osa, Lagos Island) showed 100% similarity while sample from *SU* (Surulere) is an outlier separating at similarity coefficient of 0.60. Samples MA and IS (Mainland, Isolo) were also an out group at similarity coefficient of 0.58. The cluster tree of *S. rhombifolia* (Figure 1C) shows 3 main groupings, EP and AJ (Epe, Ajeromi-Ifelodun) had 100% genetic similarity while BA (Badagry) is an out group at similarity coefficient of 0.59. In this study, we report relatively low genetic variability (43%, 42% and 48%) among species of *C. virginica L. aestuans* and *S. rhombifolia* in Lagos state. Limited genetic variability was reported by [32] among citrus cultivars similar to the findings of this work. The genetic diversity of plants is closely related to their geographic distribution (22).

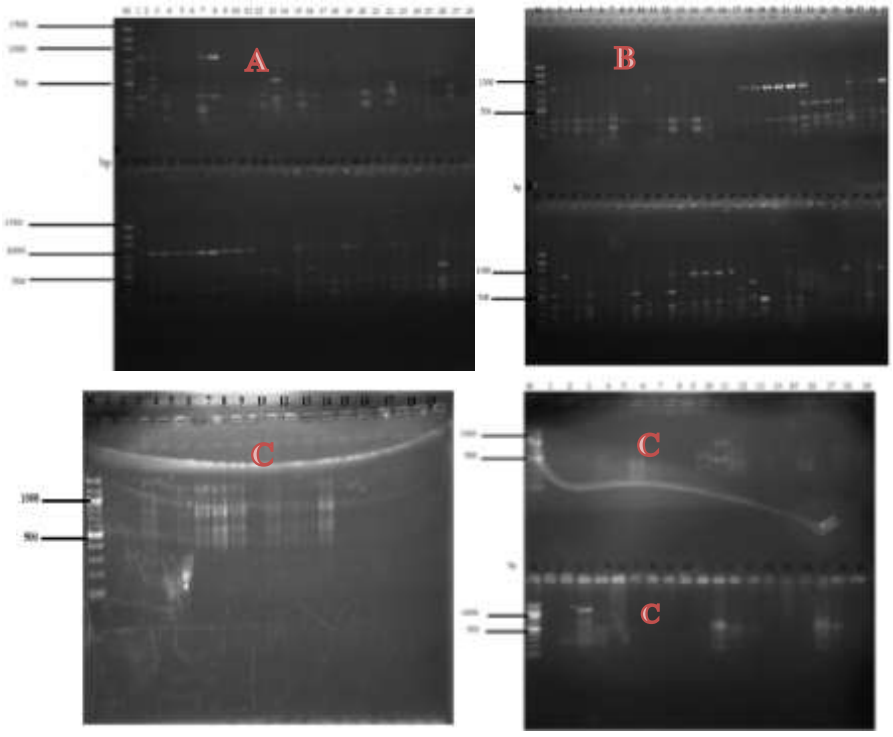

Plate 1: PCR electrophoregram of *L. aestuans, S. rhombifolia*, and *C. virginica* using RAPD Primers (A) OPA 12 (B) OPY20 and (C_1, C_2 & C_3) OPC04
Key: A and B, bands 1-19 and C_1 = *L. aestuans,* 20 to 38 and C_2= *C. virginica,* 39 to 57and C_3= *S. rhombifolia*

Table 1: Summary of percentage polymorphism, Null frequency and similarity indices

Primer/plants		No of bands	Polymorphic bands	% polymorphism	Null frequency (q)	Similarity index (SI)
OPA12	*L. aestuans*	9	7	77.8	0.13	1.13
	C. virginica	6	4	66.7	0.08	
	S. rhombifolia	6	3	50	0.05	
OPY20	*L. aestuans*	6	5	83.3	0.05	1.00
	C. virginica	7	5	71.4	0.03	
	S. rhombifolia	8	6	75	0.11	
OPC04	*L. aestuans*	5	5	100	0.32	1.00
	C. virginica	5	5	100	0.34	
	S. rhombifolia	4	4	100	0.26	

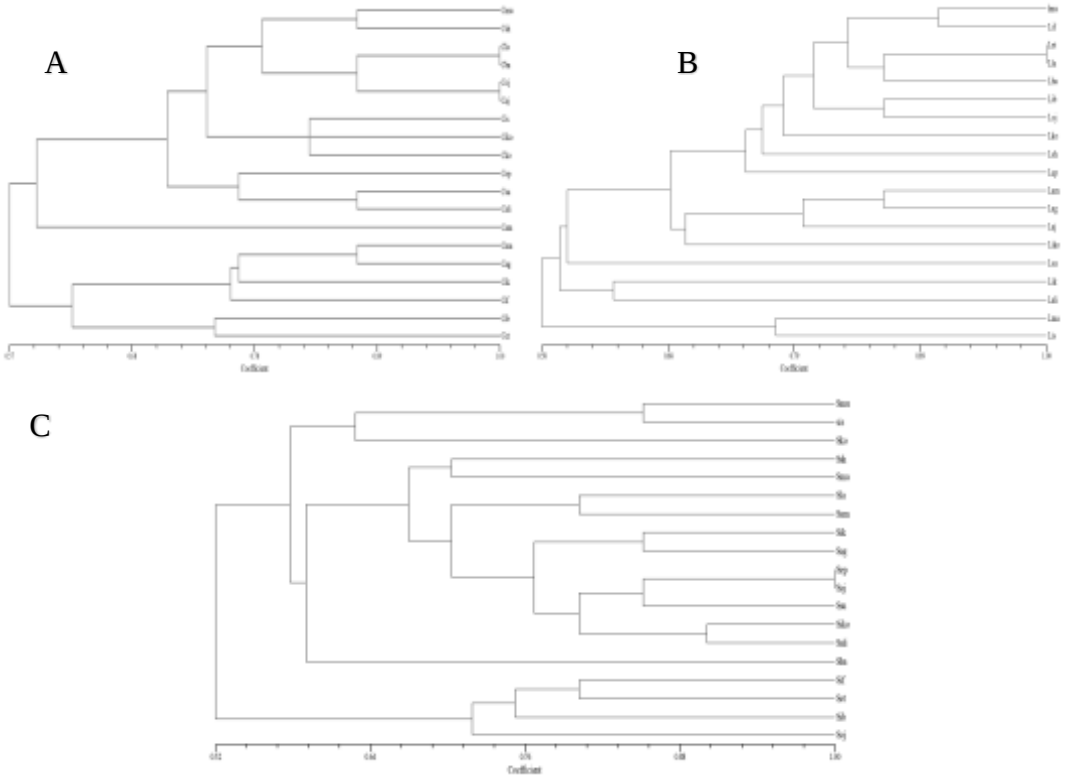

Figure 1: Similarity metrics of (A) *Commelina virginica (B) Laportae aestuans* and (C) *Sida rhombifolia* using **SM** coefficient, **UPGMA** clustering method

Key: Figure 1A (C)= *Commelina virginica*, **Figure1B** (*L*)= *Laportae aestuans*, **Figure1C** (S)= *Sida rhombifolia*. mu=Mushin, if= Ifako-Ijaye, et= Eti- osa, la= Lagos Island, ba=Badagry, ib= Ibeju-lekki, oj= Ojo, ko= Kosofe, sh= Shomolu, ep= Epe, am= Amuwo-odofin, ag= Agege, aj= Ajeromi-Ifelodun, iko= Ikorodu, su= Surulere, ik= Ikeja, ali= Alimosho, ma= Mainland, is= Oshodi/Isolo, Local government areas respectively.

Mineral, phytochemical and heavy metal composition

Results of mineral nutrition and phytochemical composition of *S. rhombifolia, C. virginica* and *L. aestuans* are presented in Tables 2 and 3 while the physicochemical composition of soil sample and the heavy metal composition of *S. rhombifolia, C. virginica* and *L. aestuans* are presented in Tables 4 and 5. Result of mineral nutrition show that *S. rhombifolia* has the highest concentration of nitrate (5415.0±2142.5), sodium (808.0±2.8),

calcium (2150.0±70.7) and magnesium (727.5±3.5) but with the lowest value of phosphate (184.5±6.4) while *L. aestuans* contain the highest amount of potassium (4125.0±35.4). Results of phytochemical analysis show that *S. rhombifolia* is particularly high in steroids I134.19±0.23), flavonoids (118.65±0.18), phenolics (125.25±1.43), alkaloids (63.56±0.11) and triterpenes (72.44±0.12) but low in saponins (23.29±0.05), glycosides (6.79±0.01) and tanins (7.10±0.02). *Laportae aestuans* however had the higher composition of phenolics (518.18±1.43). Some authors had investigated the presence of phytochemicals in *S. rhombifolia* [33, 34, 35]. In this study, *S. rhombifolia* were found to contain steroids, flavonoids, phenolics, alkaloids, terpenoids coumarins, triperpenoids, saponins and glycosides. Phytochemical content in plants and their bioavailability in human has been shown to depend upon among other thing; plant's varieties, genotypes, culture conditions, maturity, thermal processing, food matrix, structure, presence of other nutrients, site of absorption, etc. [36]. Although the concentrations of saponins (23.29±0.05)), tanins (7.10±0.02) and glycosides (6.79±0.01) were low, they were however present, contrary to the findings of [31]. Kamdoum, *et al.* [37], investigated the phytochemical composition of EtOH/H_2O (7:3) extracts of *Sida rhombifolia* L. and *Sida acuta* and found 2 previously undescribed compounds, (rhombifoliamide and xylitol dimer) in addition to other compounds. Health benefits of phytochemicals (secondary metabolites) have been outlined by [38; 36] to include: maintenance of inflammation balance, promotion of cardiovascular, neurocognitive, eye, and bone health in humans as well as help to reduce the risk of high blood pressure, cancers, diabetes, inflammation, microbial, viral and parasitic infections. Results for metal composition show that metal content of the plants are well below World Health organization (WHO) safe limits for all metals determined and for all locations (Table 5).

Table 2: Mineral nutrition of *Laportae aestuans, Sida rhombifolia* and *Commelina virginica*

Plant	$N0^-_3$	$P0_4^{3-}$	Na	K	Ca	Mg
L. aestuans	3736.0^{n}±22.6	212.0^{p}±2.8	715.0^{x}±7.1	4125.0^{u}±35.4	2070.0^{c}±42.4	695.0^{j}±7.1
S. rhombifolia	5415.0^{n}±2142.5	184.5^{o}±6.4	808.0^{y}±2.8	3425.0^{v}±35.4	2150.0^{c}±70.7	727.5^{k}±3.5
C. virginica	3675.0^{n}±35.4	215.0^{p}±7.1	642.5^{z}±3.5	3475.0^{v}±35.4	1935.0^{c}±49.5	685.0^{j}±7.1

Values are presented as mean ± SD. Mean with different superscript are significantly different from one another.

Table 3: Phytochemical composition

Compounds (mg/100g)	*S. rhombifolia*	*L. aestuans*
Saponin	23.29*±0.05	22.01±0.04
Steroids	134.19*±0.23	93.02±0.23
Flavonoids	118.65*±0.18	106.72±0.36
Phenolics	125.25±1.43	518.18*±1.43
Terpenoids	18.29*±0.11	16.88±0.08
Coumarins	72.00±0.06	72.41±0.17
Glycosides	6.79*±0.01	5.79±0.01
Alkaloids	63.56±0.11	63.90±0.03
Triterpenes	72.44*±0.12	67.69±0.23
Tanin	7.10±0.02	7.60*±0.01

*Values are presented as mean ± SD. Mean with * superscript is significantly higher at 5% level*

Table 4: Physicochemical Properties of Soil Samples

Parameters	Unit	Mean ± SD
pH	-	6.86±0.01
Ec	µ*S*	1,96±0,02
Total Organic Carbon (T.O.C.)	%	1.10±0.01
Total Organic Matter (T.O.M.)	%	1.89±0.01

Table 5: Heavy metal composition of soil and *Sida rhombifolia, Commelina virginica* and *Laportae aestuans*

Location /metals (mg/kg)	Zinc				Iron				Copper			
	Soil	SR	CV	LA	Soil	SR	CV	LA	Soil	SR	CV	LA
Isolo	47.0± 1.41	7.95± 0.99	7.05± 1.63	8.40± 0.42	286.0± 5.66	7.05± 1.63	27.5± 0.71	27.5± 2.12	17.0± 1.41	3.65± 0.07	2.70± 0.14	4.25± 0.35
Ikeja	45.5± 0.71	6.95± 0.07	6.35± 0.07	7.15± 0.21	278.0± 2.83	6.35± 0.07	34.0± 1.41	29.0± 1.41	15.5± 0.71	4.25± 0.21	1.95± 0.07	5.15± 0.07
Shomolu	55.5± 0.71	6.25± 0.21	7.10± 0.14	7.65± 0.07	287.0.5 ±3.54	7.10± 0.14	35.5± 1.71	31.0± 1.41	17.5± 0.71	31.0± 1.41	4.00± 0.14	2.65± 0.07
Agege	47.0± 1.41	6.65± 0.35	6.45± 0.35	7.30± 0.14	307.5± 3.54	6.45± 0.35	26.5± 0.71	28.5± 0.71	15.5± 3.54	2.85± 0.21	4.00± 0.14	3.20± 0.00
Apapa	49.0± 1.41	8.05± 0.21	6.40± 0.71	7.70± 0.28	246.5± 3.54	6.40± 0.71	35.0± 1.41	33.0± 1.41	17.5± 0.71	2.70± 0.14	3.90± 0.28	3.90± 0.14
Alimosho	53.0± 1.41	6.25± 0.21	6.55± 0.07	6.90± 0.14	344.0± 8.49	6.55± 0.07	32.0± 1.41	27.0± 1.41	13.5± 0.71	2.70± 0.28	2.65± 0.07	3.90± 0.14
Surulere	53.0± 1.41	6.65± 0.92	6.25± 0.07	7.60± 0.28	226.5± 0.71	6.25± 0.07	40.5± 0.71	30.5± 0.71	17.5± 0.71	2.70± 0.71	3.90± 0.14	2.90± 0.14
Badagry	51.0± 1.41	7.95± 0.21	8.05± 0.21	7.50± 0.14	289.0± 4.23	8.05± 0.21	28.5± 0.71	29.5± 0.71	14.0± 2.83	3.50± 0.28	3.70± 0.14	4.00± 0.14
Ikorodu	47.5± 2.12	7.40± 0.28	7.15± 0.50	7.45± 0.21	312.5± 3.54	7.15± 0.50	30.5± 2.12	26.5± 0.71	15.0± 0.00	3.10± 0.14	2.65± 0.07	5.25± 0.21
Epe	52.0± 1.41	7.90± 0.14	5.45± 0.50	7.00± 1.13	246.5± 3.54	5.45± 0.50	27.5± 2.12	29.5± 0.71	20.5± 2.12	3.25± 0.21	3.65± 0.07	3.60± 0.28
WHO	200.0	99.4	99.4	99.4	21000. 0	99.4	1000. 0	1000. 0	50.0	20.0	20.0	20.0

Key; SR= *Sida rhombifolia,* CV=*Commelina virginica,* LA= *Laportae aestuans*

CONCLUSION

Among the different type of molecular markers RAPD has been shown to be essential in assessment of genetic similarity and variability within plant populations. Genetic variation has been shown to play role in maintaining the developmental stability and biological potential of plant species. This study observed relatively low genetic variability among populations of *S. rhombifolia, C. virginica* and *L. aestuans.* There is therefore, the need for deliberate effort to reduce habitat destruction and fragmentation with a view to conserving genetic diversity and in turn biodiversity at large in Lagos State

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Conflict of interest

No conflict of interests exist

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