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EX-SITU Characterization of *Luffa aegyptiaca* in Lagos State, Nigeria

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Abstract:

Luffa aegyptiaca is a plant of multi-purpose importance whose usefulness cuts across virtually all areas of life. This study has characterized *L. aegyptiaca* in Lagos state and determined the mineral, proximate, phytochemical as well as the heavy metal accumulation potential. Samples were collected from the 20 Local Government Areas (LGAs) in Lagos state at 2 samples per location. Genetic similarity and intra-specific variation in 40 samples of *L. aegyptiaca* were determined using 3 Random Amplified Polymorphic DNA (RAPD) primers which yielded a total of 42 markers of which 25 was polymorphic. The maximum number of bands (14) was produced by OPC4 while the minimum (7) were produced by OPAF20. Percentage polymorphisms were 70% (OPAF20), 82.4% (OPC4) and 68.4% (OPC6) with an average value of 73.6%. The result from a genetic diversity study was scored to generate a dendrogram using NTsys (2.0j). Phytochemical, proximate, Mineral and the heavy metal study showed the presence of Flavonoid, Saponin; Carbohydrate, protein; Sodium (Na), Calcium (Ca) and Chromium, Iron, Copper, Zinc, Lead among others. The nutritional composition and the potentials of the RAPD marker in distinguishing intra-specific variation in *Luffa aegyptiaca* were highlighted in this study.

Key words: Characterization, Dendrogram, *Luffa aegyptiaca*, Phytochemical, Polymorphism.

Introduction:

The gourd is generally used to describe the crop plants in the family Cucurbitaceae. The term gourd refers to around 825 species derived from tropical and subtropical regions, out of which approximately 26 species are cultivated as vegetables including *Luffa aegyptiaca* (1). *Luffa aegyptiaca* (Mill) is a member of the Cucurbitaceae family (2). There are about nine species in the genus *Luffa* including *Luffa acutangula*, *Luffa cylindrica*, *Luffa aegyptiaca*, *Luffa operculata*, *L. graveolens* and *L. echinata* (3). *Luffa cylindrica* (found mostly in South America) is the most widely published and cultivated (4, 5). *Luffa aegyptiaca* (Mill) is found mostly in tropical Africa including Nigeria and some parts of India (6, 3, 7). *Luffa aegyptiaca* is commonly found around drainage channels, on dumpsites and uncompleted buildings clinging to nearby objects for support. The flower is yellow and blooms between August-September (2). *Luffa aegyptiaca* is a monoecious annual climber that produces fruit containing fibrous vascular system and has been shown to grow best at a pH of 6.5 and nitrogen and phosphorus being the major elements limiting the growth of the plant (4, 7).

Plate 1 : *Luffa aegyptiaca* (A) plant on a dump site (B) plant with fruit and flower, (C) seeds and (D) sponge

Generally, *L. aegyptiaca* can be used in virtually all areas. It has been suggested as a packing medium in an attached growth system (8), as an immobilization matrix for microbial cells (9). Young fruits are edible and matured fibers are generally used in washing and bathing, as packing materials, for making crafts, as filters in factories, and as a part of soles of shoes (10). *Luffa aegyptiaca* has been used in the treatment of respiratory disorders. Juice extracted from the stem and the seed has emetic action. *Luffa aegyptiaca* possess anti-inflammatory, analgesic, sedative, antifungal, expectorant and antimicrobial properties. It has been discovered that sponge gourd can supply some antioxidant constituent to the human body (5). Loofa sponge has been used as a medium for the culture of the human hepatocyte cell line (11). It possesses some nutritional properties (10), and its use as biodiesel is now gaining wide acceptance because of low CO_2 emission and other considerations (12). Two isoforms of ribosome inactivating protein (RIP), luffin-a, and luffin-b were extracted from seeds of *L. aegyptiaca* (8).

Plant molecular biology is the study of the molecular basis of plant life. It is particularly concerned with the processes by which the information encoded in the genome is structures, processes and behaviors. Molecular techniques such as DNA barcoding, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and single nucleotide polymorphisms (SNP) is being used for plant diversity studies (13). RAPD is a fast and sensitive method and is able to provide reproducible and characteristic fingerprints of complex genomes without prior sequence information. Most informative DNA bands on RAPD are usually of the 300- 3000bp range. RAPD provides a cost-effective method for the precise and routine evaluation of variability. It may also be used to identify areas of maximum diversity (13). This study aims at characterizing *Luffa aegyptiaca* in Lagos State, determine its mineral, phytochemical composition as well as the heavy metal accumulation potentials.

Materials and Methods:

Collection and Identification of Plant Materials

Fresh samples of matured *Luffa aegyptiaca* plant were randomly collected from the Twenty (20) LGAs in Lagos State, South-Western Nigeria and identified at the University of Lagos Herbarium. Forty (40) samples were collected altogether at Two (2) samples per location for the molecular study. Samples were randomly selected for mineral, proximate and phytochemical analysis. Samples from eight most industrialized LGAs were assessed for heavy metal accumulation.

Mineral, Phytochemical, nutritional and heavy metal content analysis

Collected *Luffa aegyptiaca* samples were randomly selected for mineral, phytochemical, and proximate while samples from eight most industrialized LGAs were randomly selected and assessed for heavy metal accumulation. The mineral analysis was determined using the wet digestion procedure. Calcium, Magnesium and Nitrogen contents 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) while Phosphorus (P) was obtained by the Vanadomolybdate method (14). Phytochemical analysis of ethanoic extract of *L. aegyptiaca* was done using the method of (15) while heavy metal compositions of soil and *L. aegyptiaca* were determined using Atomic Absorption Spectrophotometric (AAS) as described by (16). The physicochemical properties of the soil for heavy metal analysis were also determined.

Determination of Metal Accumulation Quotient

The translocation factor (TF) was calculated using the procedure described by (16).

$$TF = \frac{\text{Metals in shoot}}{\text{Metals in root}}$$

Biological concentration factor (BCF) was calculated as described by (17).

$$BCF = \frac{\text{Metals in root}}{\text{Metals in soil}}$$

The biological accumulation coefficient (BAC) was calculated using the method of (18).

$$BAC = \frac{\text{Metals in shoot}}{\text{Metals in soil}}$$

Genetic diversity study

Luffa aegyptiaca DNA was extracted following the method of (19, 20) while the RAPD PCR reaction was carried out using the method of (21, 22). The result from the molecular study was scored to generate a dendrogram using NTsys (2.0j).

Data analysis

Clear and repeatable amplification products were scored as 1 for present bands and 0 for absent. Polymorphism was calculated based on the presence or absence of bands as suggested by (23)

Results and Discussion:

Results for phytochemical, nutritional and mineral content analysis are presented in Tables 1, 2 and 3 respectively. From the results, Flavonoid and Alkaloid were absent in the root while Steroid and Anthraquinone were present only in the root and fruit, respectively. Tannins were present only in the leaves and fruits whereas Phlobotanin and Terpenoid were absent in the plant completely (Table 1). In this study tannins, saponin and flavonoid were present, different from the finding of (15). The medicinal, antibiotic and other health benefits of the phytochemical component of plants have been highlighted by (24, 25, 26). Nutritionally, *L. aegyptiaca* is rich in protein, carbohydrate, and fiber. The fruit which is the generally edible part of the plant is high in carbohydrate and fiber (Table 2). The mineral composition shows that the matured fruit has the highest composition of Sodium (101.94). The plant at maturity also has a high composition Potassium, Calcium, Magnesium and Iron (Table 3).

Table 1. Phytochemical composition of *L. aegyptiaca*

Compound (Mg/kg) /plant	Flavonoid	Phenol	Phlobatanin	Tannin	Steroid	Saponin	Alkaloid	Cardiac glucoside	Terpernoid	Anthraquinone
Leaves	+	+	-	+	-	-	+	+	-	-
Roots	-	+	-	-	+	+	-	+	-	-
Fruit	+	+	-	+	-	+	+	+	-	+
Stem	+	+	-	-	-	+	+	+	-	-

Key: + = present, - = absent

Table 2. Nutritional composition of *Luffa aegyptiaca*

Nutrient (%) /Plant	CHO	Protein	Crude fat	Moisture	Ash	Crude fiber
Leaves	52.58	4.90	0.86	21.47	7.96	12.23
Roots	48.71	4.24	1.41	23.79	4.52	17.33
Fruit	37.97	2.32	2.11	24.62	5.39	27.59
Stem	19.04	1.36	0.96	36.28	4.82	37.54

Key: CHO = Carbohydrate,

Table 3. Mineral composition of *Luffa aegyptiaca*

Mineral (Mg/100g)/ plant	Fe	Cu	Zn	Na	Ca	Mg	K
Leaves	41.05	1.30	21.30	73.05	192.10	58.05	89.45
Roots	43.20	1.25	25.45	92.05	214.05	62.30	72.05
Fruit	18.07	1.22	9.71	101.94	91.35	25.50	76.70
Stem	17.62	1.04	8.20	75.40	124.23	48.99	48.86

Key: Fe= Iron, Cu = Copper, Zn =Zinc, Pb = Lead, Cd= Cadmium, Na= Sodium, Ca= Calcium, Mg = Magnesium, K= Potassium.

Heavy Metal Analysis

The physicochemical properties of soil for heavy metal analysis are presented in Table 4 while the result of heavy metal accumulation potential of whole *Luffa aegyptiaca* plant is presented in Table 5. The pH of the soil is generally neutral, organic matter and organic carbon averaged 3.3 and 2.4 percent, respectively (Table 4). The maximum uptake of chromium (18.62 µg/g), copper (361.4 µg/g), Zinc (259.5 µg/g), and cadmium (16.62µg/g) were found in the plant from Kosofe with the highest Electrical Conductivity of (422 μ/CM^3). These values were well above the permissible limits for plants and indicate that the increase in EC increases uptake of these metals by plants. The same is true for organic matter, sulfate, and phosphate whose increase in values corresponds to increased uptake of chromium, copper, zinc, and cadmium in the plant (Table 5). The lowest uptake of all the metals tested was found in the plant from Ojo Local government area whose values were all below the recommended limits for plants and corresponds to the lowest value of physicochemical parameters measured with the exception of organic carbon and electrical conductivity (Tables 4 and 5). The mobility of the metals in *L. aegyptiaca* determined using the translocation factor (TF), Biological concentration factor (BCF), as well as the Biological accumulation coefficient (BAC), show that most of the metals were easily absorbed from the soil and readily transferred to the aerial parts of the plant (Fig. 1). The mobility of the metals seems to be influenced by the physicochemical characteristics of the soil similar to the finding of (27). The translocation factor (TF), the Biological concentration factor (BCF) and the Biological accumulation coefficient (BAC) of heavy metals in soil and *L. aegyptiaca* from the polluted and unpolluted environment show that Chromium, Iron, and Manganese were all within the WHOs safe limit for both soil and plant in all the sampled location. However, cadmium, lead, copper, and zinc were above the WHOs safe limit for a dump site. Attention is drawn particularly to the concentration of cadmium, lead, zinc and copper whose concentration in dump site far exceeds WHOs safe limit in both the root and shoot of *L. aegyptiaca*. This is because *L. aegyptiaca* is commonly seen growing in dumpsites from where the young fruits are plucked and eaten or sold in the market by the locals. The concentration of zinc in *L. aegyptiaca* from dumpsite apart from being greater than WHOs' safe limit is seen to be greater than that in soil. This may be due to the ability of *L. aegyptiaca* to accumulate the metal. Hence *L. aegyptiaca* is indicated to be a phyto-accumulator of heavy metals especially zinc. The adverse effect of heavy metals on human health and on plants has been documented by several authors (28, 29). Among the heavy metals mercury, lead, arsenic, and cadmium are toxic metals and have mutagenic effects even at very low concentrations. Several cases of human disease, malfunction and malformation of organs due to metal toxicity have been reported (28). Consumption of contaminated vegetables may cause immunological disorders, impair psycho-social behavior, and may retard growth due to nutrients depletion. There is an urgent need to increase awareness of the side-effects of heavy metals on human health.

Table 4. Physico-chemical properties of soil used for heavy metal assessment

Parameters	Unit	Kosofe	Ikeja	Ojo	Ikotun	Mushin	Shomolu	Surulere	Oshodi
pH	*	6.38	6.50	6.79	6.23	6.14	6.11	6.53	6.86
Ec	μ/CM^3	422.00	398.20	291.20	301.11	284.56	278.21	412.50	351.10
Organic matter	%	3.64	3.38	3.54	3.48	3.21	3.33	3.84	3.48
organic Carbon	%	3.11	1.96	2.11	2.45	2.65	2.00	2.24	1.84
Nitrate	mg/kg	9.80	6.48	2.42	3.14	2.45	3.22	5.42	8.43
Sulphate	mg/kg	29.80	14.42	6.70	14.56	9.48	24.32	30.4	12.11
Phosphate	mg/kg	69.88	54.28	10.70	65.25	15.44	41.27	20.34	10.50

Key: Ec= Electrical Conductivity, * = has no unit

Table 5. Heavy metal accumulation by *Luffa aegyptiaca*

Location/metals	Chromium		Iron		Copper		Zinc		Lead		Cadmium		Manganese	
(mg/kg)	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
Kosofe	14.21	18.62	562.40	692.3	209.40	361.4	109.1	259.5	0.82	0.93	10.42	16.62	6.87	8.16
Main Land	7.84	9.05	9.84	12.25	27.04	37.91	10.81	20.37	0.98	0.43	0.14	0.11	6.32	7.09
Ojo	0.11	0.13	0.98	16.13	0.42	0.35	2.87	2.39	-	-	-	-	0.92	0.78
Ikotun	1.24	0.26	138.42	44.61	44.10	15.71	20.17	0.86	43.10	0.21	1.04	0.05	50.10	6.10
Mushin	2.11	0.28	802.10	26.04	10.00	0.32	43.08	2.37	105.14	0.56	2.14	0.001	47.25	4.11
Surulere	4.75	1.07	407.40	37.70	15.01	6.91	50.11	3.95	64.70	0.08	2.00	0.05	18.49	1.44
Shomolu	81.4	0.45	107.81	7.73	23.32	0.37	98.73	1.48	35.87	0.19	1.41	0.08	30.43	1.29
Oshodi	57.98	0.59	385.68	41.85	34.44	0.25	44.16	2.35	81.54	0.39	1.35	0.05	49.59	1.70
WHO	400	1.30	21000	1000	50	20	200	99.4	300	0.30	3	0.20	80	30

Key: - = not detected

Genetic diversity

Plate 2 shows the PCR electropherogram of *Luffa aegyptiaca* using Random Amplified Polymorphic DNA (RAPD) Primers OPAF20 (GTCCACACGG), OPC4 (CCGCATCTAC) and OPC6 (GAACGGACTC) while the dendrograms are presented in Fig. 1. The ability of RAPD markers in distinguishing genetic diversity in plant populations has been highlighted by several authors (13, 30, 19). The use of molecular markers in genetic diversity studies has shown promise in plant breeding and systematics. In addition, estimating genetic diversity can increase the efficiency of breeding program, and thus both intra and inter specific variation may be quantified accurately (31). In this study, PCR amplification of the DNA extract of 40 samples of *L. aegyptiaca* produced 42 amplified products, of which 25 were polymorphic. Primer OPC4 produced the maximum number of the polymorphic bands (14) while the minimum number (7) was produced from OPAF20. Percentage polymorphism were 70% (OPAF20) 82.4% (OPC4) and 68.4% (OPC6) with an average value of 73.6%. The dendrogram of genetic diversity study had a genetic distance range of 0.40-1.00 and clustered at 0.44, implying 44% similarity and 56% variability. The present study obtained a higher average polymorphic bands (73.6%) compared to the previous study by (19, 32) using RAPD primers and species of cowpea.

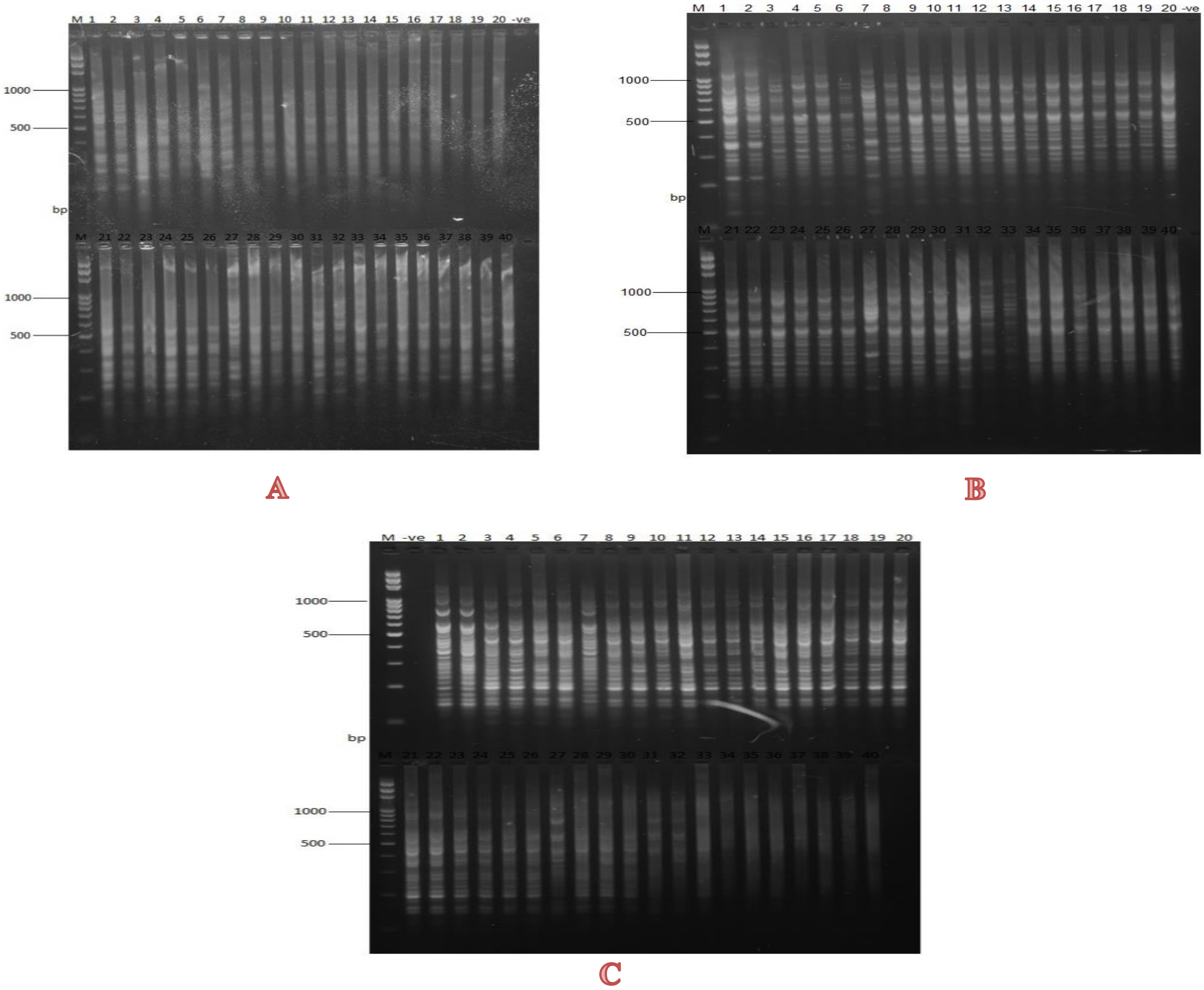

Plate 2. PCR electrophoregram of *Luffa aegyptiaca* using RAPD Primers (A) OPAF 20 (B) OPC4 and (C) OPC6

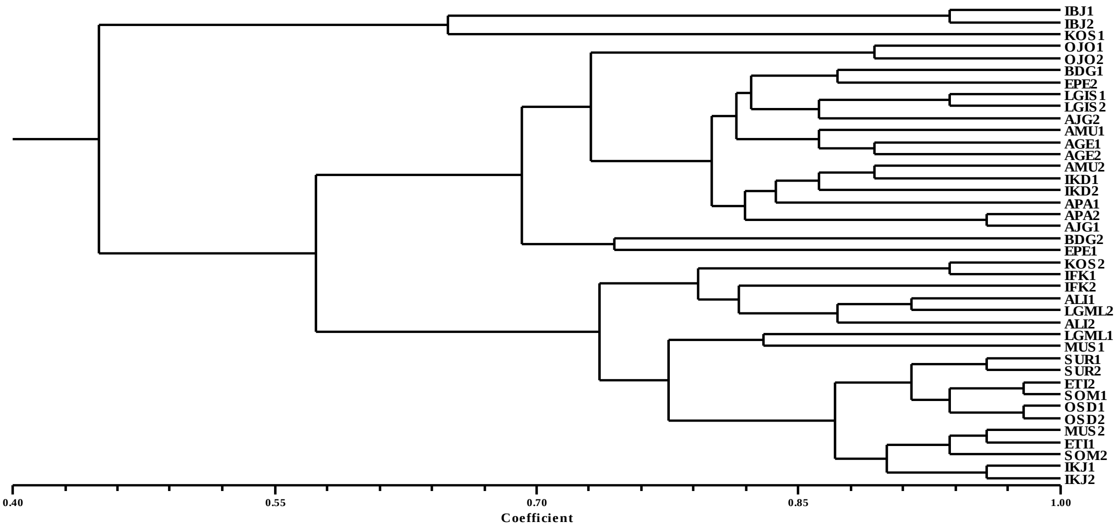

Figure 1. Dendrogram of *L. aegyptiaca* samples in Lagos state

Key: IBJ=Ibeju-Leki, KOS= Kosofe, Ojo, BDG= Badagry, Epe, LGI= Lagos-Island, AJE= Ajeromu-Ifelodun, AMU= Amuwo-odofin, AGE= Agege, IKD=Ikorodu, APA=Apapa, BDG=Badagry, KOS=Kosofe, Ifk=Ifako-ijaye, LGM=Lagos-mainland, MUS=Mushin, SUR=Surulere, ETI=Etiosa, SOM=Somolu, OSD=Oshodi, IKJ=Ikeja

Conclusion:

Luffa aegyptiaca contains saponins, glycosides, and terpenoids, phenol, tanin, flavonoid among others. The presence of those metabolites no doubt is indicative of the potential medicinal value of the plant. The potentials of RAPD markers in distinguishing inter and intraspecific variation in *Luffa aegyptiaca* is highlighted in this study.

Recommendations

The cultivation of *Luffa aegyptiaca* is highly recommended in areas with heavy metal contamination, especially in less technologically developed countries of the world.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been dully referenced

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خصائص نبات الليف (*Luffa aegyptalca*) مختبريا في ولاية لاغوس, نبجيريا

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1 قسم العلوم البيولوجية ، كلية يابا للتكنولوجيا ، يابا ، لاغوس. نيجيريا. 2 قسم النبات ، جامعة لاغوس ، أكوكا ، لاغوس. نيجيريا.

الخلاصة:

Luffa aegyptiaca نبات ذا خصائص متعددة ومهمة واستخدامه امن في جميع مجالات الحياة. درست خصائص نباتaegyptiaca L. في ولاية لاغوس والتحديد التقريبي للمعادن والمواد الكيميائية النباتية و المعادن الثقيلة. تم جمع العينات من 20 منطقة حكومية محلية (LGAs) في مدينة لاغوس عينتين من كل موقع. تم تحديد التشابه الوراثي والتباين داخل النوع ل 40 عينه من نبات.L. aegyptiaca وذلك باستخدام 3 برايمرات التضخيم العشوائي المتعدد الاشكال ل(DNA (RAPD والتي انتجت 42 معلم منها 25 كانت متعدد الأشكال.باستخدام OPC4 انتاجت اعلى عدد من النطاقات وهي 14,بينما OPAF20 انتجت اقل عدد وهي 7. كانت النسبة المئوية لتعدد الاشكال 70% (OPAF20) و82.4% (OPC4) و 68.4% (OPC6) بمتوسط قيمة 73.6%. النتيجة من دراسة التنوع الوراثي هدفت الى الرسم الشجري باستعمال NTsys(2.0...). أظهرت الدراسة التقريبية الكيميائية للنبات والمعادن والعناصر الثقيلة, الى وجود الفلافونويد والصابونيات والكربوهيدرات والبروتين والصوديوم(Na) والكالسيوم(Ca) والكروم والحديد والنحاس والزنك. تم تسليط الضوء في هذه الدراسة على الكونات الطبيعة وقدرة ال .RAPD في تمييز التباين داخل النوع في نبات Luffa aegyptiaca..

الكلمات المفتاحية: الرسم الشجيري, المركبات الكيميائية النباتية, التوصيف, تعدد الاشكال Luffa aegyptiaca .