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# Ethnobotanical survey and genetic conservation of underutilized leafy vegetables in lagos, Nigeria

Temitope Olabisi ONUMINYA<sup>\*</sup>, Oredolapo Ezekiel SHODIYA and Peace Busola EHINJU

Department of Botany, University of Lagos, Akoka, Lagos, Nigeria. \* Corresponding author; E-mail:topssy4u@yahoo.co.uk

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## ABSTRACT

Many of the leafy vegetables used for food in Nigeria are neglected and underutilized. To assess their diversity in Lagos State, an ethnobotanical survey and genetic conservation was carried out to preserve indigenous knowledge on their usage and conserve their DNA for future use. This involved collection of leafy vegetables from eight different markets in Lagos followed by semistructured questionnaire-guided interview and extraction of DNA samples. Three hundred respondents participated in the study comprising 81.67% female and 18.3% male. A total of 19 leafy vegetables belonging to 18 genera and 14 plant families were documented. About 52.6% of the documented leafy vegetables are restricted to southern Nigeria, while 47.4% are available and used nationwide. All the collected samples yielded high molecular weight DNA; these have been deposited at the University of Lagos DNA bank. The purity of the samples was also high with exception to that of *Crassocephalum cerepidioides* and *Heinsia crinata*. This work has contributed to the preservation of knowledge on leafy vegetables in Lagos and conservation of their DNA can be seen as a first step in the genetic conservation of the samples serving as a basis upon which other research can be based.

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#### **INTRODUCTION**

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked (Dhellot et al., 2006). They contain both essential and toxic elements over a wide range of concentrations (Okorondu et al., 2013) which can be successfully utilized to build up and repair the body as well as maintain alkaline reserve of the body (Okolo et al., 2015). They also act as buffering agents for acidic substances produced during the digestion process (Badau et al., 2013).

Leafy vegetables most often include short-lived herbaceous plants such as lettuce and spinach; leaves of woody plants such as

© 2018 International Formulae Group. All rights reserved. DOI : https://dx.doi.org/10.4314/ijbcs.v12i2.7 Adansonia, Aralia, Moringa, Morus and Toona as well as fodder crops (e.g. Alfalfa). They constitute the main portion of the diets of rural and urban households across most of Africa hence their conservation is of concern to both national and international agricultural research centers in the region (Gockowski et al., 2003). In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, leafy vegetables are the cheapest and most readily available sources of important proteins, vitamins minerals and essential amino acids (Aja et al. 2010; Olaposi and Adunni, 2010; Saramma and Padmaja, 2013). They are typically low in calories and fat, and high in protein per calorie, dietary, fiber, vitamin C, pro-vitamin carotenoids, A, foliate, manganese and vitamin K (Adjatin et al., 2013). They occur in a variety of colors and flavors from sweet to bitter, from peppery to earthy. Some are used in traditional medicine; for weight management, in reducing the risk of cancer and heart diseases and also to improve immune function (George et al., 2004; Chaturvedi et al., 2007; Janick, 2011).

Several researchers including Adebooye and Opabode (2004) as well as Adebooye and Ajayi (2008) have shown concern for the fast rate at which the genetic base of the World's food is being eroded due to scientific concentration on very few crop varieties. Amujovegbe et al. (2007) observed that the genetic diversity of vegetable crops in Africa generally, and in Nigeria in particular, was for long а time naturally preserved by the traditional cropping system. However, in the recent times, there has been rapid deterioration of natural resources resulting in the loss of genetic diversity due to pressure on land, as a result of human activities for industrialization and urbanization (Shebu and Sewuese, 2014). Factors such as rapid changes in land use, modernization of agricultural practices, deforestation and adoption of new varieties processing have narrowed genetic base and this has contributed to the rapid disappearance of many land races of cultivated vegetables and their wild relatives (Amujoyegbe et al.,

2007). Despite the nutritional and medicinal values of these vegetables, they have been scientifically neglected in place of the exotic ones which may even be nutritionally inferior. Although they are greatly appreciated for their taste and nutritional quality, they are often the first item to drop from the household diet when the economy of the family improves. Diouf et al. (2007) identified traditional vegetables as being associated with poor rural lifestyles and low status, hence cultural changes and urbanizations have led to further neglect in response to the decline in production, consumption and diversity of indigenous vegetables. This research therefore aimed at carrying out an ethnobotanical survey of underutilized leafy vegetables in Lagos State with a view to preserving indigenous knowledge on their usage and conserve their DNA for future use.

# MATERIALS AND METHODS Source of plant materials

Leafy vegetable samples were collected from eight different markets in Lagos State namely: Epe, Ikorodu, Yaba, Bariga, Mushin, Surulere, Oyingbo and Okeodo markets. A portion of each sample was kept in zip locked bags containing silica gel for preservation until it is ready for extraction. Voucher specimens were also prepared and deposited at the Lagos Herbarium in the Department of Botany, University of Lagos for reference purpose.

### Ethnobotanical survey of vegetable samples

ethnobotanical The survey was conducted with the full consent of all respondents with further verbal agreement and understanding that the research shall not be used for commercial purposes, but to serve as enlightenment on the diversity of leafy vegetables in Lagos State and their medicinal uses. The method used followed Otang et al. (2012) with minor modifications. A semistructured questionnaire guided interview of respondents was carried out at each collection point to document information on the plant uses, method of usage, habit, form, maturity, period of availability, extent of consumption,

degree of consumption, nutritional value, and medicinal uses of the leafy vegetables.

# Genetic conservation of vegetable samples *Extraction of DNA samples*

Extraction of DNA samples was achieved following modified CTAB protocol by Doyle and Doyle (1987) with minor modifications as follows: The mortals and pestles were autoclaved before use in order to sterilize them. About 20 ml of CTAB buffer was poured in a labeled blue cap tubes, 0.8 g of polyvinylpyrrolidone was added and 40 µl of beta-mercaptoethanol was added to the solution under a fume cupboard then the mixture was kept in the water bath at 65 °C in order to dissolve the PVP. Mortar and pestles were preheated in the water bath at 65 °C for some minutes and used to grind two leaves of the different sample with silica gel (in order to hasten the grinding) into powder and 0.10 g of the powder was transfered into newly labeled 1.5 ml Eppendorf tubes. About 800 µl of the pre-warmed (65 °C) solution (CTAB buffer, polyvinylpyrrolidone and betamercaptoethanol) in the blue cap tube was added into the tube containing the powder and the mixture was swirled in order to suspend the slurry. The slurry was incubated at 65 °C for one hour and it was mixed by inverting every 5 min. The mixture was rocked on an orbital shaker at 150 rpm (rate per minute) for 30 min and it was spun in a centrifuge at 12000 rpm for 5 min and the aqueous (top) phase was transferred into a new labeled 1.5 ml Eppendorf tube using sterile micropipettes and pipettes tips. The volume of the aqueous top phase was estimated and noted and equal volume of Sevag was added to each sample and mixed well to obtain an emulsion, it was further inverted for one minute, it was rocked on an orbital shaker for 30 min and centrifuged at 12000 rpm for 10 min and sterile micropipettes were used to transfer the upper aqueous phase into a clean new 1.5 ml Eppendorf tube. This stage was repeated. The volume of the repeated aqueous phase was added with the 0.08 volume of cold 7.5 M ammonium acetate and then multiplied by 0.54 to give the volume of cold isopropanol to

be added to the mixture; it was mixed well and kept in the freezer for 24 h to precipitate the DNA. Solution was removed from freezer and spun in the centrifuge at 12000 rpm for 20 min. The liquid was poured off and 700 µl of cold 70% ethanol was added and mixed, it was then left to stand for few minutes or until the pellet becomes free. It was centrifuged for 5 min at 12000 rpm. The liquid was poured off. The pellet was washed twice using 700 µl of cold 70% ethanol, the ethanol was poured off and the tubes were drained upside down in order to ensure complete removal of excess ethanol from the pellets and dried. The samples were eluted in 100 µl of TE buffer and it was kept in the fridge overnight.

#### Gel Electrophoresis

The method described by Adeveni and Ogundipe (2012) was followed. This was achieved using 1% agarose gel which was prepared by mixing 1.5 g agarose with 150 ml 1X TBE buffer. The mixture was dissolved in a microwave for 2 min and 4 µl of safe view was added and swirled. The gel was poured into a gel tray containing combs and allowed to stand for at least 30 min for the gel to solidify. The combs were removed after the gel has solidified and the tray was transferred into the electrophoresis tank flooded with 1X TBE buffer. About 3 µl of loading dye and 5 µl of DNA sample were spotted on a parafilm paper. The mixture was loaded into wells on the gel and this was run for 45 min at 110 amps and 60 V. The quality of the DNA was then photographed under ultraviolet light using UVIdoc System.

#### Spectrophotometric analysis

This was done to determine the purity and concentration of extracted DNA samples. It involves measuring 55  $\mu$ l of distilled water into a cuvette for blank checking, followed by the addition of 5  $\mu$ l of DNA sample and mixing thoroughly avoiding bubbles. Then the cuvette was placed in the Biophotometer to determine the concentration of the sample, the relative absorbance at different wavelength (230 nm, 260 nm and 280 nm) and the absorbance ratio were determined and recorded for each of the samples.

#### Data analysis

Data were analysed using descriptive statistics (frequencies, percentages) to generate summaries and tables.

#### RESULTS

A total of nineteen leafy vegetable samples were collected and photographed (Plate 1). The most frequently encountered of the vegetable was Amaranthus hybridus while the most frequently encountered families were Amaranthaceae and Asteraceae being represented by three species each followed by Lamiaceae represented by two species (Table 1). All the collected vegetable samples are cultivated and majority have herbaceous habit (84.2%) however only Amaranthus hybridus, Basella alba, Celosia argentea, Corchorus olitorius, Crassocephalum cerepidioides, Ocimum gratissimum, Talinum triangulare, Telfairia occidentalis and Vernonia amygdalina are indigenous to the study area. Questionnaires were administered at each collection point and a total of 300 respondents



were questioned, of these, 81.67% are female while 18.3% are male. Also, 30% of the respondents were under 35 years old while 70% were between 35 and 65 years of age. About 52.6% of the documented leafy vegetables are restricted to Southern Nigeria 47.4% are available while and used throughout the country (Table 2). Major proportions of the vegetables collected are sold fresh while some sold dried. All the collected samples yielded good quantity and quality DNA samples; these have been deposited at the University of Lagos, DNA spectrophotometric bank. The analysis showed that Ocimum gratissimum, Talinum triangulare, Vernonia amygdalina and Gnetum africanum had the highest purity with absorbance ratio of 1.90 while Heinsia crinata had the lowest purity with absorbance ratio of 1.29. Corchorus olitorius had the highest concentration of 1335 ng/µl followed by Telfairia occidentalis with 1125 ng/µl and the least concentration 27 ng/µl was seen in Pterocarpus mildbreadii (Figures 1 and 2).













**Plate 1:** Leafy Vegetables in Lagos. (a) Pterocarpus mildbreadii, (b) Gongronema latifolium, (c) Ocimum gratissimum, (d) Solanum macrocarpon, (e) Telfairia occidentalis, (f) Piper guineense, (g) Celosia argentea, (h) Amaranthus hybridus, (i) Corchorus olitorius, (j) Talinum triangulare, (k) Lasianthera africana, (l) Gnetum africanum, (m) Vernonia amygdalina, (n) Murraya koenigii, (o) Heinsia crinite, (p) Crassocephalum cerepidioides, (q) Senecio biafrae, (r) Basella alba.

<b>Table 1</b> : List of leafy vegetables studied.
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S/N Botanical names	Family names	Common names	Local names	Place of collection	Coordinates
1. Amaranthus hybridus	Amaranthaceae	Green	'Tete	Ikorodu	N6 62.121,
L.		Amaranth	abalaye'		E3 50.322
				Surulere	N06.51392 <sup>0</sup>
					E003.34893 <sup>0</sup>
2. Amaranthus viridis L.	Amaranthaceae	Slender	'Soko' green	Epe	N6 67.923,
		Amaranth			E3 57.546
3. Basella alba L.	Basellaceae	Indian spinach	'Amunututu'	Oke Odo	6°56'7.926"N
					3°13'26.31"E
<ol> <li>2. Amaranthus viridis L.</li> <li>3. Basella alba L.</li> </ol>	Amaranthaceae Basellaceae	Slender Amaranth Indian spinach	'Soko' green 'Amunututu'	Surulere Epe Oke Odo	N06.51392 E003.34893 N6 67.923 E3 57.546 6°56'7.926'' 3°13'26.31'

	Celosia argentea I	Amaranthaceae	Amaranth	'Soko' nuna	Ene	N6 67 923
-т.	cerosia argenica L.	7 marannaeede	7 unarantin	boko pupu	Lpc	F3 57 546
					Surulere	N06 51392 <sup>0</sup>
					Surviere	F003 3/893 <sup>0</sup>
5	Corchorus alitorius I	Tiliacaaa	Jew's mallow	'Ewedu'	Ikorodu	N6 62 121
5.	Corchorus oniorius L.	Tillaceae	Jew S manow	Ewedu	IKOIOdu	$F_{2} = 50.322$
					Muchin	N06 52731 <sup>0</sup>
					WIUSIIIII	E002.252750
	Courses a such a land	A	Eine and d	(Ehala)		E005.55275
0.	Crassocephaium	Asteraceae	Fire weed	EDOIO	Oke Odo	0°507.920 N
	S Maara					5 15 0.51 E
	S.Moore	Cratagogg	1 frigan	'Olragi'	Orvingha	NG 49 167
7.	Gnetum ajricanum	Glietaceae	Afficall	Okasi	Oyingbo	NO 46.107,
	weiw.		Joint_III		Oringha	$E_{3} = 30.312$
					Oynigbo	INU0.46200
0	Commence Indifalian	A	A	(T 14 a a; )	Danian	E003.38288
8.	Gongronema latifolium	Asciepiadaceae	Amaranth	Utasi	Bariga	N06.53/48°
	Benth.	D 1:	globe	( ) ( )	0 1	E003.39221°
9.	Heinsia crinata (Afzel).	Rubiaceae	English bush	'Atama'	Oyingbo	N06.48206°
	G. Taylor	<u> </u>	apple			E003.38288°
10	Lasianthera africana	Stemonuraceae	-	'Editan'	Oyingbo	N06.48206°
	Beauv					E003.38288°
1	Murraya koenigii	Lamiaceae	Curry leaf	'Efinrin oso'	Oyingbo	N06.48206°
	Spreng.					E003.38288°
12	Ocimum gratissimum	Lamiaceae	Scent leaf	'Efinrin'	Bariga	N06.53748 <sup>0</sup>
	L.					E003.39221°
1.	Piper guineense	Piperaceae	Ashanti	'Uziza'	Bariga	N06.53748 <sup>0</sup>
	Schumach.		pepper			E003.39221 <sup>0</sup>
14	Pterocarpus	Fabaceae	African	'oha'	Oyingbo	N6 48.167,
	<i>mildbreadii</i> Harms.		padauk			E3 38.312
					Bariga	N06.53748 <sup>0</sup>
						E003.39221°
1:	Solanum macrocarpon	Solanaceae	Egg plant	'Igbo'	Ikorodu	N6 62.121,
	L.					E3 50.322
					Bariga	N06.53748 <sup>0</sup>
						E003.39221°
10	Senecio biafrae Oliv. &	Asteraceae	Bologi	'Worowo'	Oke Odo	6°56'7.926''N
	Hiern.		-			3°13'26.31"E
1′	Talinum triangulare	Portulaceae	Water leaf	'Gbure'	Epe	N6 67.923,
	(Jacq.) Willd.				-	E3 57.546
					Mushin	N06.52731 <sup>0</sup>
						E003.35275°
1	Telfairia occidentalis	Cucurbitaceae	Fluted	'Ugwu'	Oyingbo	N6 48.167,
	Hook.		pumpkin	-		E3 38.312
					Bariga	N06.53748 <sup>0</sup>
					-	E003.39221 <sup>0</sup>
19	Vernonia amygdalina	Asteraceae	Bitter leaf	'Ewuro'	Epe	N6 67.923,
	Del.				1	E3 57.546
					Oyingbo	N06.48206 <sup>0</sup>
						E003.38288 <sup>0</sup>

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**Table 2:** Important leafy vegetables in Lagos State Nigeria.

Samples	Habits	Parts	Periods of	Extent of	Degree of	Nutritional	Medicinal Value
		Used	Availability	Consumption	Consumption	Value	
Amaranthus viridis	Herbs	Whole	All year round	Country wide	High	Vitamins and	Nutrient supplement
						minerals	
Amaranthus	Herbs	Whole	All year round	Country wide	High	Vitamins and	Tapeworm expellant, relief
hybridus						minerals	pulmonary problems
Basella alba	Herbs	Leaf	All year round	Restricted	Average	Vitamins and	Laxative
				(south-west)		minerals	
Celosia argentea	Herbs	Whole	All year round	Country wide	Average	Vitamins and	Laxative, Cough
						minerals	
Corchorus olitorious	Herbs	Leaf	All year round	Restricted	Average	Vitamins and	Aids child delivery, bowel
				(south-west)		minerals	movement
Crassocephalum	Herbs	Leaf	All year round	Restricted	Average	Vitamins and	Indigestion, stomach ache,
cerepidioides				(south-west)		minerals	headache, to stop nose bleeding
Gnetum africanum	Shrub	Leaf	All year round	Restricted	High	Spices	Antiseptic, childbirth, sore throat
				(south-east)			treatment
Gongronema	Herbs	Whole	All year round	Restricted (south-	Average	Spices	Stomach ache, to aid walking in
latifolium.				west)			infants
Heinsia crinata	Herbs	Whole	All year round	Restricted	Average	Vitamins and	Pain relief, in arthritis and
				(south-east)		minerals	rheumatism
Lasianthera	Herbs	Whole	All year round	Restricted	Average	Vitamins and	As antacid, analgesic, anti-
africana				(south-east)		minerals	ulcergenic,
							anti-diabetic
							and anti-malarial

Murraya koenigii	Herbs	Whole	All year round	Country wide	Average	Spices	For vomiting, dysentery and diabetes.
Ocimum gratissimum	Herbs	Leaf	All year round	Restricted (south-west)	Average	Spices	Fever, diarrhea, dysentery, pile, stomach problems, HBP
Piper guineense	Tree	Leaf	All year round	Countrywide	High	Spices	Aphrodisiac
Pterocarpus mildbreadii	Tree	Leaf	All year round	Countrywide	High	Vitamins and minerals	Skin infections
Solanum macrocarpon	Herbs	Leaf	Wet season (April)	Restricted (south)	Low	Vitamins and minerals	Treat ringworm, mouth ulcer, reduce flatulence
Senecio biafrae	Herbs	Leaf	Wet season (April)	Restricted (south)	Low	Vitamins and minerals	Heart problem, cough, wound dressing, rheumatism, tonic
Talinum triangulare	Herbs	Whole	Wet season (April- Sept)	Country wide	High	Vitamins and minerals	Potherbs, stomach problem. nutrient supplements
Telfairia occidentalis	Herbs	Whole	All year round	Country wide	High	Vitamins and minerals	Blood tonic and nutrient supplement
Vernonia amygdalina	Shrub	Whole	All year round	Country wide	High	Vitamins and minerals	Antimicrobial, antiseptic, antimalaria

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#### DISCUSSION

The result of this investigation showed that a number of vegetables retrieved from the are underutilized Lagos markets and indigenous knowledge regarding the use of these species is not uniformly distributed among the people. A high number of female respondents were recorded, this may be attributed to the fact that women are more involved in the marketing of leafy vegetables and they are generally responsible for the upkeep of the home and families (Gockowski et al., 2003; Zobolo and Mkabela, 2006). This observation is consistent with that of Ahmad and Jayed (2007), who opined that women folk provide the most valuable source of indigenous knowledge of plants. Also, the young age of the respondents shows that knowledge on the use of leafy vegetables is not yet endangered in the study area.

Owing to their weedy nature, these vegetables are mostly available all year round with exception to Talinum triangulare, Solanum macrocarpon and Scenecio biafrae which are available during the wet season. This is in line with the report of Adjatin et al. (2013), who reported that indigenous leafy vegetables are available all year round because they can be collected at the time of plenty, sun dried, stored and further used, when needed, during the long dry season. About 52.6% of the documented leafy vegetables are restricted to southern Nigeria while 47.4% are available and used throughout the country being widely known for their vitamin and mineral composition. Even so, only a fraction of the other 40% is known to the urban population and contributes to its diet. This is in contrast to Gockowski et al. (2003) who suggested that urban population are still first generation rural migrants who retain their preferences for these traditional foods and provide a base for efforts to promote the commercialization and use of these crops.

Furthermore, indigenous leafy vegetables have long been known and reported to have health protecting properties and uses (Okeno et al., 2003) and are being continually used for prophylactic and therapeutic purposes by rural communities (Smith and Eyzaguirre, 2007). This is further corroborated by the result of this study which shows a great diversity of leafy vegetables found in Lagos is of therapeutic value. It also indicates the potentials of these plants in enhancing both the nutrition and health care of average Lagosians in the face of harsh economic crisis as reported by Schippers (2000).

Also, underutilized vegetables are poorly represented in *ex-situ* gene bank collections hence special effort is required to collect genetic diversity (Padulosi et al., 2014). All the collected samples yielded good quantity and quality DNA samples; however there was a variation in the movement of DNA samples in gel; this is an indication that the DNA samples are of different molecular weight; the lower the molecular weight of the DNA, the faster the migration of gel and vice versa (Adeyemi and Ogundipe, 2012). Also, low spectrophotometric values were obtained in some of the samples and this could be as a result of contaminants present in the DNA which may also affect the visibility of the bands. Concentration of the samples also varied. The DNA extraction protocol used has proven to be advantageous due to its simplicity and quickness resulting in a high molecular weight DNA of good quality from the vegetables. According to Ayodele, the establishment of a gene bank for these vegetables will safeguard the future availability of their genetic resources which could be supplied for cultivation in gardens for subsistence and cash generation. Also, Lachkovics (2001) stated that the utilization of traditional vegetables enhances food security and the conservation of their genetic resources may contribute to new ideas and

products of value to society in the future. Again, *ex-situ* conservation of agricultural biodiversity is highly strategic to society, but its role is also effectively static in nature and needs to be integrated with *in situ*/on-farm conservation efforts for maximum effectiveness (Barbieri et al., 2014).

#### Conclusion

This study is in line with the on-going efforts previous authors towards of documentation of indigenous knowledge of vegetables leafy in Nigeria. The ethnobotanical survey has contributed to the preservation of knowledge on leafy vegetables in Lagos and conservation of their DNA can be seen as a first step in the genetic conservation of the samples serving as a basis upon which other research can be based.

#### COMPETING INTERESTS

The authors declare that they have no competing interests.

#### **AUTHORS' CONTRIBUTIONS**

TOO: Development of the concept and experiment design, sample collection and identification, compilation of manuscript. OES: Sample collection, Ethnobotanical survey of collected samples. PBE: Sample collection, preparation of reagents, extraction of samples and analysis.

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