



Morphological, Phytochemical and Molecular Characterization of five common *Jatropha* species in the Niger Delta Region of Nigeria

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ABSTRACT: The economic and medicinal important genus *Jatropha* contains many distinctly different species. To elucidate the genetic relationship of five common occurring *Jatropha* species namely *J. multifida*, *J. podagrica*, *J. tanjorensis*, *J. curcas*, and *J. gossypifolia*, thirty-nine morphological, six phytochemical features and one arbitrary marker was used to screen and explore their similarity. Morphological data was obtained from the measurement of vegetative and reproductive parts while the presence of five phytochemicals was determined using different phytochemical tests. The DNA of all five *Jatropha* species were amplified and sequenced using Ribolose 1, 5-biphosphate carboxylase molecular marker. The DNA sequences were then aligned using the Basic Local Alignment Search Tool for nucleotide 2.8.0 version of the National Center for Biotechnology Information database and phylogenetic trees were constructed using Paleontological Statistical software and Molecular Evolutionary Genetics Analysis version 7.0.26 software. From the results of the classical and phylogenetic cluster analysis, the five *Jatropha* species was separated into two major clusters. The highly distinctive *J. gossypifolia* was the only species that clustered separately from the other *Jatropha* species. Although, *J. tanjorensis* has been reported to be a hybrid from *J. curcas* and *J. gossypifolia*, the species did not segregate and cluster with these species, but segregated with *J. multifida*, and *J. podagrica*, indicating that this species is more closely related to *J. multifida*, and *J. podagrica* than *J. curcas* and *J. gossypifolia*. The result therefore provide information that would be useful in the plant improvement programs for the genus *Jatropha*.

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The genus *Jatropha* is comprised of approximately 175 species that include succulent plants, shrubs, and trees (Nwokocha *et al.*, 2012). The genus is made up of flowering plants in the spurge family Euphorbiaceae. The name '*Jatropha*' was derived from the Greek words '*iatros*', meaning "physician", and '*trophe*' meaning "nutrition", hence the common name physic nut (Heller 1996). Most of *Jatropha* species are native to the Americas, with 66 species found in Africa, Asia and Europe (Nwokocha, 2010). The major species comprise of *Jatropha curcas*, *Jatropha gossypifolia*, *Jatropha tanjorensis*, and *Jatropha podagrica*, which are closely related to economically important cultivated plants like *Manihot esculenta* [cassava], *Hevea brasiliensis* [rubber tree], and *Ricinus communis* [castor bean] (Heller 1996; El-Mewafy *et al.*, 2016). These species are widely distributed in the tropical and sub-tropical areas of the world (Li *et al.*, 2009). Although, seeds of *Jatropha* species contains compounds that are highly toxic, the plants have proven to be of economic importance, from medicinal utilization to industrial

usage, especially in the production of environmental-friendly energy products, such as bio-fuel, bio-diesel, bio-lubricants from *Jatropha curcas*, which are renewable resource and a safe source of energy and a viable alternative to petroleum products (Banerji *et al.*, 1985; Basha and Sujatha, 2007). Presently, most research on *Jatropha* species are focused mostly on the distribution areas, cultivation and nursery development of *Jatropha curcas* (Wenjun *et al.*, 2008) and research on its relatives are limited (Sudheer *et al.*, 2009). Furthermore, *Jatropha* species are incipiently domesticated with no availability of stable and commercial cultivars with high oil content, and tolerance to pests and diseases that can meet the needs of stakeholders of the value chain of these plants such as farmers and processors of the feedstock produced. Therefore, the establishment of *Jatropha* species as a commercially viable crops requires the development of a suitable genetic crop improvement program (Argolo-Marques *et al.*, 2013). However, the challenge in the improvement of *Jatropha* species has been the lack of genetic resource for the crop

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improvement program. Therefore, the aim of this study is to use three lines of taxonomic evidences namely, morphology, phytochemistry and using an arbitrary molecular marker, to provide information that will elucidate the genetic variability and explore their similarity to establish their relatedness for use in the conservation and genetic improvement of the genus *Jatropha* species.

MATERIALS AND METHODS

Plant and sample collection: Five species of *Jatropha* namely *J. multifida*, *J. gossypifolia*, *J. tanjorensis*, *J.*

podagrica and *J. curcas* were used in this study (Table 1). These *Jatropha* species were collected from the 'Fear of God Horticultural Gardens', along NTA road, Ozuoba (geographical coordinates: 4° 52' 44" North, 6° 55' 20" East), Port Harcourt, Rivers State, Nigeria. They were identified at the Research Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt. Vegetative and reproductive plant parts such as the stem, leaves, petiole, flowers and fruit respectively were collected, documented and deposited at the Herbarium.

Table 1: The taxonomy of some common *Jatropha* species in Nigeria

<i>Jatropha</i> species	Common names
<i>jatropha podagrica</i>	Buddha belly plant, bottle plant shrub, gout plant, purging-nut, Guatemalan rhubarb, and gouty-stalk nettle-spurge.
<i>Jatropha tanjorensis</i>	Catholic vegetables, Hospital too far.
<i>Jatropha curcas</i>	Physic nut, Barbados nut, poison nut, bubble bush or purging nut.
<i>Jatropha multifida</i>	Coral plant, coral bush and physic nut.
<i>Jatropha gossypifra</i>	Bellyache-bush, black physic nut, and cotton-leaf physic nut

Morphological studies: A study of the plant morphological features of the five mature plant stands of the *Jatropha* species were carried out. The description of the morphological features was conducted using the Flora of West Tropical Africa by Hutchinson and Dalziel (1958), and Taxonomy of Flowering Plants by Gill (1988). Data was collected for the length and width of vegetative parts such as the leaves, petioles and internodes, and reproductive parts such as the flowers, fruits and seeds were measured using a 30cm meter rule.

Phytochemical Studies: Fresh leaves of each of the five *Jatropha* species were collected, washed in distilled water and air-dried under the sun till they attained a constant weight. Then six different phytochemicals were extracted from each species following the protocol of Harborne (1992). This study was carried out at the Pharmacy laboratory of the University of Port Harcourt, Choba in Rivers State, Nigeria.

i. Detection of Alkaloids: To detect for alkaloids, extracts were dissolved individually in dilute Hydrochloric acid and filtered. Three tests were carried out namely:

(i.) Mayer's test in which the filtrates were treated with Mayer's reagent (i.e. Potassium Mercuric Iodide). The formation of a cream coloured precipitate indicated the presence of alkaloids.

(ii.) Dragendroff's test involved treating the filtrates with Dragendroff's reagent (i.e. solution of Potassium Bismuth Iodide). The formation of red precipitate indicates the presence of alkaloids.

c) Hager's test involved treating the with Hager's reagent (saturated picric acid solution). The presence

of alkaloids is confirmed by the formation of yellow coloured precipitate.

ii. Detection of Saponins: To detect for saponins, two tests were carried out namely:

a) Froth test involved diluting the extracts with 20ml distilled water and shaken in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam test involved shaking 0.5 gm of extract with 2 ml of water to produce foam. If the foam produced persists for ten minutes it indicated the presence of saponins.

iii. Detection of Phytosterols: To detect for phytosterols, two tests were carried out namely:

a) Salkowski's test which involved treating the leaf extracts with chloroform and filtered. The filtrates were then treated with few drops of conc. Sulphuric acid, and shaken and allowed to stand. The appearance of golden yellow colour indicated the presence of triterpenes a type phytosterols.

b) Libermann-Burchard's test involved treating the extracts with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Then conc. sulphuric acid was added. The formation of brown ring at the junction indicated the presence of phytosterols.

iv. Detection of Phenols and Tannins: To detect for phenols and tannins, Ferric Chloride Test was carried out on extracts treated with 3-4 drops of Ferric Chloride solution. Formation of bluish black colour indicates the presence of phenols. For tannins, about 0.5g of plant extract was stirred with 10ml of distilled water and filtered. 5% ferric chloride reagent was added to the filtrate. A blue-black, green or blue green

precipitate is taken as evidence for the presence of tannins.

v. *Detection of Flavonoids*: To detect for flavonoids, Shinoda reduction test was carried out. A few pieces of magnesium metal were added to 5ml of each plant extract. The solution was obtained using conc. hydrochloric acid to dissolve the extract. The formation of orange, red crimson or magenta colouration was taken as evidence of preliminary presence of flavonoids.

Molecular characterization: The young leaves of the five *Jatropha* species were taken to the Regional Centre for Biotechnology and Bioresources, University of Port Harcourt, for the genomic DNA (gDNA) extraction. The extraction of DNA from the *Jatropha* species was carried out using the ZYMO Quick-DNA™ Plant/Seed Miniprep Kit (Zymo Research Group, California, USA). The gDNA quantity and concentration were measured using the Nanodrop 2000c spectrophotometer (Thermo fisher Scientific Inc. Wilmington, Delaware, USA). The DNA purity was measured as a ratio of absorbance at 280 nanometer (nm) to that of 260 nanometer. The quality of the gDNA was further quantified using the Agarose gel electrophoresis performed according to the modified method of Saghai-Marooof *et al.* (1984). The gDNA samples were shipped to the International Institute of Tropical Agriculture (IITA) Bioscience Center, Ibadan, Nigeria for amplification and sequencing. The Ribulose-1, 5-biphosphate carboxylase (*rbcL*) primers was used; the *rbcL*-F as forward primers and *rbcL*-R as reverse primers, *rbcL*-F (5'-CCACAAACAGAGACTAAAGC -3') and *rbcL*-R (5'- GTAAAATCAAGTCCACCGCG -3') were used to amplify the fragments of nuclear ribosomal DNA (rDNA). The amplicons were sequenced using the ABI3500 capillary electrophoresis sequencer. The DNA sequence file was saved in the Bioedit file with extension .*abl*. The sequence was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0.26 software, and aligned using the Basic Local Alignment Search Tool for nucleotide (BLASTN) 2.8.0 version of the National Center for Biotechnology

Information (NCBI) database which is global database containing information on different sequences. The alignment confirmed the identities of the plants.

Phylogenetic analysis: The data collected from the morphological and phytochemical studies were arranged in a binary format in which 1 was used to indicate that the character was present and 0 was used to indicate that the character was absent using the Microsoft excel software. The data was further subjected to cluster analysis using the neighbor-joining function of the multivariate feature of the Paleontological Statistical software (PAST) by Hammer *et al.* (2001). Also, the DNA sequences from the molecular study were subjected to phylogenetic analysis using the neighbor-joining tree function in MEGA.

RESULTS AND DISCUSSION

Morphological characterization: The morphological characteristics of the five *Jatropha* species used in this study are presented in Plate 1 and Table 2. From the results, it was observed that all the five species were distinctly different from each other based on the plant habit, stem structure, the leaf margin shape, leaf colour and texture. However, they shared some features which indicated their similarity and relationship. From the results, all the species were monoecious, succulent shrub or small tree in habit with ever-green or semi-deciduous foliage.

Although all species had stems that were erect, cylindrical and stout that were unarmed with scars, and had green apices and pale brown at the bases, however, *J. podagrica* had swollen at the base (gout) while *J. gossypifolia* had purplish-red apex. Also, they all had flowers that were rosaceous in shape, unisexual, regular pentamerous and polypeptalous with contorted aestivation. However, their flower colours were different from whitish-green, yellowish-green, reddish, reddish-orange and dark red for *J. tanjorensis*, *J. curcas*, *J. multifida*, *J. podagrica* and *J. gossypifolia* respectively.



Plate 1: Vegetative morphology of the five *Jatropha* species studied. A: *Jatropha curcas*, B: *Jatropha gossypifolia*, C: *Jatropha multifida*, D: *Jatropha podagrica*, E: *Jatropha tanjorensis*

Table 2: Summary of morphological characteristics of five *Jatropha* species

S/N	Character	Code	<i>J. t</i>	<i>J. p</i>	<i>J. m</i>	<i>J. g</i>	<i>J. c</i>
1	Plant habit (1=monoecious, 2_Not monoecious)	A1	1	1	1	1	1
2	Plant growth (1=shrub> 5m, 2=small tree<5m)	A2	2	2	2	1	2
3	Foliage type (1=Evergreen, 2= not evergreen)	A3	1	1	1	1	1
4	Root system (1=tap root, 2= fibrous root)	A4	1	1	1	1	1
5	Stem type (1=erect and unarmed with scars, 2=not erect and armed)	A5	1	1	1	1	1
6	Stem shape (1=cylindrical and stout, gout, with greenish apex and brown base, 2=cylindrical and stout without gout with greenish apex and brown base, 3= cylindrical and stout without gout with purplish apex and brown base)	A6	2	1	2	3	2
7	Leaf type (1=foliage with green leaves, 2=foliage with purplish tinged leaves)	A7	1	1	1	2	1
8	Leaf oragnization (1=simple, 2=nort simple)	A8	1	1	1	1	1
9	Phyllotaxy (1=alternate, 2=opposite)	A9	1	1	1	1	1
10	Leaf shape (1=broadly ovate, 2=orbicularly ovate,	A10	1	2	1	1	1
11	Leaf lamina (1=symmetrical, 2=asymmetrical)	A11	1	1	1	1	1
12	Leaf base 1=shallowly cordate, 2=deeply cordate, 3=peltate	A12	2	3	2	1	2
13	Type of leaf apex (1=acuminate tip, 2=altenate or tailed tip, 3=acute to acuminate tip, 4=acute or short acuminate tip)	A13	1	1	2	3	4
14	Leaf texture (1=chartaceous, 2=cariaceous)	A14	1	2	2	1	1
15	Leaf surface (1=glabrous with whitish underside, 2=glabrous without whitish underside)	A15	2	1	1	2	2
16	Forms of leaf margin (1=serrated or serrulate, 2=sinulate, 3=incised with projected leaves, 4=entire and undulating)	A16	1	2	3	1	4
17	Types of leaf lobes (1=palately lobed into 3 or 5, 2=palmately lobed into 3 or 7, pinnatisect or palmately lobed into 9-11)	A17	1	1	3	1	2
18	Shape of leaf lobe (1=more or less asymmetrical with acuminate tip, 2=narrow oblanceolate with acuminate tip,3=obovate to oblanceolate with acuminate tips, 4=ovate with acute tips)	A18	1	1	2	3	4
19	Type of Inflorescence (1=multiparous or polychaial cymose, 2=biparous or dichasial cymose, 3=uniparous cymose)	A19	1	1	1	3	2
20	Peduncle type (1=long and smooth, ending with a cyme and branched end, 2= long and smooth, ending with a flowered leaf opposed cyme and branched, 3= long and smooth, with a flat topped cluster or multiparous cyme and branched)	A20	3	3	3	2	1
21	Flower description (1=rosaceous in shape, unisexual, regular pentamerous and polypeptalous, yellowish-green in colour with a faint fragrance, 2=Rosaceous in shape, unisexual, regular pentamerous and polypeptalous which is dark red in color and without fragrance,3= Rosaceous in shape, unisexual, regular pentamerous and polypeptalous which is reddish in color and without fragrance, 4=Rosaceous in shape, unisexual, regular pentamerous and polypeptalous which is reddish-orange in color and without fragrance, 5=Rosaceous in shape, unisexual, regular pentamerous and polypeptalous which is whitish-green in color and with a faint fragrance)	A21	5	4	3	2	1
22	Bract type (1=persistent, 2= leafy and foliaceous, 3=Leafy and foliaceous with serrated margins with angular tips)	A22	1	1	1	3	2
23	Aestivation (1=consorted, 2=not consorted)	A23	1	1	1	1	1
24	Form of sepal and corolla (1= petaloid and polypetalous and polyseppalous, 2=not petaloid and polypetalous and polyseppalous)	A24	1	1	1	1	1
25	Sepal shape (1=ovate to obovate in shape and non-glandular margins, 2=lanceolate to elliptical in shape with glandular margins, 3=broadly deltoid in shape)	A25	1	1	1	2	3
26	Sepal texture (1=glabrous, 2=glabrous with pubescent surface with whitish hairs)	A26	1	1	1	1	2
27	Corolla shape (1=Elliptic , 2=Obovate a, 3=Oblanceolate to elliptical in shape)	A27	1	1	1	2	3
28	Corolla texture (1=glabrous, 2=Glubrous with pubescent surface with whitish hairs)	A28	1	1	1	1	2
29	Number of floral gland (1=5 free glands, 2=no free 5 glands)	A29	1	1	1	1	1
30	Male flower more than female flower (1=yes, 2=no)	A30	1	1	1	1	1
31	Position of ovary in the flower (1=superior or hypogynous, 2=not superior or hypogynous)	A31	1	1	1	1	1
32	Pistil type (1= Elliptic syncarpous, 3-celled, triovulate, 3 spreading bifurcate style that is fused at the base with 2-lobed stigma per style, 2= Globose syncarpous, 6-ripped and 3-celled, triovulate, 3 spreading style that is fused at the base with 2-lobed stigma per style)	A32	2	2	1	2	1
33	Fruit type (1=simple, 2=not simple)	A33	NA	1	1	1	1
34	Fruit shape (1=globrose capsule and 3-seeded, 2=ellipsoid capsule and 3-seeded)	A34	NA	1	2	1	2
35	Fruit texture (1= glabrous, 2=pubescent with white hair)	A35	NA	1	0	1	0
36	Fruit dehiscence (1=septicidal, 2=septifrugal)	A36	NA	1	2	1	2
37	Seed description (1=Ellipsoid with dark brown aril, 2=Ellipsoid with to triangular convex with dark brown aril, 3=oval shape with dark brown aril)	A37	NA	1	3	1	2
38	Number of seed per fruit (1=3, 2=more than 3)	A38	NA	1	1	1	1
39	Seed dispersal (1=Fruit dehisces and the seed are released by explosion, 2=Fruit dehisces but seeds remain intact till fruit drops to the ground)	A39	NA	1	2	1	2

J. t: *Jatropha tanjorensis*, *J. p:* *Jatropha podagrica*, *J. m:* *Jatropha multifida*, *J. g:* *Jatropha gossypifolia*, *J. c:* *Jatropha curcas* NA: not available

Phytochemical characterization: The results of the phytochemical studies are presented in Table 3. From the results it was observed that all the six phytochemicals namely alkaloids, tannins, flavonoids, saponins, phytosteroids, and phenols were present in the leaves of the five *Jatropha* species but at different levels of concentrations. For alkaloids, *Jatropha multifida* had more alkaloids than the other four

Jatropha species. For tannins, *Jatropha curcas* and *Jatropha gossypifolia* had more tannins than the other three *Jatropha* species. For flavonoids, *Jatropha curcas* had more flavonoids than others, however, it was not found in *Jatropha multifida*. The leaf of *Jatropha podagrica* had more saponins than the other *Jatropha* species. Phytosteroids and phenols were present in all the *Jatropha* species.

Table 3: Phytochemicals present in the leaves of five *Jatropha* species studied

<i>Jatropha</i> species	Plant part	Alkaloids	Tannins	Flavonoids	Saponins	Phytosteroids	Phenols
<i>Jatropha podagrica</i>	Leaf	++	++	++	+++	++	+
<i>Jatropha tanjorensis</i>	Leaf	++	++	+	++	+	+
<i>Jatropha curcas</i>	Leaf	++	+++	+++	++	+	++
<i>Jatropha multifida</i>	Leaf	+++	++	-	++	++	+
<i>Jatropha gossypifolia</i>	Leaf	++	+++	++	++	++	++

Key: +-present, ++-deeply present, +++-very deeply present

Cluster analysis based on morphological and phytochemical attributes: The result of the cluster analysis of the relationship of the five *Jatropha* species based on the morphological and phytochemical characteristics are presented in Figure 1 and Table 4. From the result, it was observed that the five common *Jatropha* species were divided into two main clusters. The first cluster included four *Jatropha* species namely *J. curcas*, *J. multifida*, *J. tanjorensis*, *J. podagrica* while *J. gossypifolia* belonged to the second cluster. The species *J. gossypifolia* had a distance indices of 6.226, 6.164, 6.928 and 6.708 from *J. tanjorensis*, *J. podagrica*, *J. multifida* and *J. curcas* respectively. The first cluster was further sub-divided into two subgroups in which *J. curcas* was in the first subgroup while *J. multifida*, *J. tanjorensis*, and *J. podagrica* clustered in the second subgroup. The second sub-group was further divided into two subgroups in which *J. multifida*, and *J. tanjorensis*, clustered together into a group. The highest distance index was 7.280 which was between *J. curcas* and *J. podagrica*, thus indicating that they were highly dissimilar, which *J. multifida*, and *J.*

tanjorensis had the lowest distance index of 5.050 indicating that they were may be more closely related than the other species.

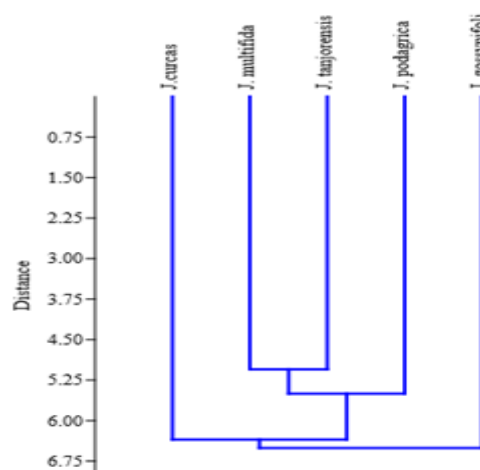


Fig 1: Cluster (classical) analysis indicating the relationship among five common *Jatropha* species based on the morphological and phytochemical characters.

Table 4: The similarity and distance indices of the five *Jatropha* species

	<i>J. tanjorensis</i>	<i>J. podagrica</i>	<i>J. multifida</i>	<i>J. gossypifolia</i>	<i>J. curcas</i>
<i>J. tanjorensis</i>	0				
<i>J. podagrica</i>	5.344	0			
<i>J. multifida</i>	5.050	5.657	0		
<i>J. gossypifolia</i>	6.226	6.164	6.928	0	
<i>J. curcas</i>	5.532	7.280	6.245	6.708	0

Molecular Analysis: The result of the phylogenetic analysis of the DNA sequences of the five *Jatropha* species is presented in the dendrogram in Figure 2. From the dendrogram, it was observed that the five *Jatropha* species were separated into two clusters: Cluster 1 was made up of four *Jatropha* species namely *J. multifida*, *J. podagrica*, *J. tanjorensis*, and

J. curcas, while Cluster 2 had only *J. gossypifolia*. This indicated that the *Jatropha* species in cluster 1 are more closely related than *J. gossypifolia* in cluster 2. Also, cluster 1 was further divided into two sub-clusters A and B. *J. multifida*, *J. podagrica*, *J. tanjorensis* were grouped into sub-cluster A while *J. curcas* was grouped into sub-cluster B. This indicates

that although the four *Jatropha* species namely *J. multifida*, *J. podagrica*, and *J. tanjorensis* are more closely related. However, sub-Cluster A was further sub-divided into two groups, with *J. multifida* and *J. podagrica* clustering together and *J. tanjorensis* grouped into a separate group. This finding indicates that among the five *Jatropha* species, *J. multifida* and *J. podagrica* are more closely related.

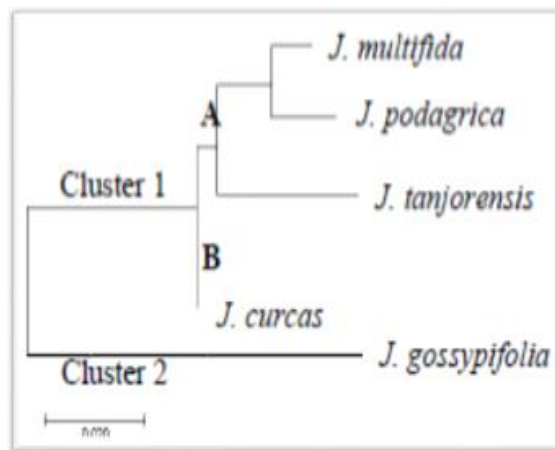


Fig 2: A dendrogram illustrating the phylogenetic relationships of the five *Jatropha* species based on Ribulose-1, 5-biphosphate carboxylase (rbcL) molecular marker.

The genetic diversity of the highly distinctly different *Jatropha* species is critical to establish strategies for conservation and genetic breeding for the improvement of these income security plants. Therefore, the genetic relationship among the different *Jatropha* species needs to be cataloged. The present study was aimed to investigate the morphological, phytochemical and molecular phylogenetic affinity among the five *Jatropha* species. These three taxonomic lines of evidence are important and had been used in the elucidation of *Jatropha* species in Egypt (El-Mewafy *et al.*, 2016). From the gross morphology, it was observed that all the five species were distinctly different from each other based on their vegetative and reproductive features. However, they shared some features which indicated their similarity and relationship. For instance, all species had stems that were erect, cylindrical and stout that were unarmed with scars, and were pale brown coloration at the stem bases, however, *J. podagrica* had swollen base (gout) while *J. gossypifolia* had purplish-red apex. Also, all species had different flower colours from whitish-green, yellowish-green, reddish, reddish-orange and dark red for *J. tanjorensis*. *J. curcas*, *J. multifida*, *J. podagrica* and *J. gossypifolia* respectively. However their flowers were rosaceous in shape, unisexual, regular pentamerous and polypeptalous with contorted aestivation. These characteristics have been reported as important traits

for the elucidation of *Jatropha* species (Ratha and Paramathma, 2009, Nwokocha *et al.*, 2012). Phytochemical analyses for medicinal properties have shown that numerous compounds in plant traditionally used for medicinal purpose have chemical properties that are effective in treating illness. Phytochemicals are chemical compound formed during the plant normal metabolic process and are often referred to as secondary metabolite of which there are several classes including alkaloids, flavonoids, saponins, coumarins, steroids, glycosides, gum, phenol, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004). The presence of secondary metabolites in the different *Jatropha* species at different levels of concentrations are taxonomically useful; and indicates that *Jatropha* species are potential sources of these important phytochemicals (Nwokocha *et al.*, 2011). In this study, six phytochemicals namely alkaloids, flavonoids, tannins, saponins, phytosteroids and phenols were present but at different levels of concentrations in the leaves of the five *Jatropha* species studied. The presence of these phytochemicals confirmed the relatedness of these five *Jatropha* species. However, the variability in the concentrations of these phytochemicals confers individuality on the species. Also, from this study, *Jatropha curcas* and *Jatropha gossypifolia* were observed to have more tannins than the other three *Jatropha* species. While *Jatropha curcas* had more flavonoids than the rest. Also, from this study, it was observed *Jatropha tanjorensis* had a very high concentration of alkaloid. Alkaloids are very important component that explains why this plant is being used for the treatment of illnesses such as malaria since most alkaloidal plants have high antimalarial activity (Viswanathan *et al.*, 2012). These secondary metabolites identified in these *Jatropha* species have been reported in several other plants used for curative activity against various pathogens, used traditionally as analgesic and soothing herbs (El-Mewafy *et al.*, 2016). The use of molecular marker technique has been employed to authenticate the identification and relationship of organisms (Gontia-Mishra *et al.*, 2013; Bechem and Afanga, 2017). The use of molecular marker has been employed in the taxonomic elucidation of *Jatropha* species (Tanya *et al.*, 2011, El-Mewafy *et al.*, 2016). In the study, this technique showed the relatedness of five distinctly different *Jatropha* species, by comparing their DNA sequence. The phylogenetic analysis resulted in a dendrogram that revealed the affinity and diversity of the five *Jatropha* species studied. This is reported for the first time in Niger Delta and further builds on the works reported by Nwokocha *et al.*, (2011; 2012). From the two cluster analysis conducted in this study, it was observed that the five *Jatropha* species were segregated into two

major clusters. El-Mewafy *et al.* (2016) had made similar observation among the commonly occurring *Jatropha* species in Egypt. Among the five *Jatropha* species, *J. gossypifolia* was the only species that clustered separately. This may be as a result of its unique and distinctive purplish colour of its leaves, stem and flower. Also, from the first groups of the two clusters, it was observed that *J. tanjorensis*, *J. multifida*, and *J. podagrica* clustered in the same subgroup, while *J. curcas* segregated into another group. Despite the dissimilarity of *J. curcas* and *J. gossypifolia*, a natural interspecific hybrid between had been found and classified as *J. tanjorensis* (Prabakaran and Sujatha 1999). Yet in this study, *J. tanjorensis* did not segregate and cluster with *J. curcas* or *J. gossypifolia*. Therefore, further investigation is needed to establish the phylogeny of *J. tanjorensis*. Moreover, the segregation of *J. tanjorensis* with *J. multifida*, and *J. podagrica* into one group may indicate that this species is more closely related to *J. multifida*, and *J. podagrica* than *J. curcas* and *J. gossypifolia*. Hybridization among these three species will contribute to the improvement program of *Jatropha* species.

Conclusion: The comparison of five common but distinctly different *Jatropha* species found in some parts of Niger Delta was done using morphological, phytochemical and molecular marker methods to establish their affinity. From the cluster analysis, it was observed that the five *Jatropha* species, are divided into two major groups. The species, *J. gossypifolia* was the only species that clustered separately from the other *Jatropha* species. The species, *J. tanjorensis*, *J. multifida*, and *J. podagrica*, clustered together indicating that they are more closely related.

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