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European Journal of Experimental Biology, 2014, 4(6):71-76

ISSN: 2248 –9215
CODEN (USA): EJEBAU

A numerical approach to the taxonomy of the genus *Jatropha* Linn. using quantitative phytochemical constitutents

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ABSTRACT

The systematic relationship existing among five members of the genus Jatropha namely Jatropha curcas Linn.; Jatropha gossypifolia Linn.; Jatropha integerrima Jacq.; Jatropha podagrica Hook. and Jatropha multifida Linn. found in Nigeria were studied using quantitative phytochemical parameters. The study was aimed at elucidating the taxonomic importance of the phytochemical parameters in the leaves of the five species of the Jatropha. The species were screened to determine the quantity of the biologically active compounds using standard methods. The active compounds screened for were tannins, flavonoids, phytosteroids, cardiac glycosides, alkaloids, saponins, terpenoids and phenols. Results revealed that the leaf contains tannins, flavonoids, phenols and cardiac glycosides in sizable quantities. Sokal and Sneath coefficient of similarity revealed generally low level of similarity in the quantitative phytochemical parameters of leaves of the five species, ranged from 2.19 and 12.31%.

Keywords: Quantitative Phytochemical Parameters, Cluster analysis, leaves, taxonomy, *Jatropha* species

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INTRODUCTION

Morphometrics can be defined as the quantitative analysis of biological form that has being widely used in a lot of discipline including systematics (Henderson, 2006). The practice of numerical taxonomy embraces a number of fundamental assumption and philosophical attitudes towards taxonomic work. It has the ability to integrate data from a variety of sources of forms such as anatomy, cytology, ecology, genetics, geography, physiology, palynology, chemistry etc. (26).

Generally, chemical identification of specific compounds will provide a greater insight into the relationships and differences among plant taxa (5). David (1994) reported that physiochemical data provide much useful information concerning relationship both within the Euphorbiaceae and between this family and relatives. The presence or absence of secondary metabolites and the biosynthetic pathways responsible for their production are useful for establishing taxonomic and phylogenetic relationship (11). Earlier attempts have been made in several fields of Biology to place the taxonomic relationships of species upon a firm physico-chemical foundation (30).

Earlier taxonomic treatments of the genus *Jatropha* were evaluated on the basis of morphological (10; 9), wood anatomy (20) and leaf and seed electrophorensis (21; 22), leaf epidermal features (1).

Hegnaeur (1989) observed that for the family Euphorbiaceae, secondary metabolites such as alkaloids, cyanogenic glycosides, diterpenes, glucosinolates, tannins and triterpenes are the most common metabolites of taxonomic importance at the suprageneric levels. In view of the complex taxonomic status of *Jatropha* species, this study

attempted a quantitative phytochemical screening of the genus *Jatropha* in Nigeria with a view to investigating the taxonomic relationship of members of the genus.

MATERIALS AND METHODS

Fresh specimens of *Jatropha curcas*, *Jatropha gossypifolia*, *Jatropha integerrima*, *Jatropha podagrica* and *Jatropha multifida* were collected in different locations in Nigeria and were identified as the Forest Herbarium, Ibadan (FHI). Voucher specimens were prepared according to the established protocol of (26). Each specimen was assigned with a specific voucher number (Table 1). Voucher specimens were deposited at the Forest Herbarium, Ibadan (FHI).

2.1 Phytochemical screening

Freshly collected leaves of the specimens were air dried for 15 days until devoid of moisture. The dried leaves were ground into fine powder and transferred into airtight containers with proper labelling. They were subjected to phytochemical screening which was carried out at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria.

The secondary metabolites screened for were tannins, flavonoids, phytosteroids, cardiac glycosides, alkaloids, saponins, terpenoids and phenols. The phytochemical screenings were carried out using established standard procedures (4; 15; 15; 28).

2.1.1 Determination of tannins: Tannin content was determined using the method outlined by (29). Five hundred mg of the leaf sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and then shaken thoroughly for 1 hour in a mechanical shaker. The solution was filtered into a 50 ml volumetric flask and made up to the mark. Five ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (12).

2.1.2 Determination of flavonoids: To determine the flavonoid content in the leaves of three *Jatropha* species, the aluminium chloride colorimetric method was employed. One ml of each plant extract was mixed with 3 ml of methanol, 0.2 ml of 10 % aluminium chloride, 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 min, while the absorbance was measured at 420 nm. The total flavonoid content in each plant part was expressed in terms of standardized quercetin equivalent (mg/g of each extracted compound) (3).

2.1.3 Determination of phytosteroids: For this purpose, the crude extract of each *Jatropha* leaf was mixed with 2 ml of chloroform and concentrated sulphuric acid (H_2SO_4) was added sidewise. The presence of steroids was noticed from the red colour produced in the lower layer of chloroform. To confirm further the presence of this phytochemical, another test was performed by mixing each crude extract of the plant materials with 2 ml of chloroform. Two ml of concentrated H_2SO_4 and of acetic acid were then poured into the mixture and the development of greenish coloration indicated the presence of steroids.

2.1.4 Determination of cardiac glycosides: Buljet's reagent (13) was used to evaluate the cardiac glycoside content in the examined *Jatropha* species. For this purpose, 1 g of each powdered sample was soaked in 100 ml of 70 % alcohol for 2 hrs before filtration. Using lead acetate and Na_2HPO_4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

2.1.5 Determination of alkaloids: A weighed amount (5 g) of each powdered sample of the *Jatropha* leaf was transferred into a 250 ml beaker. Two hundred ml of 20 % acetic acid was added and then covered to stand for 4 hrs. Filtration was done, and concentration of the extracted content to one quarter of original volume was applied using a water bath. Drop-wise addition of concentrated ammonium hydroxide to the extract followed until the precipitate was complete. The entire solution was allowed to settle and collection of the precipitate was done by filtration (15; 19) and then weighed.

2.1.6 Determination of saponins: A spectrophotometric method described by (6) was used for saponin analysis. One gram of the finely ground sample of the *Jatropha* species was weighed into a 250 ml beaker and 100 ml of Isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5 hr to ensure uniform mixing. Subsequently, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40 % saturated solution of Magnesium carbonate was added. The obtained mixture with saturated $MgCO_3$ was again

filtered to obtain a clear colourless solution. One ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5 % $FeCl_3$ solution was added and made up to the mark with distilled water and then allowed to stand for 30 min for the development of red colour. Standard saponin solutions of 0–10 ppm were prepared from saponin stock solution and each standard solution was treated similarly with 2 ml of 5 % $FeCl_3$ solution. The absorbances of the sample as well as the standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer, at a wavelength of 380 nm, and percentage of saponin was calculated.

2.1.7 Determination of Terpenoids: The method of (14) was used to evaluate the total terpenoid contents of the leaves of the studied species. 10g of plant powder were taken separately and soaked in alcohol for 24 hours. Then filtered, the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

2.1.7 Determination of Phenol: Total phenol content in the sample was determined using Folin-Ciocalteu's method as modified by (23). 0.5 mL of the extract was added to 10 mL of deionized distilled water and 2.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2 mL of 2% sodium carbonate was added. The absorbance of the solution was measured at 780 nm after 10 mins. Quercetin was used as standard for calibration curve.

All tests were carried out in triplicate for each sample and results were presented as means±SD. The contents were estimated and expressed in mg/g.

2.2 Numerical analysis

Each species were treated as Operational Taxonomic Units (OTU's). Single Linkage Cluster Analysis (SLCA) was carried out on the data using Palentological Statistics, Ver. 2.17c (PAST). Sokal and Sneath (1963) was used to show the level of similarity of the phytochemical parameters.

RESULTS

The mean and standard deviation of the eight quantitative phytochemical parameters of the studied *Jatropha* species is presented in Table 2 and the Sokal and Sneath similarity index based on the quantitative phytochemical parameters are shown in Table 3. Sokal and Sneath coefficient of similarity revealed generally low level of similarity in the quantitative phytochemical screening of leaves of the five species ranged from 2.19 and 12.31% (Table 3). The highest coefficient of similarity occurred between *Jatropha curcas* and *Jatropha integerrima* (12.31%) while the lowest was between *Jatropha podagrica* and *Jatropha multifida* (2.19%). The Single Linkage Cluster Analysis (SLCA) dendrogram of quantitative phytochemical parameters of *Jatropha* species was presented in Figure 1. The SLCA dendogram shows the five species separated into two main groups with *Jatropha gossypifolia* and *Jatropha integerrima* representing the taxa of the first cluster while *Jatropha curcas, Jatropha podagrica* and *Jatropha multifida* are the taxa formed by the second main cluster. *Jatropha podagrica* and *Jatropha multifida* appears to be the most closely related and the cluster to the highest level.

DISCUSSION

The two techniques (Principal Component Analysis and Cluster Analysis) used in this work are commonly used in Numerical taxonomy. Soladoye *et al*., (2008) employed these techniques in the phytochemical and morphometric analysis in the genus *Acalypha*. Oladipo and Illoh (2012a) applied these techniques to analyse the total protein banding patterns of six species of the genus *Jatropha* using gel electrophorensis while (21) investigated the systematic values of the comparative wood anatomical characters of the five species of *Jatropha*. The various research results revealed the hierarchical classification and visual interpretation of the taxonomic relationship within the studied species and also sub-sectional discrepancies in the existing traditional classification of the genus. The techniques are regarded as unbiased indicators of the similarity or differences existing between the taxa, which are in turn used to arrange taxa in hierarchical order (21).

The groupings observed as a result of the Principal Component Analysis provide some strength to the existing classification of (10; 8; 9; 21). The pattern of clustering in SLCA and PCA plots conforms to the current subgeneric delimitation of the taxa. The closeness observed between *Jatropha podagrica* and *Jatropha multifida* in the dendogram is in line with their current subgeneric and sectional delimitation (i.e *Jatropha* and *Peltatae* respectively). The grouping of *Jatropha integerrima* and *Jatropha gossypifolia* conforms to the morphological resemblance of the leaf and fruit morphology of the two species and also in line with the clustering pattern of the SLCA dendogram of the study on comparative wood anatomical characters observed by (21) conducted on the five *Jatropha* species.

Table 1: Voucher information of the Studied *Jatropha* species

Species	Place of Collection	Date of Collection	Voucher number
***Jatropha curcas* Linn.**	Celestial area, Oyo town, Oyo State	28/6/2013	FHI 109865
***Jatropha gossypifolia* Linn.**	Along Akoda-Ede road, Ede, Osun State.	8/5/2013	FHI 109863
***Jatropha integerrima* Jacq.**	DVC RTI Premises, Faculty of Agriculture area, UNILORIN, Kwara State.	18/4/2013	FHI 109864
***Jatropha podagrica* Hook.**	Residential Building, behind Union Baptist Church, Upper Gaa Akanbi area, Ilorin, Kwara State	16/4/2013	FHI 109871
***Jatropha multifida* Linn.**	Ogbondoko town, Along Afon road, Asa LGA, Kwara State.	28/4/2013	FHI 109872

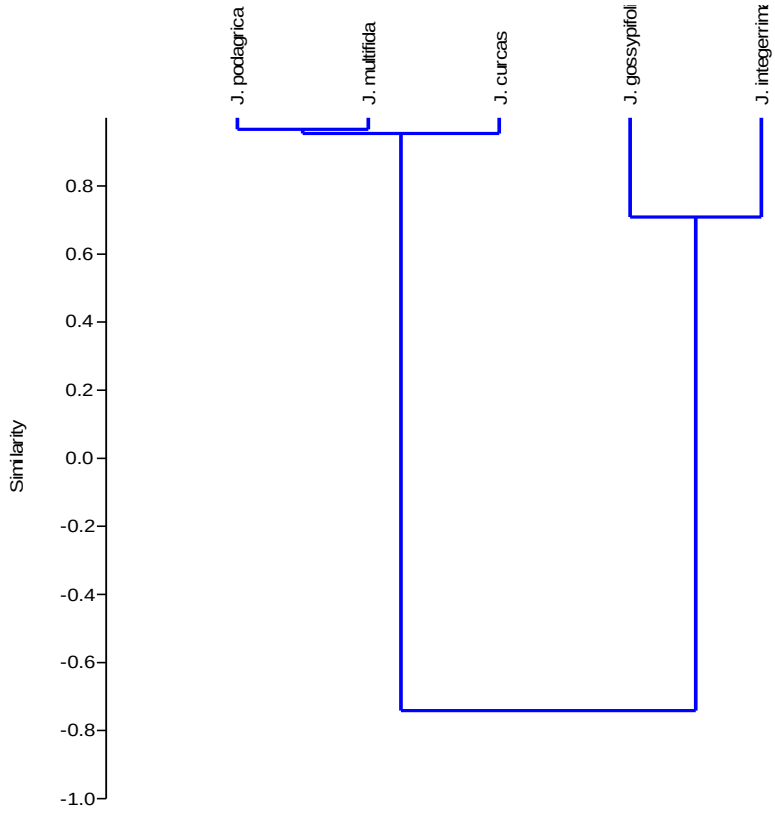

Figure 1: Single Linkage Cluster Analysis (SLCA) dendrogram of quantitative phytochemical in the five species of *Jatropha* studied

Table 2: Mean±SD of the eight quantitative phytochemical paramaters of *Jatropha* species studied

Plant Species	Tannins	Flavonoids	Phytosteroids	Cardiac glycosides	Alkaloids	Saponins	Terpenoids	Phenols
***Jatropha curcas* Linn.**	2.77±0.16	5.28±0.48	0.32±0.003	1.06±0.095	0.69±0.065	0.363±0.048	0.13±0.02	5.92±0.06
***Jatropha gossypifolia* Linn.**	8.87±0.015	6.31±0.43	0.31±0.011	1.27±0.085	1.11±0.015	0.55±0.015	0.10±0.004	16.17±0.515
***Jatropha integerrima* Jacq.**	8.13±0.60	11.61±0.55	0.42±0.009	2.35±0.115	1.21±0.07	0.81±0.351	0.14±0.005	14.90±0.835
***Jatropha podagrica* Hook.**	4.43±0.05	6.29±0.23	0.42±0.009	1.27±0.05	0.98±0.04	0.49±0.02	0.14±0.003	7.93±0.55
***Jatropha multifida* Linn.**	3.18±0.175	7.87±0.37	0.32±0.055	1.58±0.075	0.88±0.04	0.44±0.02	0.11±0.002	8.72±0.315

Table 3: Sokal and Sneath similarity index for *Jatropha* species based on the quantitative phytochemical parameters

Plant Species	*Jatropha curcas*	*Jatropha gossypifolia*	*Jatropha integerrima*	*Jatropha podagrica*	*Jatropha multifida*
Jatropha curcas	-				
Jatropha gossypifolia	11.983	-			
Jatropha integerrima	12.312	5.61	-		
Jatropha podagrica	2.82	9.36	9.59	-	
Jatropha multifida	3.88	9.51	8.81	2.19	-

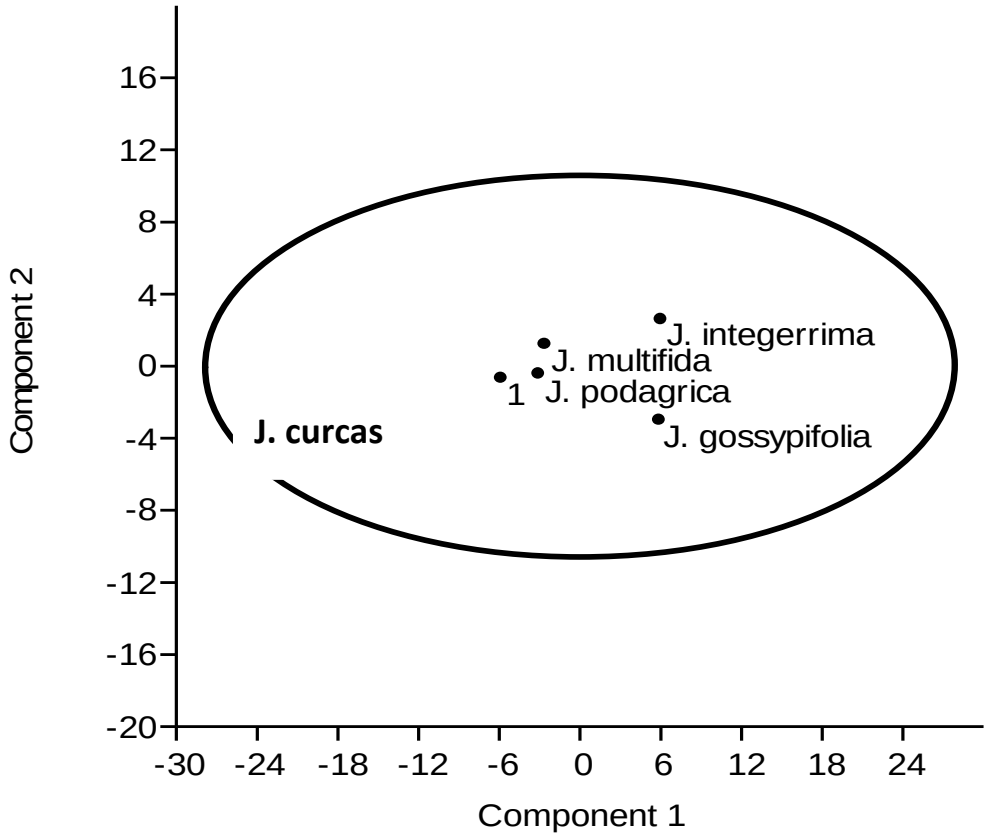

Figure 2: Principal Component Analysis Cluster Plot of the *Jatropha* species using the quantitative phytochemical parameters

The differences observed in the leaf quantitative phytochemical screening results demonstrate close relationship of the members of the genus studied and could therefore be employed in the taxonomic treatment of other genus and also in their infra-generic delimitations.

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