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MORPHOMETRIC IDENTIFICATION OF *Calotropis procera* (Aiton) Dryand and *Jatropha curcas* L. IN NORTHERN NIGERIA

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ABSTRACT

Phenetic analysis is a method for comparing plant species to establish their degree of genetic similarity. To identify and classify the enormous variety of plant life, morphological traits are essential. As the primary characteristics of *Calotropis procera* and *Jatropha curcas* species, subspecies, and variety were inadequately documented, there has been long-standing misunderstanding and controversy concerning the taxonomic designations of these species. This study aimed to use morphometric data to taxonomically identify *C. procera* and *J. curcas* using multivariate analysis. From the wild of Randagi, Sabon Gero, and Kabara in Kaduna state, Northern Nigeria, twelve samples of *C. procera* and *J. curcas* each were collected. SIMCA-P (V.14.1, Umetrics Sweden) was used for unsupervised multivariate analysis along with numerical phenetic analysis. The analysis of this phenetic data yielded a spectrum filter model with the strongest predictive power (fitness of the model) (Q2 (cum) 0.977) and the maximum variation (R2X (cum) 0.988). Principal component analysis (PCA) showed clustering between the species under examination along PCs 1 and 2, with *C. procera* clustering along the Y axis and *J. curcas* clustering along the X axis. A useful technique for avoiding adulteration is phenetic analysis-based taxonomic identification of plants in conjunction with multivariate analysis. However, to specifically analyse their relationships, a combination of molecular and developmental datasets is still required.

Keywords: *Calotropis procera*, discrimination, *Jatropha curcas*, plant taxonomy, phenetic

INTRODUCTION

Plants are crucial to human survival because they produce oxygen, food, and medicine. Further, plants are essential to preserve the planet's natural state. Plant identification is the foundational science from which modern medicine, gastronomy, farming, and botany have developed. The dispersal units in this family come in a wide range of shapes, sizes, and surface sculpturing, making them excellent taxonomic tools for plant identification (Harish *et al.*, 2013). The necessity of accurately naming a plant gave rise to the field of taxonomy. As per conservative estimates just one third of the world's plant species have formally been described and named (Fatihah *et al.*, 2012). No botanist could be familiar with more than a minuscule proportion of the known species (Harish *et al.*, 2013). Therefore, it is a crucial and challenging task to identify every plant species that exists around us. The identification and categorization of plant can be determined by using the morphological characteristics of various plant parts such as leaves, fruit, flower, etc. Each plant has unique leaf characteristics that helps botanists to distinguish different plant species and gauge the health of individual plants. The development and geographic distribution pattern of plants depend on the environmental factors like temperature, rainfall, topography, solar energy availability, altitude, soil texture, soil fertility, evaporation, light intensity, wind speed, etc. (Mildawati *et al.*, 2022). Recent scientific advancements have considerably helped the area of biosystematics, which is the study of systematic classification of living things. This is directly related to the vast morphological diversity observed in the natural world. Although the usefulness of a morphological method for classify plants has been contested, it nevertheless plays a significant role in recent classification schemes (Schneider *et al.*, 2013).

A drug plant's botanical identity, the quality of herbal preparation, and pharmacognostic standardization all rely on the pharmacopoeia literature of crude pharmaceuticals' morphological and microscopic structural descriptions (Alamgir, 2017; Mahmoud Abba, 2021; Jumare *et al.*, 2022). Morphological features are crucial for quick and accurate identification (Oktavianingsih *et al.*, 2019). Thus, the process of plant classification and identification based on their leaves is essential due to its fascination and the variety of applications it possesses. Leaf characteristics like shape, colour, and texture, play a vital role in plant identification (Kadir *et al.*, 2012, 2013). Because of their shared physical qualities, *Calotropis procera* (Apocynaceae) and *Jatropha curcas* (Euphorbiaceae) are often misidentified. Species taxonomy and evolutionary analysis are further confounded by the complex nature of interspecies relationships and the wide range of variation within each species. The unbroken leaf specimens are best suited for morphological method of identification because of its simplicity, speed, and ease of use (Ren *et al.*, 2018). The latex of *C. procera* has been used to heal boils, infected wounds, and other skin problems in humans, as well as to exterminate parasites in animals (Mahmoud Abba, 2021). Dried and chewed, the entire plant has tonic, anthelmintic, and expectorant properties (Mainasara *et al.*, 2012; Al-Rowaily *et al.*, 2020).

The leaves of *J. curcas* have been used for several medical ailments, like scabies, paralysis, rheumatism, and even hard tumours. They have also been used as antiparasites and rubefacient (Airaodion *et al.*, 2020). *C. procera* grows naturally in dry and semiarid regions all over the world (Al-Rowaily *et al.*, 2020). *J. curcas* plant grows naturally in both tropical and subtropical regions, so is widespread throughout the globe (Airaodion *et al.*, 2020). Phenetics is the study of evolutionary relationships among organisms based on shared phenotypic features which include shared morphological, anatomical, biochemical and phenotypic characteristics (Fatihah *et al.*, 2012). Statistical taxonomy or taximetrics is gold standard for analyzing phenetic relationships (Ningrum and Chasani, 2021).

Neither naturalists nor environmentalists have focused on the misidentification of *C. procera* and *J. curcas* from their natural habitat in Northern Nigeria. The success of agricultural and pharmaceutical endeavours, as well as environmental sustainability, depends on a deeper familiarity of plants in our environments. The taxonomical status of *C. procera* and *J. curcas* has become somewhat muddled. The rationale and criteria for determining the species restrictions are still up for discussion. For visual distinction, the species' morphologies are too similar. To better understand the distinctions between *C. procera* and *J. curcas*, additional research based on morphological evidence of each species is required. Botanical studies in underexplored regions, such as Kaduna state of Northern Nigeria, and the neighboring states, is necessary. We have chosen a few characteristics that have been particularly instructive. The purpose of this work was to employ multivariate analysis and generate morphometric data to taxonomically identify *Calotropis procera* and *Jatropha curcas*.

MATERIALS AND METHODS

Study area

All plant specimens were collected from wild in Kaduna state of northwest region of Nigeria. The state of Kaduna is located at latitude 10°20' North and longitude 07°45' East, with a total land area of about 46,053 km^{-2}. The rich plant diversity in Kaduna state is expected to serve as a starting point for any taxonomic research in Northern Nigeria. Specimens were obtained from Kabara (Soba), Randagi (Birnin Gwari), and Sabon Gero (Chikun) (Fig. 1), and were identified and placed at the Ahmadu

Bello University herbarium under the voucher numbers *Calotropis procera* (V/N ABU900218) and *Jatropha curcas* (V/N ABU2347).

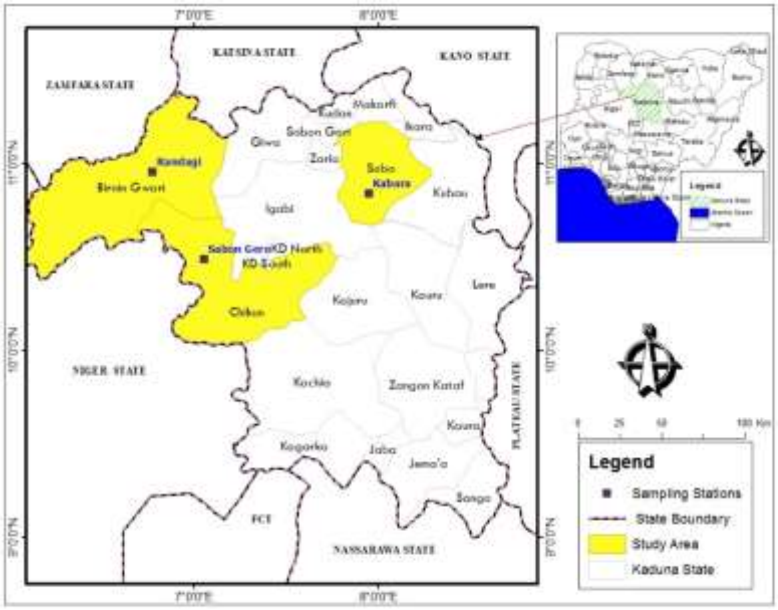

Fig. 1: Map of Kaduna State showing sampling location
Source: GIS Lab Department of Geography Using Arc GIS 10.3 Software

Sample sampling

Purposive samplings were used to compile the samples. Twelve samples were selected from each of the study area (Kamsurya *et al.*, 2022).

Morphometric analysis

For morphometric analyses, a total of 72 specimens from the research location were collected, 12 samples from each site for each species (Table 1) (Fatihah *et al.*, 2012). The analysis of twenty (20) characters was thorough. While qualitative characters were classified, continuous characters were counted. The leaves served as the source for observing, measuring, and encoding 20 distinct morphological features (Table 1) (Fatihah *et al.*, 2012).

Table 1: Character code of the attributes selected for morphometric analysis of *Calotropis procera* and *Jatropha curcas*

S. No.	Characters	Character coding
1	Plant height	$0 \leq 3$ m, $1 \geq 6$ m
2	Leaf length	$1 \leq 18$ cm, $1 \geq 19$ cm
3	Leaf width	$0 \leq 14$ cm; $1 \geq 16$ cm
4	Primary midrib	0 = yes; 1 = no
5	Petiole length	$0 \leq 5$ cm; $1 \geq 14$ cm
6	Petiole morphology	0 = Base swollen; 1= Pulvinate
7	Petiole attachment	0 = Marginal; 1 = Peltate central
8	Leaf attachment	0 = Opposite; 1 = Alternate
9	Lamina shape	0 = Obovate; 1 = Elliptic
10	Leaf organisation	0 = Simple; 1 = Palmately compound
11	Leaf base angle	0 = Obtuse; 1 = Wide obtuse
12	Leaf apex angle	0 = Obtuse; 1 = Odd-lobed obtuse apex
13	Leaf base shape	0 = Cordate; 1 = Sagittate
14	Petiole position	0 = Margin; 1 = Peltate eccentric
15	Leaf apex shape	0 = Lobed; 1 = Convex
16	Lamina margin	0 = Erose; 1 = Serrate
17	Leaf lobation	0 = Unlobed; 1 = Palmately lobed
18	Flower type	0 = Single colour; 1 = Multicolour
19	Vein category	0 = Pinnate; 1 = Actinodromous
20	Vein spacing	0 = Uniform; 1 = Abruptly increasing toward base

Multivariate analyses

The association between *C. procera* and *J. curcas* was determined by performing an unsupervised pattern of multivariate analyses with SIMCA-P (V.14.1 Umetrics Sweden) program, which included principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Abdulrahman *et al.*, 2021).

Principal components analysis

Through principal component analysis, a correlation matrix between researches can be derived. Specifically, it intended to examine pairs of variables that were linearly related. PCA is used to separate closely related species and to pin point the causal state of character.

Hierarchical cluster analysis

Using the acquired data, the hierarchical cluster analysis, a sort of multivariate analysis, categorizes the data. By grouping individuals with similar traits into clusters and emphasizing similarities and differences within and between the clusters, HCA develops a similarity matrix between the research subjects (Abdulrahman *et al.*, 2021). The most closely related species are those that form a clan. The cophenetic correlation coefficient between the distance matrix and the tree matrix was discovered in order to assess how well the cluster analysis matched the distance matrix (Abdulrahman *et al.*, 2021).

RESULTS AND DISCUSSION

The morphological characteristics of each species evaluated in this study effectively showed some variance among the test species and may be used to comprehend the differences between the species of *C. procera and J. curcas* (Fig. 2 and 3). To simplify data variables and minimize data dimension, the principal component analysis (PCA) typically involves transforming them into new variables known as principal components (Oktavianingsih *et al.*, 2019; Ningrum *et al.*, 2021). The use

Fig. 2: Wild picture of *Calotropis procera*; Whole plant, branch, leaf; and flower (from left to right)

Fig. 3: Wild picture of *Jatropha curcas;* Whole plant, branch, leaf; and flower (from left to right)

of macro-morphological characteristics. This is advantageous in taxonomy. The numerical categorization applications are widely used (Xiao *et al.*, 2004; Herdiyeni and Santoni, 2012; Chen *et al*, 2020). Morphological information based on multivariate analysis, an unsupervised analysis, was used to distinguish and classify plant species. This is because the PCA and HCA methodologies are impartial.

The fit plot of principal component analysis (PCA) revealed that the percentage of variation in the training data set for PCA is shown by R2 value. How well the model matches the data is shown by the fit statistic R2. To demonstrate how well the data-set fits the model, an R2 that is large (close to 1) is necessary. The model below was discovered to have 1. Having a high Q2 indicates a high level of predictability. (Fig. 4). The fitness of model demonstrated its potential as a quick approach to distinguish between plants of various species when paired with chemometrics.

The analysis of this phenetic data yielded a spectrum filter model with the strongest predictive power (fitness of the model) (Q2 (cum) 0.977) and the maximum variation (R2X (cum) 0.988). The Fig. 4 displays PCA scatter plot and major component value, which revealed the

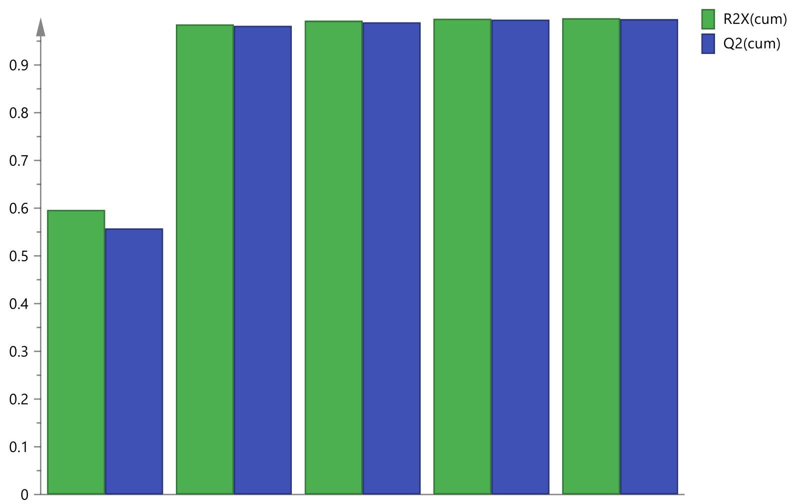

Fig. 4: Fitness model. As a measure of fit, it displays that the model well fits the data

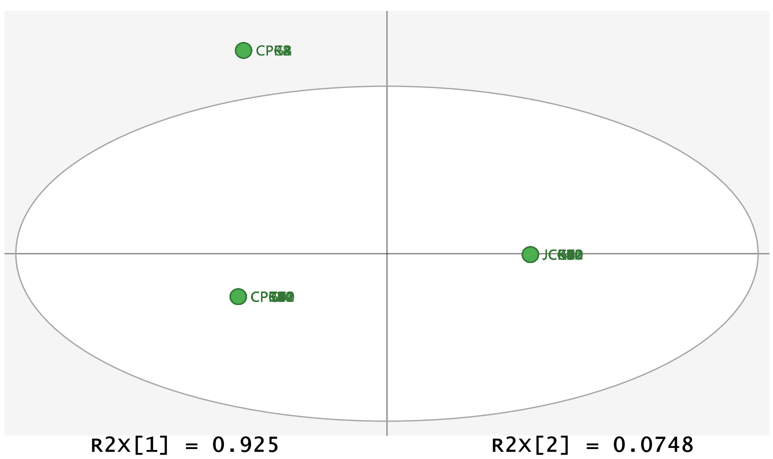

Fig. 5: PCA score plot of *C. procera* and *J. curcas;* It displays how the two species are situated with respect to each other

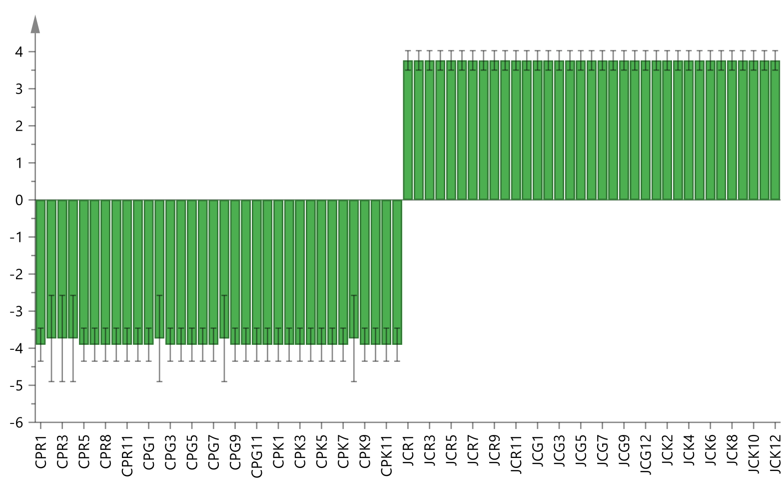

Fig. 6: Loading score plot of *C. procera* and *J. curcas* It displays how the observations contributed to the discrimination of the species

clustering based on the most salient characteristics. With *C. procera* clustering along Y-axis and *J. curcas* clustering along X-axis, there was a clear differentiation between the examined species along X-axis of PC 1 and 2 (Fig. 6).

Table 2: Mean contribution of variables responsible for the discrimination of examined species

S. No.	Variables	Mean
1.	Leaf length (LL)	1.54133
2.	Leaf width (LW)	0.57710
3.	Petiole length (PL)	0.88487
4.	Primary midrib (PMR)	0.88487
5.	Petiole morphology (PM)	0.88487
6.	Leaf organisation (LO)	0.88487
7.	Leaf base angle (LBA)	0.88487
8.	Leaf apex angle (LAA)	0.88487
9.	Leaf base shape (LBS)	0.88487
10.	Lamina shape (LS)	0.88487
11.	Petiole position (PP)	0.88487
12.	Leaf apex shape (LAS)	0.88487
13.	Lamina margin (LM)	0.88487
14	Flower type (FT)	0.88487
15	Vein category (VC)	0.88487
16	Vein spacing (VS)	0.88487
17	Leaf lobation (LLo)	0.88487
18	Petiole position (PP)	088487

To group them into separate categories and learn about their connections and distinctions, the model was used to create hierarchical cluster analysis (HCA), which divides the species into several clades according to their relatedness. A dendrogram serves as the output of a hierarchical cluster analysis (tree plot). The branching diagram indicated the number of separate groups (Fig. 7). They separated into two major clades, with first major clade displaying variance from *Calotropis procera* in the species gathered from Saxon Gero area (Fig. 7). While the second major clade showed all collected *J. curcas*, regardless of wherefrom they came (Fig. 7). Environment impacts the growth and development of plants. Phenotypic characteristics are significantly influenced by environmental changes, while environmental variations are significantly impacted by geographic and climatic conditions. Additionally, it caused variation in phenotypic traits of plants, both morphological or data with chemometrics could be a quick way to distinguish between different kinds of plants.

Conclusion: The morphological characteristics are "gold standard" for elucidating the evolutionary relationship of plants, and their use in tandem with multivariate analysis has proven quite fruitful. *Calotropis procera* and *Jatropha curcas* have fully been distinguished from one another in terms of their relationships and differences, with the additional caveat that there is intra-variation within the same species of *C procera* gathered from the same area. Discriminating between the test species was well explained by PCA and HCA. The traits discovered as biomarkers were flower type, vein category, and vein spacing. We tried to putforth a *C. procera* and *J. curcas* chemometrics phylogenetic framework for the first time, which may serve as the basis for further pharmacological research.

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