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## NUMERICAL TAXONOMIC STUDY OF SOME EUPHORBIACEAE SPECIES WITHIN AMBROSE ALLI UNIVERSITY MAIN CAMPUS, EDO STATE, NIGERIA

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#### ABSTRACT

Numerical taxonomic studies have been described as highly effective in plant classification and delimitation even in difficult cases. This study employed numeric taxonomic methods in studying different Euphorbiaceae species within Ambrose Alli University campus with the goal of identifying similarities and differences between different species within the family. Twenty-five species comprising thirteen genera were studied using thirty-five morphological characters which included plant habit, colour of leaf, fruit shape, presence of sap, pubescence of leaves, presence of three-cell ovary, and inflorescence type. Data generated from the different morphological characters were scored and converted to binary numbers used for generating clusters of the Operation Taxonomic Units (OTUs) according to their overall similar Euclidean distance. Using both single and complete linkage, dendrograms were drawn to illustrate the relationship among the species. The dissimilarity shown in the OTUs were split into two clusters, C1 and C2 each comprising 10 and 15 species respectively. Species within the same cluster had greater similarity than those in the other cluster. Amongst diverse findings from the study is a great affinity between Jatropha curcas and Jatropha tanjorensis, Alcornea laxifolia and Mallotus oppositifolius; Hevea brasilensis and Croton zambesicus; and Acalypha hispida and Alcalypha wikisiena. Great dissimilarity was observed between Riccinodendron heudelotti and Euphorbia milli. The results from this study have shown that there is great similarity, and at the same time variety and heterogeneity among the different species of Euphorbiaceae considered.

Key words: Dendrogram; Euphorbiaceae; Numerical Taxonomy; Species.

## INTRODUCTION

Euphorbiaceae, also known as the spurge family, is a large family of flowering plants with over 300 genera and more than 7,500 species (Secco et al., 2012; Webster, 1994). It is one of the most complex and diverse family among the angiosperms. Members of the spurge family exhibit diversity in growth forms varying from small ephemerals to herbaceous annuals or perennials, shrubs, small trees and cactus-like succulents (Pahlevani, 2017). They occur mainly in the tropics, however, some of them are well represented in non-tropical areas such as the Mediterranean Basin, the Middle East, South Africa and southern United States (Rahman and Akter, 2013; Davis et al., 2007).

Some plants of tremendous economic importance belong to the Euphorbiaceae family, e.g. cassava (Manihot esculenta), considered to be one of the thirteen most important human foods in the world (Secco et al., 2012); the Para rubber tree (Hevea brasiliensis), a natural source of rubber; castor oil plant (Ricinus communis) and Barbados nut (Jatropha curcas). Many of them such as poinsettia (Euphorbia pulcherrima) and leafy spurge (Euphorbia esula) are also grown as ornamentals. Their leaves are alternate, seldom opposite, with stipules. They are mainly simple, but where compound, are always palmate, never pinnate. Stipules may be reduced to hairs, glands, or spines, or in succulent species are sometimes absent. The radially symmetrical flowers are unisexual, with the male and the female flowers usually occurring on the same plant. Also, there is a wide variety in the structure of their flowers. They can be either monoecious or dioecious. The stamens can number from 1 to 10 or even more and the female flowers have superior ovaries (Davis *et al.*, 2007). A milky latex is a characteristic feature of the subfamilies Euphorbiodeae and Crotonoideae.

Although the Euphorbiaceae is one of the largest dicot families and conspicuous in the tropics, Webster (1994) pointed out that they had been relatively neglected by systematists in the 20th century. Other families such as Compositae, Leguminosae, and Solanaceae gained more attention as they were the subjects of various symposia. The very first international conference which focused on the Euphorbiaceae family was held at Kew in 1986 (Jury et al., 1987). According to Webster (1994), the 1986 Kew symposium, on the Euphorbiales, was heavily biochemical in orientation and focused more on relationships between the Euphorbiaceae and other families. Another symposium held years later in St. Louis was the first in which the classification of the family and its constituent taxa was the major focus of intrafamilial attention (Webster, 1994).

One of the most important benefits of taxonomic studies is that they help to provide a fast and accurate way to identify organisms (Quicke, 1993). A taxonomic key, also referred to as a diagnostic key, consists basically of a series of questions about the characteristics of an unknown organism. By carefully working through these questions, and answering each in order, the user is led to a correct identification of the unknown organism. Numerical taxonomy is a system of grouping of species by numerical methods on the basis of their character states. It establishes classification of organisms based on their similarities. It utilizes many equally weighted characters and employs clustering and similar algorithms to yield objective groupings. It can be extended to give phylogenetic or diagnostic

systems, and can be applied to many other fields (Sneath and Sokal, 1973). Numerical taxonomic methods besides providing help in identifying organisms, have been emphasized as a reliable and effective way of resolving conflicting taxonomic issues. In plant systematics studies where it is often difficult to get the desired results conventional taxonomic through methods. numerical taxonomic techniques have proven to be effective. Several researchers by their works have shown the importance of numerical taxonomic methods in plant classification and delimitation. For example, Kolawole et al. (2016) used numerical taxonomic methods to observe the differences and similarities in the morphological characters of several species of the Genus Jatropha belonging to the family Euphorbiaceae.

The numerical taxonomic methods proved to be more sensitive in the delimitation of the studied taxa, as it provided a greater discrimination along the spectrum of taxonomic differences among the Jatropha species, and helped to reveal more information on the level of relationship within the genus. Also, Bello et al. (2013) in attempts to resolving conflicting taxonomic issues resulting from gross morphological variability between and within several species of Solanum, employed numerical techniques to evaluate the taxonomic status of some species in the genus Solanum (Solanaceae) vegetative using and floral characters. Their study succeeded in highlighting a few morphological markers for distinguishing these species.

Other studies that employed numerical taxonomic techniques include the work of Illoh and Olorede (1991) involving the use of numerical taxonomic techniques in classification of *Magnifera indica;* Folorunsho and Jayeola (2009); El-Gazzar (2008) and Rhodes *et al.* (1970).

However, it must be stated here that more work needs to be done using numerical taxonomic techniques to better expatiate the pattern of relationship existing in the family Euphorbiaceae. This study was aimed at contributing to what is currently known about the taxonomic relationship of species within the family Euphorbiaceae. The objective of the study was to confirm the phenetic relationships among some species of Euphorbiaceae using numeric data, and to also identify the similarities and differences between different species in the family Euphorbiaceae within Ambrose Alli University main campus, based on diverse morphological characters.

# MATERIALS AND METHODS

# Study Area

The study was conducted in Ambrose Alli University Campus (Lat.  $6^{\circ} 42^{1}$ N, Long.  $6^{\circ} 08^{1}$ E), in Ekpoma, Edo state, Nigeria. Ekpoma has a tropical climate characterized by two distinct conditions of wet and dry seasons. Often, while April to October is wet with a brief dry spell in August, November to March is dry. About 1666 mm of precipitation falls annually, and the average annual temperature in Ekpoma is 24.8 °C (Climate-data, 2019; Nigerian Meteorological Agency, 2017). The area is characterized by rainforest vegetation.

## **Plant Material**

The specimens were collected from different sites within Ambrose Alli University campus (Fig. 1). A total of twenty-five Euphorbiaceae species composing of thirteen genera were sighted within the study area, and they were collected from their natural habitat (Table 1). Specimen identification was done at Botany Herbarium, Ambrose Alli University (AAU), and compared with those of the Herbarium specimens of Forestry Herbarium Ibadan (FHI), Nigeria for authentication. The fresh specimens collected were prepared and deposited in Botany Herbarium, Ambrose Alli University, Ekpoma as voucher specimens.

## **Morphological Characters**

Characters such as the colours of leaves, midrib, petiole length, presence and absence of sap and shape of fruit, among others were recorded on the field. Other qualitative characters such as pubescence of the leaves, stem, petiole and the presence of stipules and three-cell ovary were noted in laboratory with the aid of a hand lens. The information of floral parts such as the numbers of inflorescence and number of leaves at stem base were determined by counting physically. Other characters like leaf shape, leaf base, leaf apex, leaf margin, fruit shape, leaf texture and leaf spine, stem spine and dropping leaves were recorded. The quantitative attributes of the vegetative and reproductive parts were measured and scored. The length and girth of the stem, leaves and flowers together with the length of petiole, style and stamen were measured to the nearest centimetre using metric ruler. Thirty-five characters in all were used for the numerical analysis.

## **Numerical Analysis**

Numerical Taxonomical Analysis was based on the method of Sheath and Sokal (1973). The hierarchical cluster analysis for morphological characters was performed using binary squared Euclidean distance; the dendrogram was drawn using single and complete linkage. The similarity coefficient Euclidean distance were measured using clustered by un-weighted. Pair-group clustering was measured using SPSS version 20 software. Data generated from the different morphological characters were scored and converted to binary numbers of 1 or 0. i.e., present or absent and entered into a computer which generated clusters of the Operational Taxonomical Units (OTUs) according to their overall similar Euclidean distance. Dendrogram represented was constructed. which the diagrammatic illustration of the relationship among the species based on their degree of similarity in which the OTUs are linked together at various levels of resemblance.



Fig. 1. Map of Ambrose Alli University, Ekpoma, Edo State

Keys:

- Main road
  - Foot path
- Abundant species

<b>1</b> a	ble 1: List of the Flant Species	conected and used for the Study	
S/N	Plants collected	Common/Local Names	Habit
1.	Euphorbia hirta L.	Asthma plant (English); Azugben	Herb
		(Esan/Benin)	
2.	Euphorbia heterophylla L.	Mexican fire plant, Milkweed (English)	Herb
3.	Alchornia laxifolia (Benth.)	Lowveld bead-string (English);	Shrub
	Pax and Hoeffm. K.	Uwenuwen (Esan/Benin)	
4.	Euphorbia hyssopifolia L.	Hyssop spurge (English)	Herb
5.	Mallotus oppositifolius	Ogheghe (Esan/Benin)	Shrub
	(Geisel.) Mull. Arg.		
6.	Alchornia cordifolia (Schum	English Christmas Bush (English);	Shrub/small
	and Thonn) Mull. Arg.	Akowo, uwanwe (Esan/Benin)	tree
7.	Alcalypha ciliata Forssk.	Ifoki (Esan/Benin)	Herb
8.	Hevea brasilensis Muell. Arg.	Para rubber tree (English)	Tree
		Alrhaba-nofua (Esan/Benin)	
9.	Manihot esculenta Crantz.	Cassava (English); Igai (Esan/Benin)	Shrub
10.	Ricinus communis L.	Castor oil plant (English)	Shrub/small
			tree
11.	Alcalypha wikisiena Mull.	Jacob's coat (English)	Shrub
	Arg.		
12.	Alcalypha hispida Burm. F.	Chenille plant (English)	Shrub
13.	Cordieum veriagatum (L.) A.	Garden croton (English)	Shrub
	Juss.		
14.	Euphorbia millis Des Moul.	Christ plant; crown of thorns (English)	Shrub
15.	Jatropha curcas L.	Barbados nut (English)	Shrub
		Ujavade/okokoiko (Esan/Benin)	
16.	Jatropha tanjorenses Ellis and	Jatropha, Hospital too far	Herb
	Saroja.		_
17.	Hura crepitans L.	Sandbox tree (English)	Tree
18.	Bryenia nivosa W. Bull.	Snow Bush (English)	Herb
<b>19.</b>	Euphorbia postrata Aiton.	Prostrate spurge (English)	Herb
20.	Jatropha gossypifolia L.	Bellyache bush (English)	Shrub
21.	Ricinodendron heudelotti	African wood-oil nut tree (English);	Tree
	(Baill.) Pierre ex Heckel	okhuen-n´fua (Esan/Benin)	<b>TT</b> 1
22.	Croton hirtus L. Herit	Hairy croton (English)	Herb
23.	Jatropha multifida L.	Coral plant (English)	Shrub
24.	Croton zambesicus Muell.	Ajekobale (Yoruba)	Shrub
	Arg.		<b>TT</b> 1
25.	<i>Euphorbia graminea</i> Jacq.	Grassleaf spurge (English)	Herb

## RESULTS

Thirty-five vegetative and reproductive characters were identified for numerical analysis of species belonging to the family Euphorbiaceae. These characters are listed in Table 2. Two types of dendrograms were obtained: single linkage (Figure 2) and complete linkage (Figure 3), based on morphological character code numerically inputted in SPSS version 20. These characters and character states generated were used for the cluster analysis. From the characters

employed in the numerical analysis, the similarity and dissimilarity of the species of Euphorbiaceae are represented in the complete linkage of the dendrogram. The dissimilarity shown in the operational taxonomic units (OTU) was separated into two clusters. Cluster 1 contains 10 species which is further split into two sub clusters (c3 and c4); c3 contain 5 species namely: Riccinodendron heudelotti, Jatropha *multifida*, Ricinus communis, Manihot esculenta and Jatropha gossypifolia. c4 also contain 5 species namely;

Cordiaeum variegatum, Bryenia nivosa, Euphorbia heterophylla, Euphorbia graminea and Euphorbia millis. Cluster 2 contains 15 species with two major sub clusters (c5 and c6), c5 contain 5 species while c6 contain 10 species within two clusters (c7 and c8). c7 contains 2 species while c8 contain 8 species.

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S/No.	Character	Character State	Number of
			States.
1.	Habit	tree or shrub	2
2.	Growth habit	prostrate, erect	2
3.	Leaf tip hair	present or absent	2
4.	Leaf apex	sharp or blunt	2
5.	Leaf base shape	cordate, acute, truncate or rounded or oblique.	3
6.	Leaf margin	entire or undulate, crenate	2
7.	Leaf shape	palmate, elliptic, obovate, ovalto ovate, cordate.	5
8.	Leaf spines	present or absent	2
9.	Leaf texture	tender and stiff	2
10.	Leaf pubesence	present or absent	2
11.	Leaf attachment	alternate or opposite	2
12.	Leaf arrangement	simple leafs, compound leafs, whorled leafs	3
13.	Midvein thickness	thick or thin	2
14.	Midvein colour	green or red	2
15.	Pinnate leaf	present or absent	2
16.	Stem colour	green or purple	2
17.	Stem pubescence	present or absent	2
18.	Stem spines	present or absent	2
19.	Stem scent	obvious or little to absent	2
20.	Stem habit	woody or herbaceous	2
21.	Petiole thickness	thick or thin	2
22.	Petiole length	long or short	2
23.	Petiole colour	green or purple	2
24.	Petiole pubescence	present or absent	2
25.	Inflorescence at node of a leaf	present or absent	2
26.	Inflorescence type	racemose or cymose	2
27.	Fruit shape	three sided or others	2
28.	Stem shape	round or others	2
29.	Presence of sap	present or absent	2
30.	Presence of three cell ovary	present or absent	2
31.	Stipules	present or absent	2
32.	Inflorescence number at leaf base	many or few	2
33.	Leaf number at stem base	many or few	2
34.	Colour of leaves	green or red	2
35.	Dropping leaves	present or absent	2

## Table 2: List of Characters Used in the Numerical Analysis.



Figure 2: Dendrogram using single linkage to show the species relationship.



# Dendrogram using Complete Linkage

Figure 3: Dendrogram using complete linkage to show the species relationship.

# DISCUSSION

Numerical methods according to Illoh et al. (1992) have been successfully used in various plant systematic studies where classification and delimitation prove difficult to achieve by conventional taxonomic methods. In this study, numerical taxonomy has proven helpful in showing similarities and dissimilarities among the different Euphorbiaceae species. Each cluster was divided to form a clade that anchor species that are phylogenetically related based on Hutchinson and Dalziel (1958) classification scheme. The relative closeness between these species is attributed to their similar morphological features in single linkage as shown in Figure 2. In the first cluster Riccinodendron heudelotti is grouped with Jatropha multifida indicating that they are closely allied, and this is evidenced by the presence of palmate leaves (shape), radiating or whorled leaves (arrangement), alternate leaves (attachment), entire margin, growth habit, plant habit and leaf apex.

Two major clusters (C1 and C2) were derived from the complete linkage (Dendrogram) with C1 having 10 species and two sub-clusters (c3 and c4), with five species in each. This gives an indication that the 10 species in C1, are distinct from those in the second cluster (C2), and they are more closely related to each other. The first chunk of cluster 1 species (i.e. c3) are: Riccinodendron heudelotti, Jatropha multifida, Ricinus communis, Manihot esculenta and Jatropha gossypifolia. These species are closely related, and this is evidenced by the

presence of several similar characters. However, two major characters present in these five species namely: radiating or whorled leaves in their leaf arrangement pattern, and their palmate leaf shape, distinguish them from the other sub-cluster (c4) in cluster 1. The five species in c4 also have their major similarities which separate them from species in the first chunk (c3). These similarities include leaf margin (entire or undulate) and leaf Among these species, texture. Euphorbia heterophylla and Euphorbia graminea show evidence of being more closely related as indicated by the characteristics they share: the same undulating leaves and leaf shape or supporting structure. These findings agree with previous findings documented in literature (Hutchinson and Dalziel, 1958; Heywood, 1993; Aigbokhan and Ekutu, 2012).

A close association between Alcalypha ciliate, Croton hirtus, Euphorbia prostrata, Euphorbia hyssopifolia and Euphorbia hirta is evidenced in the second cluster by the common characters shared by these species which include similar leaf arrangement, leaf colour and leaf texture. In the sub-cluster, the highest similarities have been observed among Euphorbia hyssopifolia, Euphorbia prostrata and Euphorbia hirta with the presence of sap or latex, prostrate growth habit, blunt leaf apex, pinnate leaves, presence of stipules, cymose inflorescence, leaf colour, inflorescence at node of a leaf, petiole length and petiole thickness. These findings agree with earlier reports, such as that of Rahman and Akter (2013) on the taxonomy and medicinal uses of the plant family Euphorbiaceae in Rajshahi, Bangladesh. Also, the results indicated that the species: Jatropha curcas and Jatropha tanjorensis have great affinity (Kolawole et al., 2014).

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The results from this study also showed great dissimilarity between Riccinodendron heudelotti and Euphorbia milli but show great affinity Alcornea laxifolia between and Mallotus oppositifolius. The eight species under cluster 7 namely; Hevea brasilensis, Croton zambesicus, Alcornea laxifolia, Mallotus oppositifolius, Alcalypha hispida, Alcornea cordifolia, Alcalypha wikisiena and Hura crepitans have common characters linking them together but the division into sub-clusters is based on the dissimilarities even amidst their similarities. Hura crepitans is the only species in its chunk because of its unique character, i.e. the presence of more than three-cell ovary (Park and Backlund, 2002; Webster, 1994). Hevea brasilensis and Croton zambesicus are closely related. Acalypha hispida and Alcalypha wikisiena are also closely related with similar characters such as leaf shape, dropping leaves, leaf texture, petiole length, stem shape and infloresence type, but these two species cut across Alcornea laxifolia, Mallotus oppositifolius and Alcornea cordifolia, and the five species have one major similarity, which is the presence of dropping leaves (Soladoye et al., 2008; Webster, 1994).

## CONCLUSION

The results from this study have shown that among the twenty-five Euphorbiaceae species studied, there exists interesting similarities, and at the same time great variety and heterogeneity. To identify more similarities and dissimilarities within the Euphorbiaceae family, more numerical taxonomic studies covering broader vegetation zones and utilising more types of characters such as anatomical, cytological and molecular base markers, should be conducted.

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