



# Journal of Applied Sciences

ISSN 1812-5654

**science**  
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## Phytochemical and Morphometric Analysis of the Genus *Acalypha* Linn. (Euphorbiaceae)

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**Abstract:** The relationship among five species of *Acalypha* Linn. (Euphorbiaceae) is described using phytochemical and quantitative morphological parameters based on herbarium and field collections. Preliminary phytochemical study revealed the presence of alkaloids, tannins, saponins and cardenolides in the five species. These secondary metabolites might be responsible for the frequent use of these species of *Acalypha* in Traditional medicine. The results of the multivariate analyses (Principal Component Analysis (PCA) and Cluster analysis) show that two of the nine quantitative parameters account for the differences among the taxa. *A. hispida* was found to be more closely related to *A. fimbriata*. *A. hispida* and *A. wilkesiana* share some resemblances. The affinity between *A. ornata* and *A. fimbriata* was stronger with 21.082 coefficient of agglomeration than the affinity between *A. ornata* and *A. hispida* with 1224.099 coefficient of agglomeration.

**Key words:** *Acalypha*, phytochemical-screening, morphometrics, numerical taxonomy

### INTRODUCTION

The genus *Acalypha* Linn. belongs to the family Euphorbiaceae with 462 species. It is the fourth largest genus of the family and is found in tropical and warm temperate regions. *Acalyphaeae* is the largest tribe in the uniovulate subfamily *Acalyphoideae* (Angiosperm family Euphorbiaceae). It consists mainly of trees and shrubs distributed in paleotropics. The tribe is made up of several economical, ecological and ornamental groups of plants (Govaerts *et al.*, 2000). Thirteen species are found in West Tropical region (Hutchinson and Dalziel, 1958). Ten of the species are found in the South Western region of Nigeria out of which five are abundant and widespread throughout the region. The species are monoecious or dioecious and are either herbs or shrubs, but rarely trees or lianas. The plant has contoured leaves which are curl shaped, olive green in colour with creamy margin, with the exception of *Acalypha wilkesiana* which is often copper red in colour. The leaves of *Acalypha* species are succulent with sappy stalks which tend to lose sappiness with age, alternate, stipulate, characterised with serrated edges, obvious mid-ribs and veins. The staminate flowers have four valvate sepals, four to eight stamens and vermiform anthers. The pistillate flowers are often prominently bracteate with 3 (4, 5) sepals (2) 3 carpels, 1 ovule per carpel and divided styles. Many species of

*Acalypha* share the characteristic of allomorphic pistillate flowers and fruits (Levin *et al.*, 2007).

The species of *Acalypha* are prominent in the Traditional medicinal practice of most tribes in Africa and Asia (Mothana *et al.*, 2008; Duraipandiyani *et al.*, 2006; Sofowora, 1982). *Acalypha wilkesiana* is used in West Africa for the treatment of headache and cold and in Nigeria, the cold extract of the leaves is used to bath babies with skin infection (Adesina *et al.*, 2000). The leaf poultice is deemed good for headache, swellings and colds in Trinidad. Its leaf extract is active against Gram+ve bacteria. The extracts from the seeds have immunomodulating properties that work against some tumors (Büssing *et al.*, 1999). *Acalypha ornata* has a wide range of uses and it is considered to be non toxic. It is reported as having medicinal applications in Central Africa. The cooked leaf is taken to relieve post partum pains and a root decoction as a laxative. *A. ornata* has been reported to be used for cutaneous and subcutaneous parasitic infections (Sabrina *et al.*, 2005). The leaves are compounded with the leaves of other drug-plants into a drug for children with rabies in Southern Nigeria. *A. hispida* has medicinal uses in South Eastern Asia.

However, inspite of the great relevance of the species of *Acalypha* in traditional medical practice in Africa (Adesina *et al.*, 2000; Akinyemi *et al.*, 2005; Wiart *et al.*,

2004; Motsei *et al.*, 2003), there has been very little work done on the herbarium study, collection and classification of the species of this genus found in South Western Nigeria. It is hoped that the methods of numerical taxonomy (Boratynski *et al.*, 2008; Henderson, 2006; Sonibare *et al.*, 2005) that are employed in this study will produce a hierarchical classification of the taxa with a visual interpretation of the taxonomic relationship between taxa.

This research analyses the phytochemical constituents of *Acalypha* species and its relationship with morphological characteristics in delimiting the species. The species include *Acalypha ornata* Hochst. *Acalypha hispida* Burm.f, *A. racemosa* Wall., *A. wilkesiana* Mull.-Arg and *A. fimbriata* Schum and Thonn.

### MATERIALS AND METHODS

In keeping with conventional taxonomic practice, both fresh and herbarium specimens of *A. racemosa*, *A. fimbriata*, *A. ornata*, *A. hispida* and *A. wilkesiana* were used in this study. Fresh specimens were collected in Ibadan city and Villages close to Ibadan in March, 2007. Herbarium specimens were those that were previously collected from different regions of Nigeria (Table 1). Each specimen was subjected to standard herbarium practice for preservation. The fresh specimens were placed inside absorbent paper (news paper) individually and pressed using pressing boards tied with strings. This was dried in the sun for 5 days. Plant identification and authentication were done at the Forest Herbarium Ibadan (FHI) by Mr. T.K. Odewo. Dried specimens were brushed with mercury chloride solution to reduce fungal load and mounted on mounting sheets. Field notes indicating location of collection, habit, collector's name,

determinative, name of specimen and plant description were also attached to the cardboards on which the specimens were mounted. Voucher specimens of all the species were deposited at FHI.

**Phytochemical methods:** Freshly collected specimens were dried at room temperature for 2 weeks. The dried specimens were finely blended using an electric blender and were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as alkaloids, saponins, anthraquinones, cardenolides and tannins. The phytochemical screening was carried out using standard procedure (Ajaiyeoba *et al.*, 2003; Trease and Evans, 1989).

**Morphometric analysis:** With the aid of a line ruler and twine, measurements such as petiole length, lamina length, leaf width, leaf length and length of apex were taken and recorded. The number of serrations and lateral nerves were also counted. The quantitative morphological measurements were compiled on recording sheets using ten specimens for each taxon. Values for each Operational Taxonomic Unit (OTU) were entered into Microsoft Excel spreadsheet and raw data were then coded to allow analysis using Unistat at 4.0 windows. The Mean and Standard deviations were calculated for all the entries. Analysis of variance (ANOVA) was carried out for nine selected quantitative measurement: leaf length, leaf width, lamina length, petiole length, Apical length, number of serrations, number of lateral nerves, lamina length/stalk length ratio, leaf length/leaf width ratio. These parameters were also subjected to Cluster analysis. The quantitative characters considered most helpful for distinction were put together into pictorial diagrams and a reduced sample was subjected to PCA ordination.

Table 1: Voucher information and distribution of representative Fresh and Herbarium specimens examined

Species	Distribution
<i>A. fimbriata</i> Schumm and Thonn	S. Nigeria - Oyo road: Akinyele, Latilo and others FHI 102046 Ibadan: Jericho Rosanwo and others FHI 107889
<i>A. racemosa</i> Wall	E. Nigeria - Cross - River: Obubura, Onochie FHI 89054 S. Nigeria: Abeokuta: foot of Olumo, Odewo FHI 35980 Ondo: Ado-ekiti secondary forest, Daramola and others FHI 91945 Oyo: Ibarapa, inside Anarcadium plantation, Akilla FHI 89478 Ogun: Olokemeji, Rosanwo and others FHI107891
<i>A. ornata</i> Hochst	N. Nigeria: Niger; Abuja, Kennedy FHI 7794 Kogi: Kabba secondary forest, Onochie FHI 88416 S. Nigeria: Oyo: Iseyin Ado-awaiye high forest, Daramola FHI 34691 Ogun: Olokemeji high forest, Rosanwo and others FHI 107890
<i>A. hispida</i> Burm. F.	E. Nigeria: Benin: Benin forest reserve, Onochie FHI 14600 S. Nigeria: Ibadan, Forestry research Institute, Odewo FHI 50283 Ibadan, Jericho, Ekundayo FHI
<i>A. wilkesiana</i> Mull.- Arg.	S. Nigeria: University of Ibadan, Indi hall, Odewo FHI 18133 Ibadan, : Amy barracks, Daramola FHI 47218 Ibadan: Forestry Research Institute, Latilo FHI 107050 Ibadan: Forestry Research Institute, Rosanwo and others FHI 107892

N: North, S: South, E: East

Table 2: Summary of the presence of the metabolites in the *Acalypha* species studied

Plant species	Secondary metabolites				
	Alkaloids	Cardenolides	Anthraquinones	Saponins	Tannins
<i>Acalypha fimbriata</i>	++	+	-	++	++
<i>A. wilkesiana</i>	++	++	-	++	++
<i>A. hispida</i>	++	+	-	++	++
<i>A. ornata</i>	+	+	-	++	-

+: Present in low concentration, ++: Abundant, -: Absent

Table 3: Mean±SD of the 9 Quantitative characters of *Acalypha* species examined

Char/Species	La. leng	P. leng	N. serr.	L. width	L. leng	La. nerve	Lt/stalk	L/width	L. apex
<i>A. ornata</i>	8.0±1.88	5.0±1.89	33.3±7.53	4.6±1.30	13.1±3.72	3.7±1.16	1.70±0.37	2.8±0.30	0.90±0.50
<i>A. hispida</i>	14.4±4.29	5.1±2.37	54.8±15.45	9.5±2.65	19.5±5.91	4.0±0.00	3.20±1.13	1.8±0.32	0.80±0.28
<i>A. fimbriata</i>	6.0±1.69	3.8±2.19	33.7±3.06	3.1±1.21	9.8±3.46	3.3±0.95	3.10±4.65	3.2±0.38	0.60±0.23
<i>A. wilkesiana</i>	14.8±4.15	1.8±0.33	76.6±25.24	8.8±3.66	16.6±4.12	3.7±0.48	8.70±4.15	2.3±0.51	0.40±0.20
<i>A. racemosa</i>	6.7±2.21	4.2±2.63	38.3±10.48	4.2±1.62	10.9±4.67	3.8±0.63	1.95±1.10	2.6±0.41	0.47±0.20

La. leng = Lamina length, P. leng = Petiole length, N. serr. = Number of serration, L. width = Leaf width, L. leng = Leaf length, Lt. Nerve = Lateral nerve, Lm./stalk = Lamina/stalk ratio, L/width = Leaf length/width ratio, L. apex = Length of apex

## RESULTS AND DISCUSSION

The results of the phytochemical screening of the plant species are presented in Table 2. All the secondary metabolites tested for (alkaloids, cardenolides, saponins, tannins) are present in abundance in all the *Acalypha* species except Anthraquinones. The mean and standard deviations of the quantitative characters employed are shown as Table 3. According to the PCA, 93% of the variance is expressed by two factors (lineal combinations of parameters). For each factor, parameter with maximum discriminating power and the percent of variance they account for are expressed as indicated in Table 4.

Figure 1 shows the component plot on rotated axes for the nine quantitative parameters while Table 5 shows the result of principal components of the factors analyzed. Only two (the lamina length and petiole length) contributed greatly to the delimitation of the species. Table 6 shows the correlation coefficients of the nine quantitative parameters. It is shown that there is high significant negative correlation between: petiole length and lamina length, number of serrations and petiole length, leaf width and petiole length, lamina length/Stalk length ratio and petiole length, lamina length/stalk length ratio and number of lateral nerves, leaf length/leaf width length, leaf length/leaf width and number of serrations, leaf length/leaf width and leaf width, leaf length and leaf length/leaf width, leaf length/leaf width and number of lateral nerves, lamina length/stalk length ratio and leaf length/leaf width ratio. They are highly significant. The degree of relationship between the five species of *Acalypha* is shown in Table 7 and Fig. 2.

The description and taxonomy of the genus *Acalypha* have been attempted at different times by various authors (Sagun and Levin, 2007; Qin *et al.*, 2006; Levin, 1999). The use of morphometric technique combined with phytochemical screening for the presence

Table 4: Factor loading of 9 quantitative characters in principal component analysis

Characters/axes	PRIN 1	PRIN 2
Lamina length	0.985	0.064
Petiole length	-0.395	0.913
No. of serrations	0.933	-0.358
Leaf width	0.980	0.173
Leaf length	0.911	0.375
Lateral nerves	0.632	0.629
Lamina/stalk ratio	0.723	-0.664
Leaf length/width ratio	-0.877	-0.423
Length of apex	0.200	0.839

Table 5: Cumulative principal component analysis (PCA)

Characters	Eigenvalue	Variance (%)	Cumulative
Lamina length	5.519	61.325	61.325
Petiole length	2.856	31.732	93.057
No. of serrations	0.553	6.140	99.197
Leaf width	0.072	0.803	100.000
Leaf length	4.52E-016	5.02E-015	100.000
Lateral nerves	8.51E-017	9.46E-016	100.000
Lamina/stalk ratio	-3.65E-017	-4.06E-016	100.000
Leaf length/width ratio	-8.12E-017	-9.02E-016	100.000
Length of apex	-4.07E-016	-4.52E-015	100.000

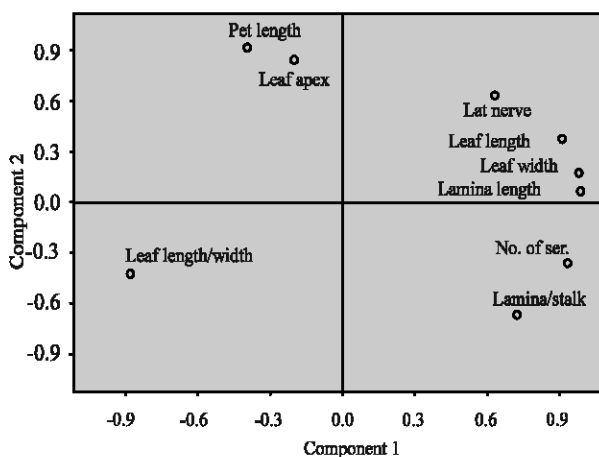


Fig. 1: Component plot in rotated space for 9 quantitative parameters

Table 6: Similarity matrix based on correlation coefficient of *Acalypha* species

Characters	La. leng	P. leng	N. serr.	L. width	L. leng	La. nerve	Lt./stalk	L/width	L. apex
L.a. leng	1	0.332	0.719	0.99	0.949	0.593	0.697	-0.86	-0.068
	ns	ns	ns	ns	ns	ns	ns	sig	sig
P. leng	0.332	1	-0.699	0.225	-0.016	0.316	-0.901	-0.052	-0.825
	ns	ns	sig	ns	sig	ns	sig	sig	sig
N. Serr.	0.9	-0.699	1	0.852	0.719	0.359	0.919	-0.658	-0.468
	ns	sig	ns	ns	ns	ns	ns	sig	sig
L. width	0.99	0.225	0.852	1	0.973	0.686	0.604	0.923	-0.021
	ns	ns	ns	ns	ns	ns	ns	ns	sig
L. leng	0.949	-0.016	0.719	0.973	1	0.734	0.439	0.927	0.209
	ns	sig	ns	ns	ns	ns	ns	ns	ns
La. Nerve	0.593	0.316	0.359	0.686	0.734	1	0.026	-0.893	0.217
	ns	ns	ns	ns	ns	ns	ns	sig	ns
Lm./stalk	0.697	-0.901	0.919	0.604	0.439	0.026	1	-0.311	-0.605
	ns	sig	ns	ns	ns	ns	ns	sig	sig
L/width	-0.86	-0.052	-0.658	0.923	0.927	-0.893	-0.311	1	-0.066
	sig	sig	sig	ns	ns	sig	sig	ns	sig
L. apex	-0.068	-0.825	-0.468	-0.021	0.209	0.217	-0.605	-0.066	1
	sig	sig	sig	ns	ns	ns	sig	sig	ns

L.a. leng. = Lamina length, P. leng. = Petiole length, N. serr. = Number of serration, L. width = Leaf width, L. leng. = Leaf length, Lt. Nerve = Lateral nerve, Lm./stalk = Lamina/stalk ratio, L/width = Leaf length/width ratio, L. apex = Length of apex, Result significant at  $p \leq 0.05$ , ns = not significant, sig = significant

Table 7: Agglomeration schedule

Stage	Cluster 1	Cluster 2	Coefficient
1	1: <i>A. ornata</i>	3: <i>A. fimbriata</i>	21.082
2	1: <i>A. ornata</i>	5: <i>A. racemosa</i>	29.401
3	2: <i>A. hispida</i>	4: <i>A. wilkesiana</i>	525.611
4	1: <i>A. ornata</i>	2: <i>A. hispida</i>	1224.099

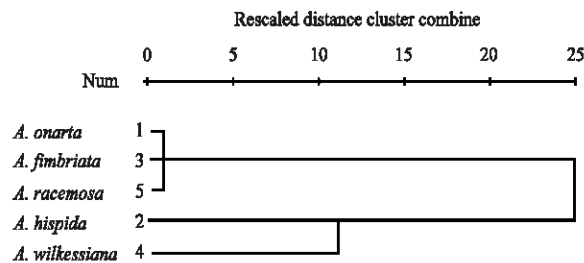


Fig. 2: Dendrogram showing the relationship among *Acalypha* species

of secondary metabolites in the species of *Acalypha* represents a new approach in the taxonomy of the genus in Nigeria (Neustupa and Stastny, 2006; Sonibare *et al.*, 2004; Gomez-Campo *et al.*, 2001; Chiapella, 2000; Sneath and Sokal, 1973). The methods of Numerical taxonomy have been used by many authors in classifying plants as well as interpreting results of taxonomic studies (Fabio, 2007; Borazan and Babac, 2003). The results of these methods are considered to be unbiased indicators of the similarity or difference between the taxa, which are in turn used to arrange taxa in hierarchical order (Agbagwa and Okoli, 2005; Quike, 1993).

In the present study, two out of the nine quantitative parameters employed have been shown by PCA and Cluster analysis to be accountable for the differences among the taxa. *A. fimbriata* and *A. hispida* are more

closely related. *A. ornata* shared some resemblances with *A. fimbriata*, *A. hispida* and *A. racemosa*. The affinity between *A. ornata* and *A. fimbriata* (with 21.082 coefficient of agglomeration) was found to be stronger than that between *A. ornata* and *A. hispida* (with 1224.099 coefficient of agglomeration). This result permits a visualization of the degree of affinity among the species. The two characters: lamina and petiole lengths which have contributed greatly to the delimitation of the species can be efficiently employed in the taxonomic treatment of the other species of *Acalypha* that are not included in the present study. Morphologically, *Acalypha hispida* and *A. wilkesiana* are closely related. They both have terminal red female inflorescence and characteristic mid ribs. But the quantitative study of these species explains their differences. The dendrogram, using average linkage between groups for hierarchical analysis reveals that *A. hispida* is more closely related to *A. fimbriata* although also sharing some resembles with *A. wilkesiana*. The difference in the values of mean and standard deviation of the nine quantitative parameters shows that both species differ from each other. The factor loading of the parameters also showed that some characters such as lamina and petiole length carried more weight in variation than others. This is probably accounting for the variation within the species.

The variation within species may also be due to age difference of the plants as younger leaves are succulent and their stalks are sappy but tend to lose their sappiness with development or age (Delzon and Loustau, 2005; Jongbloed *et al.*, 2004). Leaf shapes and sizes have been shown by the work of previous authors to vary within the same plant due to the light intensity acting on the leaves,

thus affecting the carbohydrate balance which in turn affects the length of the cells in the direction of the long axis thereby giving rise to differences in the length, shapes and width of the leaves (Campey *et al.*, 2000; Wilson *et al.*, 1998).

Varying intensities of light could produce leaves of different shapes, sizes and width. Environmental factors and geographical localities are known to contribute to the variation within plant species. The observations made in the species of *Acalypha* may likewise be attributed to environmental factors, genetic variation or interaction among plants, competition for space and minerals which can also bring about morphological discrepancies. If the environment of plants varies, reproductive isolation and thus racial variation is an inevitable result (Tsukaya, 2006; Kim *et al.*, 2005). Distribution maps and many literatures show that *Acalypha wilkesiana*, *A. ornata*, *A. racemosa*, *A. hispida*, *A. fimbriata* are wide spread and abundant in the South Western zone of Nigeria (Hutchinson and Dalziel, 1958; Keay, 1989). As a result, morphological variation among species is inevitable.

The presence of Alkaloids and Tannins in all the species may be taken as an indication of their common ancestry and also as scientific evidence of their medicinal application (Adesina *et al.*, 2000). The presence of these typical metabolites has been reported in many plants to be responsible for the pharmacological activities of medicinal plants (Gutierrez-Lugo *et al.*, 2002; Okoli *et al.*, 2007; Mothana *et al.*, 2006). The presence of alkaloids and Tannins in all the species of *Acalypha* studied is an indication of a close relationship among the species and is presenting a further evidence for the development of new antimicrobials from these species. In addition to demonstrating the value of morphometric methods combined with phytochemical screening in the taxonomy of the genus *Acalypha*, the study presents greater and more detailed information on level of relationship within the genus. We suggest an application of this method in future elaborate taxonomic treatment of the genus *Acalypha*.

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