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Chemotaxonomic Significance and Environmental Implications of the Phytochemical Constituents of four *Mussaenda* L. (Rubiaceae) taxa in Nigeria

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ABSTRACT

This work investigated the phytoconstituents of some *Mussaenda* taxa (Rubiaceae) collected from Nsukka (Derived Savanna) and Uyo (Tropical Rainforest) ecological zones of Nigeria to establish their contribution as possible taxonomic and environmental monitoring markers. Fresh leaf samples used in this study were collected from plants of the same age, air-dried, and made into powder for further use. Histochemical and phytochemical tests were carried out by following the standard procedures. Results of the comparative phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, phenols, hydrogen cyanide, reducing sugars, soluble carbohydrates, saponins, steroids, terpenoids, and tannins in varying proportions. Results of the phytochemical constitute analysis revealed the presence of the cystoliths from the *M. elegans* (MEL) and *M. erythrophylla* (MER) which were absent in *Mussaenda* " Doña Aurora" (MDA) and *Mussaenda* "Doña Luz"(MDL). Further, the presence of the Raphides was unique to MEL while Gum and mucilage were reported only in MDA. Quantitatively, MEL had the highest value of terpenoids (650.88 mg/100g) while MDA had the highest values of phenols (899.27 mg/100g), alkaloids (311.01 mg/100g), reducing sugars (967.35 mg/100g), steroids (2.89 mg/100g), soluble carbohydrates (27.68 mg/100g) and tannins (393.16 mg/100g), and MDL was richest in glucosides (339.64 mg/100g), flavonoids (69.34 mg/100g) and hydrogen cyanides (1.34 mg/100g). The cluster analysis based on obtained phytochemical data revealed three (3) distinct clusters with MEL in cluster 1; MDA and MDL in cluster 2 while cluster 3 had MER. The evolutionary closeness

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of the two infraspecific and exotic species (MDA and MDL) were confirmed and their taxonomic relationship with the indigenous infrageneric taxa (MEL and MER) was established. The results also highlight the opportunity of employing plant metabolomics in ecological studies and environmental monitoring.

1 Introduction

'Phytochemotaxonomy' is an expanding area of study that focuses on utilizing chemical characteristics to improve plant classification. Phytochemical characteristics often correlate well enough with other types of characters and provide help in clearer insights into taxonomic relationships (Pandey and Misra 2008; Nwafor and Orabueze 2019). Recognition of chemical evidence in plants started, in fact, since early man and plants were classified based on their color, odor, and efficacy, obviously as a result of their chemical compositions (Nwafor and Orabueze 2019). A wide spectrum of useful biomolecules synthesized from different metabolic pathways exists in plants (Harvey, 2000). Chemical data of taxonomic value include micromolecules (both primary and secondary metabolites) and macromolecules, both non-semantide (not involved in information transfer such as starches, cellulose etc.), and semantides (molecules responsible for carrying information such as protein, RNA and). Primary metabolites are thosecompounds that take part in essential metabolic pathways, these include amino acids, aconitic acid, and citric acid. On the other hand, secondary metabolites are by-products of metabolism which are involved in non-essential roles such as protection against herbivores, insects, and harsh environmental conditions. These compounds include phenolics, alkaloids, terpenes, glucosinolates, etc. (Pandey and Misra 2008, Salim et al. 2008).

Specific chemicals or groups of compounds have been proven good chemical markers for the taxonomic delineation of plants (Nwafor and Orabueze 2019). A good example is lathyrine, which is isolated only from species of *Lathyrus* (Fabaceae), and as such, its distribution has contributed to a successful taxonomical grouping of the genus into seven distinct intrageneric taxa (Pandey and Misra 2008). Phytochemicals that are ubiquitous such as flavonoids and alkaloids are more extensively studied in chemotaxonomy. Flavonoids have been proven very useful in the classification and delimitation of the families like Asteraceae, Cactaceae, Molluginaceae, and Rubiaceae (Pandey and Misra 2008; Bhargava et al. 2013) while alkaloids are proven useful in taxonomic delimitations of the members of Apocynaceae, Papaveraceae, Solanaceae, and Fabaceae. The three genera of the family Fabaceae namely *Genista, Ammodendron,* and *Adenocarpus* contains ammondendrine-hystrine alkaloids which help in the identification of these genera. Further, morphine is present only in *Papaver somniferum* (Pandey and Misra, 2008). The presence or absence of latex vessels, resins, gums, and crystals in the wood are also features of taxonomic significance (Gott et al. 2006; Singh 2016). Many cellular contents, for example, albuminoids (*Laportea*), starch grains (*Solanum tuberosum*), protein bodies (Cactaceae), large silica bodies in epidermal cells (Arecaceae, Musaceae), calcium oxalate crystals (*Allium, Eichhornia*), cystoliths (Cannabinaceae, Moraceae, Urticaceae) and tanniniferous cells (Raptaceae, Xyridaceae) also have some bearing in plant systematics (Pandey and Misra 2008; Ekeke and Agbagwa 2014).

The genus *Mussaenda* L. is comprised of a group of flowering plants classified under the family Rubiaceae. They constitute approximately 200 individual species distributed across Asia, Australia, and Africa. It has also been introduced into Europe, South America, and North America (Nwafor et al. 2019). Most of the members of this genus are popular in the landscape industry as ornamental plants, largely due to their very attractive and colorful blooms. It is claimed that only a few ornamentals can compete favorably with *Mussaendas* when in full bloom. For instance, *M. philippica* is amongst the most cultivated ornamental plants around the globe. Hybridization and other breeding methods have further given rise to so many cultivars for improved aesthetic values, most of which bloom all seasons. Some other species have also found usefulness in other areas of human endeavor. These are also used in ethnomedicine for the treatments of cough, jaundice, liver diseases, dropsy, swellings, oedema, gout, and as febrifuge and appetizers (Burkill 1985; Stuart 2016). Phytochemicals reported from various parts of *Mussaenda* species had diuretic, antiphlogistic, antipyretic, anti-fertility (Venkatesh et al. 2013), antimicrobial (Kim et al. 1999; Jayasinghe et al. 2002), anti-tumor, analgesic, diuretic, anticonvulsant, antioxidant activities (Yaolan et al. 2004; Vidyalakshmi et al. 2007), antiviral (Sunit et al. 2003) and cytotoxic activities (Jing-Qiu et al. 2002).

Notwithstanding the multipurpose utilization of these species and their potential, especially in the area of drug discovery, there still exists a paucity of information on their taxonomic classification, and the fact that new cultivars have emerged in the last decades makes the situation even more difficult (Nwafor et al. 2019). In this study, therefore, we assessed the qualitative and quantitative phytochemical constituents of the four *Mussaenda* taxa in Nigeria (*M. elegans, M. erythophylla,* and two cultivars of *M. philippica*) for their possible contribution as chemical markers for taxonomic delimitation, ecological studies, and environmental monitoring.

2 Materials and Methods

2.1 Collection of Plant Samples

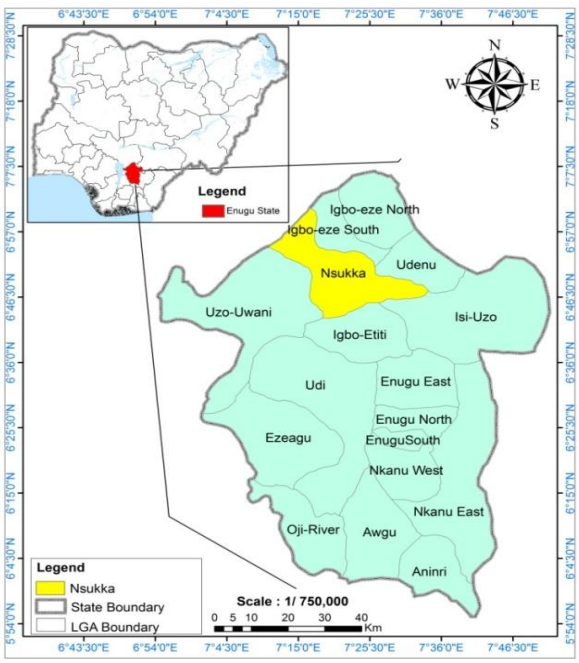
Fresh leaves of *M. elegans* (MEL), *M. erythophylla* (MER), *M.* "Dona Aurora" (MDA), and *M.* "Dona Luz" (MDL) used in this study were collected from Nsukka (Derived Savanna) and Uyo (Tropical Rainforest) ecological zones in Nigeria (Figure 1). Sampling and collection were carried out in July 2017. Nsukka is located in Enugu State, south-eastern Nigeria between Latitude 7°9'30" to 7°35'0" E and Longitude 6°41'15" to 7°5'20" N. It has a tropical climate with a mean annual temperature of 25°C and a mean annual rainfall of 1580 mm. Its vegetation type is derived savanna. Uyo is situated in Akwa Ibom State in the Niger-Delta region of Nigeria. It has a tropical rainforest climate with a mean annual temperature of 26.4 °C and a mean annual rainfall of 2500 mm. The vegetation type is rainforest and mangrove swamps.

2.2 Histochemical Studies

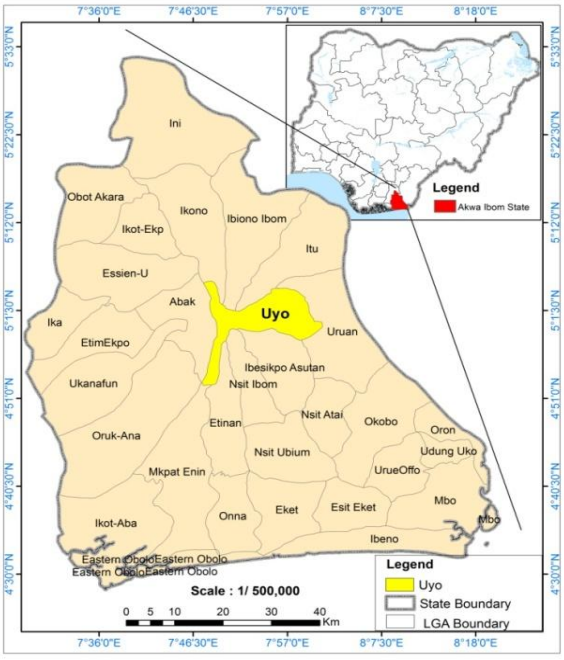
The leaves were dried under shaded conditions and pulverized with mortar and pestle. Chemo-microscopy was conducted on the powders to determine the presence of starch, calcium oxalate crystals, and lignified vessels. A judicious quantity of the sample was dropped on a glass slide. One drop of chloral hydrate was dropped and passed over a Bunsen burner repeatedly until bubbles formed. This signified the successful clearing of the tissues. The presence of lignin was tested by dropping phloroglucinol and concentrated hydrochloric acid (1:1) on a little quantity of the cleared powder on a glass slide, and glycerin was added to aid observation under a light Olympus Tokyo (Japan No.271961) microscope at ×100 magnifications. To test for starch, a drop of iodine was added to a little quantity of the cleared leaf powder on a glass slide and observed under a light Olympus Tokyo (Japan No.271961) microscope at ×400 magnification. A drop of Iodine solution and concentrated acetic acid (1:1) added to a little quantity of the cleared leaf powder on a glass slide revealed the presence or absence of calcium oxalate crystals, raphides, and cystoliths. Sudan IV reagent was used for fats and oil test, Ruthenium red for gum and mucilage, and Biuret reagent, ninhydrin for protein (Nwafor et al. 2019).

2.3 Phytochemical Studies

Qualitative phytochemical screening was carried out by following the standard methods. The presence of the alkaloid was tested by using Dragendorff's test (Sofowora 1993). Fehling's test was carried out for the presence of reducing sugars while the Frothing test was carried out for the estimation of Saponins, and Molisch's test was carried out for the presence of carbohydrates (Sofowora 1993). Liebermann-Buchard's test was carried out to evaluate the presence of Steroids and Terpenoids (Sofowora 1993). Ferric chloride was used for the estimation of the presence of Tannins, and a sodium hydroxide test was carried out for flavonoids (Trease and Evans 2002). Legal's Test was carried out for the estimation of glycosides, while the Ferric Chloride test was used for Phenols (Vijisaral and Subramanian 2013). Quantitative tests were also conducted on the phytochemicals that showed a



Nsukka in Enugu state, Nigeria



Uyo in Akwa Ibom State, Nigeria

Figure 1 Study locations

positive results. This was determined spectrophotometrically according to El-Olemyl et al. (1994) and Nwokonkwo (2009). AUv-Vis spectrophotometer (Shimadzu – UV 1800) was used to measure absorbance and values were calculated from the standard curves.

2.4 Statistical Analyses

Data obtained from the results were subjected to the Analysis of Variance (ANOVA) on the Statistical Package for Social Sciences (SPSS version 20) platform and a significant difference was tested

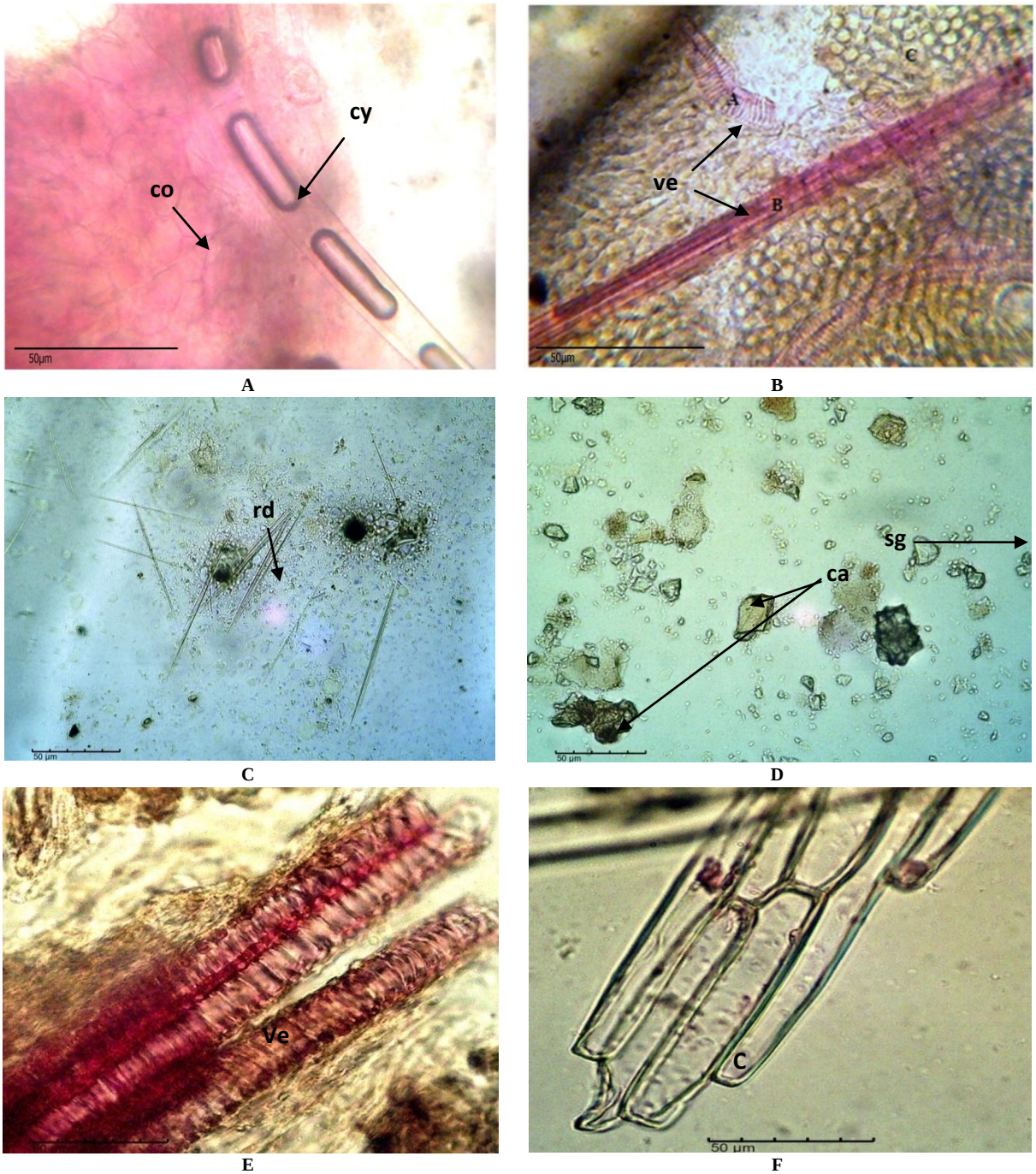


Figure 2 Chemomicrophotographs of the leaves of the *Mussaenda* taxa: A=co (lignified collenchyma cell) and cy (cystolith); B = ve (lignified vessel element) and c (cork cell); C = rd (raphide); D = ca (calcium oxalate crystal) and sg (starch grain); E = ve (lignified vessel element); F = c (cork cell)

at 95% probability (at $P \leq 0.05$) whereas Duncan's Multiple Range Test (DMRT) was used as post hoc test for separation of means. Students' independent sample T-test was used to test for significance (at $P \leq 0.05$) and to compare means of data from the two locations. Principal Component Analysis (PCA) was used to establish the taxonomical relationship among the taxa.

3 Results

3.1 Chemomicroscopy

The chemomicroscopy of the leaf powder of the four studied taxa of *Mussaenda* showed the presence of the various histochemicals (Table 1). The histochemicals were observed at different proportions across the studied four species. Among the studied taxa, lignin was reported at a higher concentration across all four taxa while fats, oil, and protein were absent. The presence of cystoliths was reported from the MEL and MER while it was absent in MDA and MDL. Further, Raphides were unique to MEL while gum and mucilage were reported from the MDA (Table 1). The chemomicrophotographs of the *Mussaenda* taxa are presented in Figure 2 and showed the presence of lignified collenchyma cell, cystolith, lignified vessel element, raphide, calcium oxalate crystal, starch grain, and cork cell.

3.2 Qualitative and Quantitative Estimation of Phytochemicals

The phytochemical analysis results of the four studies on taxa of *Mussaenda* showed the presence of various phytochemicals such as alkaloid, flavonoid, glycoside, hydrogen cyanide, phenol, and reducing sugar, saponin, soluble carbohydrate, steroid, tannin, and terpenoid in different proportions. MEL was unique with the high presence of HCN while MER was unique with the lower presence of reducing sugars and

Table 1 Histochemicals present in the leaves of the four taxa of *Mussaenda*

Histochemical	MEL	MER	MDA	MDL
Lignin	+++	+++	+++	+++
Starch	++	++	++	++
Calcium oxalate crystals	++	+	+	+
Raphides	+	-	-	-
Cystoliths	+	+	-	-
Fats and oil	-	-	-	-
Gum and mucilage	-	-	+	-
Protein	_	_	_	_

+++: Highly present; ++: moderately present; +: present; - : Absent

Table 2 Qualitative phytochemical constituents of the four *Mussaenda*taxa

Phytochemicals	MEL		MER		MDA		MDL	
	DS	RF	DS	RF	DS	RF	DS	RF
Alkaloids	++	++	++	++	++	++	++	++
Carbohydrates	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Glycosides	++	++	++	++	++	++	++	++
HCN	++	++	+	+	+	+	+	+
Phenols	++	++	++	++	++	++	++	++
Reducing sugars	++	++	+	+	++	++	++	++
Saponins	+	+	+	+	+	+	+	+
Steroids	++	++	+	+	++	++	+	+
Tannins	++	++	++	++	++	++	++	++
Terpenoids	++	++	+	+	++	++	++	++

+ = present; ++ = highly present

Table 3 Quantitative phytochemical constituents of the four *Mussaenda*taxa

Phytochemical (mg/100 g)	MEL	MER	MDA	MDL
Alkaloids	238.93 ± 7.92^{b}	230.55 ± 4.55^{b}	311.01 ± 0.37^{a}	223.33 ± 6.26^{b}
Flavonoids	34.13 ± 0.32^{c}	45.08 ± 0.04^{b}	21.17 ± 0.05^{d}	69.34 ± 0.15^{a}
Glycosides	321.25 ± 0.08^{b}	306.04 ± 0.38^{c}	278.89 ± 0.00^{d}	339.64 ± 0.22^{a}
HCN	0.61 ± 0.01^{c}	0.56 ± 0.01^{c}	1.00 ± 0.07^{b}	1.34 ± 0.18^{a}
Phenols	659.75 ± 0.45^{c}	649.82 ± 0.00^{d}	899.27 ± 0.04^{a}	843.07 ± 0.03^{b}
Reducing sugars	603.85 ± 0.22^{b}	478.14 ± 0.06^{d}	976.35 ± 0.21^{a}	584.05 ± 0.03^{c}
Saponins	0.60 ± 0.02^{d}	0.68 ± 0.00^{c}	0.82 ± 0.00^{a}	0.74 ± 0.01^{b}
Carbohydrates	12.86 ± 0.02^{d}	14.33 ± 0.00^{c}	27.68 ± 0.01^{a}	19.58 ± 0.00^{b}
Steroids	2.34 ± 0.01^{b}	0.76 ± 0.02^{d}	2.89 ± 0.03^{a}	0.83 ± 0.00^{c}
Tannins	304.11 ± 2.26^{d}	311.35 ± 2.24^{c}	393.16 ± 0.45^{a}	323.38 ± 0.06^{b}
Terpenoids	650.88 ± 0.36^{a}	178.53 ± 2.24^{d}	467.62 ± 0.25^{c}	524.65 ± 0.14^{b}

*Means with different superscript alphabets across each row differ significantly at $P \leq 0.05$

Table 4 Quantitative comparative phytochemical constituents of the four *Mussaenda* taxa across the study sites

Phytochemical (mg/100 g)	MEL		MER		MDA		MDL	
	DS	RF	DS	RF	DS	RF	DS	RF
Alkaloids	256.63 ± 0.10*	221.23 ± 0.15	238.22 ± 0.01*	222.89 ± 6.69	311.84 ± 0.01	310.18 ± 0.00	237.33 ± 0.03*	209.33 ± 0.03
Flavonoids	34.85 ± 0.01*	33.42 ± 0.06	45.05 ± 0.05	45.11 ± 0.05	21.28 ± 0.00	21.06 ± 0.02	69.67 ± 0.01	69.01 ± 0.00
Glycosides	321.43 ± 0.01	321.08 ± 0.01	306.89 ± 0.01*	305.18 ± 0.01	278.88 ± 0.01	278.88 ± 0.01	340.14 ± 0.01*	339.14 ± 0.01
HCN	0.63 ± 0.00*	0.60 ± 0.00	0.58 ± 0.03*	0.54 ± 0.01	1.15 ± 0.01*	0.85 ± 0.01	1.74 ± 0.00	0.94 ± 0.00*
Phenols	660.75 ± 0.00*	658.75 ± 0.00	649.82 ± 0.01	649.82 ± 0.01	899.33 ± 0.01	899.20 ± 0.07	843.14 ± 0.01	843.00 ± 0.00
Reducing sugars	604.35 ± 0.00*	603.35 ± 0.00	478.00 ± 0.00	478.27 ± 0.01	976.68 ± 0.01*	976.01 ± 0.33	584.09 ± 0.04	584.00 ± 0.00
Saponins	0.65 ± 0.00*	0.55 ± 0.00	0.68 ± 0.00	0.68 ± 0.00	0.83 ± 0.00*	0.81 ± 0.00	0.77 ± 0.00*	0.71 ± 0.00
Carbohydrates	12.89 ± 0.00*	12.82 ± 0.04	14.33 ± 0.00	14.33 ± 0.00	27.68 ±0.01	27.68 ± 0.01	19.58 ± 0.00	19.58 ± 0.00
Steroids	2.37 ± 0.00*	2.32 ± 0.00	0.75 ± 0.03	0.78 ± 0.00	2.96 ± 0.01*	2.82 ± 0.00	0.83 ± 0.01	0.83 ± 0.01
Tannins	309.17 ± 0.00*	299.05 ± 0.02	316. 35 ± 0.01*	306.35 ± 0.01	394.16 ± 0.01*	392.16 ± 0.01	323.52 ± 0.01	323.23 ± 0.02
Terpenoids	651.68 ± 0.00*	650.08 ± 0.00	183.53 ± 0.01*	173.53 ± 0.01	468.17 ± 0.00*	467.07 ± 0.03	524.35 ± 0.00	524.95 ± 0.02

* = significantly higher at $P \leq 0.05$

terpenoids. Also, the high presence of steroids in MDA differentiates it from MDL (Table 2). The amounts of the phytochemicals significantly ($P < 0.05$) varied across the different taxon (Table 2). MDA recorded significantly ($P < 0.05$) higher amounts of alkaloid, phenol, reducing sugar, saponin, soluble carbohydrate, steroid, and tannin (Table 3). Significant differences in the amounts of phytochemicals evaluated across the two study locations in each of the taxa were also recorded (Table 4). Generally, the phytochemicals were significantly higher in plants collected from the derived savannah as compared to the rainforest. *M. elegans* collected from the derived savannah had a significantly higher concentration of alkaloid, flavonoid, hydrogen cyanide, phenol, reducing sugar, saponin, soluble carbohydrate, steroid, tannin, and terpenoid contents as compared to those collected from the rain forest (Table 4).

3.3 Cluster analysis of the taxa

The cluster analysis which was based on correlations using Ward's method grouped the accessions into 3 clusters for histochemical and phytochemical attributes (Figure 3). MEL was in cluster 1, cluster 2 contained MDA and MDL while cluster 3 had MER. However, MDA and MDL were more related to MEL than MER. The low concentrations of reducing sugars and terpenoids could be the distinguishing factor of MER.

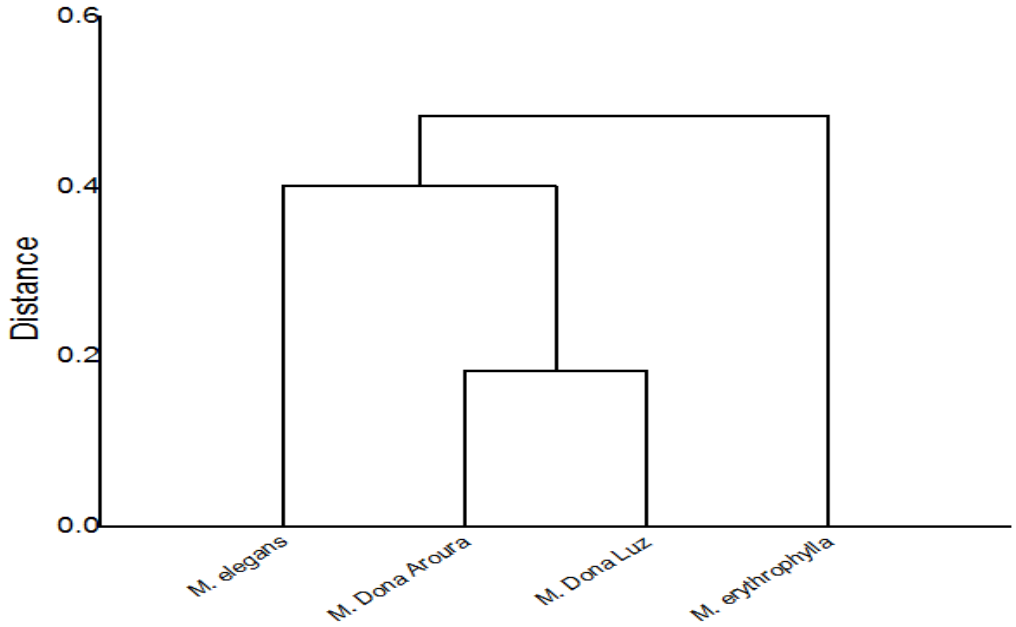


Figure 3 Cluster pattern of the histochemical and qualitative phytochemicals analysis of the four taxa of *Mussaenda* generated from Hierarchical cluster analysis using the ward's correlation method

4 Discussion

Results of the chemomicroscopy showed that lignified tissues, starch granules, calcium oxalate crystals, and cystoliths were present in all studied taxa. These histochemicals are important diagnostic features in the identification and standardization of crude drugs even when in powdered form (Sonibare and Adeniran 2014; Erst et al. 2021). *M. elegans* stood out by possessing rod-like calcium oxalate crystal forms raphides. Calcium oxalates and cystoliths are often found in plant tissues, especially in trichomes to protect them from harsh environmental conditions and herbivores. This could be a reason why the use of these plant species for grazing and animal feed has not been reported in the literature.

Further phytochemical screening revealed the presence of phenols, glycosides, tannins, and terpenoids like secondary metabolites in higher concentrations. These were closely followed by reducing sugars and alkaloids. The considerable quantities of flavonoids and phenolics reported here could an indication that the examined taxa could be investigated for potential anti-oxidative stress agents (Suksungworn and Duangsrisai 2021). The higher contents of alkaloid, tannin, and terpenoid as observed are indications of possible antiparasitic, antiviral and antimicrobial properties of the species (Akiyama et al. 2001; Kolodziej and Kiderlen 2005; Soladoye and Chukwuma 2012; Nweze and Nwafor 2014; Kolawole et al. 2017).

The steroid composition of these samples suggested their possible use in the development and production of sex hormone-related drugs (Okwu, 2001). Asl and Hossein (2008) emphasized the contribution of saponins in orally administered herbal formulations and their ability to hydrolyze glycosides from terpenoids. Interestingly, unhealthy phytoconstituents such as hydrogen cyanide and soluble carbohydrate were minutely present to prove the safety of these plants in the treatment and management of ailments as already been reported in the literature (Manandar and Manandar 2002; Vidyalakshmi et al. 2007).

Specific plant chemicals or groups of compounds have been proven good chemical markers for the taxonomic delineation of plants (Nwafor and Orabueze 2019). In this study, it was reported that the four taxa were successfully distinguished based on the differences and similarities in their phytochemical make-up. *M. elegans* was in cluster 1, cluster 2 contained *M.* "Dona Aurora" and *M.* "Dona Luz" while cluster 3 had *M. erythrophylla*. The two infraspecific and exotic taxa (MDA and MDL) were confirmed to be most closely related based on cluster analysis. It also shows that they are more related to MEL than MER (both of which are indigenous species) (Burkill 1985). The low concentrations of reducing sugars and terpenoids could be the distinguishing factor of MER.

Generally, the phytochemicals were significantly higher in plants collected from the derived savannah as compared to their counterparts collected from the rainforest (wetter) region. This could be explained by the fact that plants produce phytochemicals (secondary metabolites) in response to varying environmental stress conditions, to survive under harsh situations such as drought, diseases, pest attacks, etc. (Liu et al. 2016; Nwafor and Orabueze

2019). This also opens up the opportunity of employing plant metabolomics in ecological studies and environmental monitoring. Results of the study also reported the environmentally-influenced changes in the wood anatomical features of the same species (Nwafor et al. 2021).

Conclusion

The results of the study can be concluded that phytochemical content can be used as an important diagnostic tool in the delineation and identification of the studied taxa. For instance, only MEL had raphides while gum and mucilage were observed in MDA. Their evolutionary relationship was also established based on cluster analysis of data obtained from the qualitative and quantitative phytochemical studies. The evolutionary closeness of the two infraspecific and exotic species (MDA and MDL) were confirmed and their taxonomic relationship with the indigenous infrageneric taxa (MEL and MER) was established. The results also highlight the opportunity of employing plant metabolomics in ecological studies and environmental monitoring.

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