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Taxonomic Values of Secondary Metabolites (Hydrocarbons and Flavonoids) in the Genus *Euphorbia* L. from Niger Delta, Nigeria

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ABSTRACT

Taxonomic value of secondary metabolites in the Euphorbia L. has been carried out. This study aimed at using hydrocarbon skeletons and flavonoids in improving the classification of the genus in Nigeria. Eleven species of this genus occurring in Niger Delta, Nigeria were identified based on field collection and herbarium specimens deposited in Forestry Herbarium Ibadan (FHI). Fresh plant specimens from these species of Euphorbia were subjected to Gas and paper chromatography. Results of this study showed that these species contained aliphatic hydrocarbons (C_8 , C_9 , C_{10} , C_{11} , C_{12} , C_{13} , C_{14} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{22} , C_{26} , C_{28} , C_{30} , C_{32} , C_{34} and C_{36} and flavonoid classes (biflavonyl, flavone, flavonol, anthocyanin and glycosylflavone). C_8 to C_{11} are widespread among the species of Euphorbia studied, while C_{21} to C_{30} are restricted to E. heterophylla, E. splendes, E. hyssopifolia, E. milli, E. prostata, E. thymifolia and E. tirucalli and C_{31} to C_{40} are not found in E. hirta and E. desmondi but are widely distributed among the other species studied. The hydrocarbons and flavonoids screening showed differences among the species and could be used to classify the genus. This result confirmed that E. splendes is distinct from E. milli.

Key words: Taxonomy, hydrocarbons, flavonoids, *Euphorbia*

INTRODUCTION

Euphorbia L. belongs to the tribe Euphorbieae in the family Euphorbiaceae. This family is highly heterogeneous and occurs mainly in the tropics, warm temperate and sub-tropic zones of the world. The members of this family are trees, shrubs or herbs producing milky latex. This family is spread over 290 genera and 7500 species and is well represented in Africa and Tropical America (Willis, 1973). In West Africa, about 65 genera and 266 species are represented in this family (Gill, 1988; Hutchison and Dalziel, 1954). However, among the 65 genera, Euphorbia L. is the most popular genus and is widespread all over the tropical, temperate and subtropical zones and includes about 22 species recorded in Nigeria. Attempts have been made by several authors to document the origin and distribution of most Euphorbia species (Hutchison and Dalziel, 1954; Keay, 1989).

In Nigeria, some members of this genus are used for different purposes. For instance Euphorbia heterophylla and E. pulcherrima are used for ornamental and landscaping. Also, Euphorbia hirta enhances mammary development and milk secretion in young guinea pig and as a galactogogue by women in Nigeria (Aworinde et al., 2009).

Earlier works done on this genus were based on propagation, growth and physiology (David and Ruth, 1988; Kuhn et al., 1996; Schurr, 1998; Buckeridge et al., 2000; Qin et al., 2004), developmental anatomy (Heckenberger et al., 1998; Oparka and Turgeon, 1999), embryology and carpology (Raja Rajeswari Rao and Prakasa-Rao, 1975; Gori 1987; Bhanwra, 1987; Carmichael and Selbo, 1999), medicinal potentials of members of the family (Aworinde et al., 2009; Anselm, 2004) and the application of morphology and anatomy in phylogeny, ecology and systematics (Tokuoka and Tobe, 1995, 2002; Chaturvedi and Dalal, 2000; Esser, 2003; Aworinde et al., 2009).

The use of data generated from secondary metabolites and numerical methods in resolving the taxonomic problems in some taxa has gained much recognition for a long time. Some of the prominent works that have solved taxonomic problems among plant taxa with secondary metabolites include (Hsiao and Lin, 1995; Vicente et al., 2007; Weiller et al., 1994; Griesbach and Santamour, 2003; Slimestad, 2003; Andrey and Long, 1996; Gerard and Hendrik, 1980; Evans et al., 2008; Davies, 1960; Zuloaga et al., 1993). Mostly the sequence of proteins, flavonoid, amino acids and peptides are used (Stace, 1980; Harborne, 1973).

This present study described the importance of secondary metabolites (hydrocarbon and flavonoids) in some members of the genus *Euphorbia* with the aim of providing useful taxonomic data that would give further insight into proper classification, delineation and identification of the studied taxa.

MATERIALS AND METHODS

Sources of material: Plant materials used for this investigation were obtained from Field collections conducted between September, 2006 to December, 2008 in Niger Delta, Nigeria. These plant specimens were taken to Forestry Research Institute of Nigeria, Ibadan (FHI) and University of Port Harcourt Herbaria for proper identification.

Hydrocarbon screening: Five (5.0) grams of fresh plant material (cut into 5 mm size) was dipped into a clean, dry beaker containing 10 mL of hexane and allowed to stand for 30 sec. The extract was filtered to remove dirt. The filtrate was then passed through Al_3O_3 (alumina column) and eluded with petroleum (hexane). The samples (extract) was saponified with methanolic KOH and Ketonic materials by reacting them with 2-4-dinitrophenylhydrozine in aqueous 2 M HCl. The extract was then subjected to Gas Chromatography (GC) (Englinton and Hamilton, 1963b).

Flavonoid screening: The procedure employed in the survey of the plant tissues for flavonoids (flavones and flavonois) is as stated by Bate-Smith (1962) and Harborne (1973) who have surveyed over 1000 angiosperm species for their flavonoids.

Cluster analysis: The results were scored 1 (presence) or 0 (absence) and the Euclidean distance (similarity distances) clustered using Past software to generate dendrogram which represented the diagrammatic illustration of the relationship among the species based on their degree of similarities.

RESULTS AND DISCUSSION

The results and findings of this study are presented in Table 1-3 and Fig. 1.

The occurrence of different hydrocarbon backbones is presented in Table 2. The result showed that C_8 - C_{11} occurred in all the *Euphorbia* species studied. However, C_{12} occurred in all the species except *E. pulcherima*, C_{18} , C_{14} and C_{17} were not identified in *E. splendes*, *E. hirta* and *E. pulcherima*

Table 1: Occurrence and distribution of different aliphatic hydrocarbon backbones in the Euphorbia species

Species name	Alip	Aliphatic hydrocarbon backbones																		
	C ₈	C ₉	C_{10}	C ₁₁	C ₁₂	C ₁₃	C_{14}	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C_{20}	C_{22}	C_{26}	C_{28}	C ₃₀	C ₃₂	C ₃₄	C ₃₆
E. heterophylla	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	-
E. splendes	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
E. hirta	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. pulcherima	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
E. desmondi	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
E. hypossifolia	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-
E. kamerunica	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	+	-
E. milli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E. prostrata	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	+	-	+	-
E. thymifolia	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-
E. tirucalli	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	-

^{+:} Present, -: Absent

Table 2: Occurrence and distribution of different flavonoid groups in the Euphorbia species

	Different flavonoids groups identified in the species												
Species name	Iso-Orientin	Azaleatin	Myricetin	Cyanidin	Quecertin	Isorhamnetin	Vitexin	Luteolin	Delphinidi				
E. heterophylla	-	-	+	+	+	-	+	+	-				
E. splendes	-	-	+	-	-	-	+	+	+				
E. hirta	-	-	+	+	+	-	+	-	+				
E. pulcherima	-	-	+	+	+	-	-	+	-				
E. desmondi	-	-	+	+	-	-	-	-	-				
E. hypossifolia	-	+	+	+	+	-	-	-	-				
E. kamerunica	+	-	+	+	-	+	+	-	+				
E. milli	+	-	+	-	+	+	+	-	+				
E. prostrata	-	-	+	-	+	+	+	-	+				
E. thymifolia	+	-	+	-	+	-	+	-	+				
E. tirucalli	+	-	-	-	+	-	+	-	-				

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Species name	Penidin	Pelargonidin	Apigenin	Kayaflavone	Kaempferol	Chrysoriol	Tricin	Alkaloid
E. heterophylla	+	-	+	+	-	-	-	+
E. splendes	+	+	-	+	-	-	-	+
E. hirta	+	-	-	+	+	+	-	+
E. pulcherima	-	-	+	-	-	-	-	+
E. desmondi	-	-	-	-	+	-	-	+
E. hypossifolia	+	+	-	-	-	+	+	+
E. kamerunica	-	-	-	+	-	+	+	+
E. milli	-	-	+	-	-	-	+	+
E. prostrata	-	-	-	+	-	-	+	+
E. thymifolia	-	-	-	-	-	-	-	+
E. tirucalli	+	-	-	-	-	-	+	+

^{+:} Present, -: Absent

while C_{15} and C_{18} did not occur in four (4) species namely E. prostrata, E. pulcherima, E. hirta and E. splendes. C_{16} occurred in E. heterophylla, E. desmondi, E. hypossifolia, E. milli and

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 $\ \, \text{Table 3: Different flavonoid groups, spectral range, Rf values and colour in UV/UV+Ammonia in the \textit{Euphorbia} species } \\$

	RF value×	100				
Species			Colour in	Spectral Max	Compound	Class of
name	BAW	FORESTAL	UV/UV+NH₃	in EtOH (nm)	identified	Flavonoid
E. heterophylla	44	27	Yellow	256, 378	Myricetin	Flavonol
	-	50	Reddish-Brown	535	Cyanidin	Anthocyanin
	41	64	Yellow	255, 374	Quercetin	Flavonol
	41	-	Brownish	269, 336	Vitexin	Glycosylflavone
	78	67	Brownish	252, 268, 350	Luteolin	Flavone
	70	62	Brownish	352	Peonidin	Anthocyanin
	88	82	Brownish	269, 336	Apigenin	Flavone
	98	-	Brownish	269, 336	Kayaflavone	Biflavonyl
E. splendes	-	30	Mauve	546	Delphinidin	Anthocyanin
	-	29	Yellow	256, 378	Myricetin	Flavonol
	=	67	Yellow	520	Pelargonidin	Anthocyanin
	78	65	Brownish	252, 268, 350	Luteolin	Flavone
	98	-	Brownish	252, 269, 350	Kayaflavone	Biflavonyl
	40	-	Brownish	269, 336	Vitexin	Glycosylflavone
	70	62	Brownish	352	Peonidin	Anthocyanin
E. hirta	-	48	Reddish-Brown	535	Cyanidin	Anthocyanin
	-	31	Mauve	546	Delphinidin	Anthocyanin
	70	62	Brownish	352	Peonidin	Anthocyanin
	82	57	Yellow	268, 368	Kaempferol	Flavonol
	42	27	Yellow	256, 378	Myricetin	Flavonol
	65	42	Yellow	255, 374	Quercetin	Flavonol
	81	76	Yellow	252, 269, 350	Chrysoeriol	Flavone
	98	-	Brownish	269, 336	Kayaflavone	Biflavonyl
	40	-	Brownish	269, 336	Vitexin	Glycosylflavone
E. pulcherima	26	43	Yellow	256, 378	Myricetin	Flavonol
-	65	40	Yellow	255, 374	Quercetin	Flavonol
	78	67	Yellow	252, 268, 350	Luteolin	Flavone
	90	82	Brownish	269, 336	Apigenin	Flavone
	-	50	Reddish-Brown	535	Cyanidin	Anthocyanin
E. desmondi	_	52	Reddish-Brown	535	Cyanidin	Anthocyanin
	82	55	Yellow	268, 368	Kaempferol	Flavonol
	45	28	Yellow	256, 378	Myricetin	Flavonol
E. hyssopifolia	42	30	Mauve	546	Delphinidin	Anthocyanin
y.r - ,	-	48	Brownish	535	Cyanidin	Anthocyanin
	50	48	Yellow	254, 369	Azaleatin	Flavonol
	-	62	Brownish	532	Peonidin	Anthocyanin
	44	28	Yellow	256, 378	Myricetin	Flavonol
	40	64	Yellow	255, 374	Quercetin	Flavonol
	72	72	Yellow	248, 269, 355	Tricin	Flavone
	80	-	Brownish	520	Pelargonidin	Anthocyanin
	85	76	Yellow	252, 268, 350	Chrysoeriol	Flavone
E. kamerunica	_	49	Brownish	535	Cyanidin	Anthocyanin
. namerwield	- 43	49 29	Yellow	256, 378	Myricetin	Flavonol
	45 83	29 76	Brownish	250, 57 6 252, 268, 350	Chrysoeriol	Flavonoi
		10	Yellow		Iso-Orientin	
	41	- 98	ченоw Brownish	252, 268, 350 269, 336	iso-⊖rienun kayaflavone	Glycosylflavone Biflavonyl

Table 3: Continue

	RF value×	100				
Species			Colour in	Spectral Max	Compound	Class of
name	BAW	FORESTAL	UV/UV+NH ₃	in EtOH (nm)	identified	Flavonoid
	74	52	Yellow	254, 369	Isorhamnetin	Flavonol
	73	71	Yellow	248, 269, 355	Tricin	Flavone
	43	-	Reddish-Brown	546	Delphinidin	Anthocyanin
E. milli	-	27	Yellow	256, 378	Myricetin	Flavonol
	41	41	Yellow	252, 268, 350	Iso-Orientin	Glycosylflavon
	43	-	Reddish-Brown	546	Delphinidin	Anthocyanin
	40	42	Brownish	269, 336	Vitexin	Glycosylflavon
	64	40	Yellow	255, 374	Quercetin	Flavonol
	89	82	Brownish	269, 336	Apigenin	Flavone
	74	52	Yellow	254, 369	Isorhamnetin	Flavonol
	73	71	Yellow	248, 269,355	Tricin	Flavone
E. prostrata	-	31	Mauve	546	Delphinidin	Anthocyanin
	63	42	Yellow	255, 374	Quercetin	Flavonol
	98	-	Brownish	269, 336	kayaflavone	Biflavonyl
	42	27	Yellow	256, 378	Myricetin	Flavonol
	41	42	Brownish	269, 336	Vitexin	Glycosylflavon
	74	52	Yellow	254, 369	Isorhamnetin	Flavonol
	73	70	Yellow	248, 269,355	Tricin	Flavone
E. thymifolia	-	33	Mauve	546	Delphinidin	Anthocyanin
	43	26	Yellow	256, 378	Myricetin	Flavonol
	66	40	Yellow	255, 374	Quercetin	Flavonol
	40	41	Yellow	252, 268, 350	Iso-Orientin	Glycosylflavon
	41	42	Brownish	269, 336	Vitexin	Glycosylflavon
E. tirucalli	40	41	Brownish	269, 336	Vitexin	Glycosylflavon
	69	-	Brownish	532	Peonidin	Anthocyanin
	40	42	Yellow	252, 268, 350	Iso-Orientin	Glycosylflavon
	64	43	Yellow	255, 374	Quercetin	Flavonol
	72	71	Yellow	248, 269,355	Tricin	Flavone

EtOH: Ethanol, BAW: Butanol, acetic acid and water (4:1:5), UV: Ultra violet, Forestal: Concentrated hydrochloric acid, Acetic acid and water (3:30:10), NH₃: Ammonia

E. thymifolia while C_{19} occurred in five species namely E. heterophylla, E. desmondi, E. hypossifolia, E. milli and E. tirucalli. C_{20} , C_{22} C_{26} and C_{36} are restricted to E. milli. The hydrocarbon C_{28} is restricted to E. hypossifolia, E. milli, E. thymifolia and E. tirucalli while C_{34} occurred in all the species except E. hirta and E. desmondi. In the same vein, C_{30} did not occur in E. heterophylla, E. desmondi, E. hirta, E. pulcherima, E. kamerunica and E. tirucalli while C_{32} did not occur in E. heterophylla, E. desmondi, E. hirta, E. pulcherima and E. prostrata.

The variation and occurrence of these hydrocarbons is of taxonomic value since some of them are restricted to some species of this genus while others are widely distributed. This aspect of the phytochemical result is in line Harborne (1973), who reported alkanes with carbon backbone of C_{25} to C_{35} in angiosperms. It also confirms the report of Englinton and Hamilton (1963a and 1963b) who reported the occurrence of C_{33} , C_{31} , C_{30} , C_{29} , C_{28} , C_{27} , C_{26} and C_{25} in Euphorbia aphylla and Sorensen (1968) who reported the occurrence of acetylenes with carbon chain mostly C_{14} to C_{18} in higher plants. The hydrocarbon screening indicated that most of these Euphorbia species under investigation are distinct but some are more closely related and supports Laurence (1934) who used

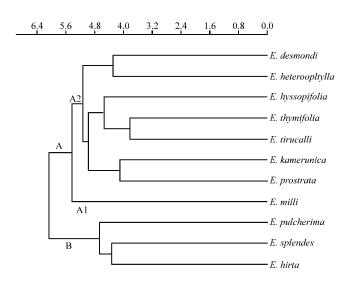


Fig. 1: Dendrogram showing the relationship among the Euphorbia species studied

the isoelectric of the proteins in Euphorbia to distinguish E. splendes from E. milli. This hydrocarbon results are also in line with the reports of Sonibare et al. (2005) and Evans et al. (2008) who reported the occurrence and taxonomic values of hydrocarbon in higher plants and their occurrence in the resin dots of different species of plants. The classes and types of different flavonoids identified in the Euphorbia species under different chromatograms and the occurrences are as shown in Table 2 and 3. Biflavonyl occurred in all the species except E. pulcherima, E. desmondi, E. hyssopifolia, E. thymifolia and E. tirucalli. Also, flavone did not occur in E. pulcherima, E. desmondi and E. thymifolia while glycosylflavone was not identified in E. pulcherima, E. desmondi and E. thymifolia. E. heterophylla, E. splendes, E. hirta, E. kamerunica and E. prostrata contain all the classes of flavonoid identified in the species studied. The implication of this is that the species that has similar flavonoid classes are more closely related compared to those that do not have such class of flavonoid. The occurrence of these flavonoids in the plants is in line with the previous reports by Harborne (1973), Vicente et al. (2007), Griesbach and Santamour (2003) and Slimestad (2003) who reported that these compounds are widely distributed in plants and are of taxonomic value.

The dendrogram as shown in Fig. 1 shows the clustering of the Euphorbia species based on the different chemicals (hydrocarbons and flavonoids) identified in them. These species studied formed two maim clusters A and B. Cluster A contains eight species among the eleven species studied. E. milli is distinct from all other species and formed a separate sub-cluster A1 while the other seven species are contained in A2. E. desmondi and E. heterophylla are more closely related, E. hyssopifolia, E. thymifolia and E. tirucalli are closely related while E. kamerunica and E. prostata are more closely related. However, cluster B contained E. pulcherima, E. splendes and E. hirta. The findings of this work is in consistent with the previous studies conducted by Keay (1989), Priti and Shital (1979), Davis and Heywood (1973), Hutchison and Dalziel (1954) and Laurence (1934).

CONCLUSION

This study has established the occurrence of different aliphatic hydrocarbon backbones and different classes of flavonoids in the genus *Euphorbia* in Nigeria and conclude that these secondary metabolites identified in this study are restricted to some of the species and are of great taxonomic value.

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