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Vol. 8(7), pp. 343-352, July 2014 DOI: 10.5897/AJPS2014.1161 Article Number: FECF47D46355 ISSN 1996-0824 Copyright © 2014 Author(s) retain the copyright of this article http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Chemical profiles as chemotaxonomic tools for Loranthaceae in Nigeria

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Received 10 February, 2014; Accepted 26 June, 2014

The Loranthaceae species are widespread throughout most regions of the world, and are used for various medicinal and ethnopharmacological purposes. However, the species vary in their pharmacological activity, sometimes in correlation with the species from same ecological region or host plant, due to variation in the chemical profiles. This has led to great emphasis on caution in identification and collection for use. The wide array of secondary metabolites in Loranthaceae species are believed to be of chemotaxonomic importance. In this study, the leaves of seven Nigeria species from different ecological locations were screened for the profiles of their secondary metabolites with a view towards establishing chemotaxonomic significance. The results show the complete absence of alkaloid from all the species. Over 80% of the species tested positive for balsam, flavonoids and phenols, more than 70% tested positive for tannins, 60% for saponins and about 50% tested positive for glycosides and volatile oils. Resins, phlobatannin, terpenes, sterols and anthraquinones were present in less than 50% of the species. Some metabolites were completely absent in one or more species. The patterns displayed could be of chemotaxonomic importance for Loranthaceae in Nigeria.

Key words: Loranthaceae, chemotaxonomy, secondary metabolites, Nigeria.

INTRODUCTION

Mistletoes are widespread throughout Africa, North America, Asia, Europe, Australia and Malaesia, with the American mistletoe (*Phoradendron serotinum*) and the European mistletoe (*Viscum album*) particularly well known. Different species growing on different hosts may synthesize toxic compounds and protein such as lectins

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and alkaloids with varying pharmacological activities (Preston et al., 2010). Thus, both the mistletoe and its host have shared responsibility in determining the pharmacological activity of the species. The distributions of these compounds or metabolites in different parts of a plant also vary (Preston et al., 2010). These pharmacological effects are due to variation in the chemical profiles especially the profiles of secondary metabolites. Secondary metabolites are proteins, glycosides, phenolics, steroids, saponins, terpenes, alkaloids and other chemical substances. Takhtajan (1973) suggested that secondary metabolites are compounds that may have taxonomic relevance.

Eighty percent or more of the world's population is estimated to depend primarily on traditional medicine for the treatment of ailments (Cunningham, 1993), and as a matter of fact, the use of medicinal plants is the main means of treatment by traditional healers. Also, many useful compounds, which are today used for treatment of life threatening diseases, were isolated from medicinal plants e.g. Artemisinin from Artemisia annua L. and Vincristine from Catharantus roseus (L.) G. Don (Dana, 2012; Aslam et al., 2010). The Loranthaceae, a parasitic family with mistletoes members are often considered useful as medicinal plants. It has been documented that mistletoes have immeasurable medicinal and traditional uses (Burkil, 1995; Erturk et al., 2003). The biological activities of immunomodulatory and antitumor effect of some mistletoe may be attributed to the presence of metabolites like lectins, viscotoxins and alkaloids found in the parasites (Stirpe et al., 1982; Bussing et al., 1996; Fernandez et al., 1998; Stein et al., 1999; Mengs et al., 2002).

metabolites The wide array of secondary in Loranthaceae sp. is believed to be of chemotaxonomic importance. Chemotaxonomic studies of 12 Loranthaceae and Viscaceae species namely, Viscum Viscum capensis L.f., rotundifolium L.f., Viscum combreticola Engl., Viscum obovatum Harv., Viscum obscurum Thunb., Viscum verrucosum Harv., Loranthus dregei Eckl. & Zehy., Loranthus minor Sprague, Loranthus oleifolius (Wendl.) Cham. & Schltdl., Loranthus rubromarginatus Engl., L. zeyheri Harv. and Loranthus sp. were carried out in South Africa (Tilney and Lubke, 1974), and chlorogenic acid was found in all the species. Gedalovich-Shedletzky et al. (1989) analyzed and compared the chemical composition of viscin mucilage from three mistletoe species. Chemical analyses of different extracts from Agelanthsu dodoneifolius yielded components such as triterpenes, sterols, carotenoides, saponosides, anthracenosides, anthocyanosides and tannins (Traoré, 2000). However, chemotaxonomic information on the West African or Nigerian species is unavailable. To clarify the status of Loranthaceae in the region, a revision of the Nigerian species was carried out recently and about 15 species were documented for the region (Ibrahim and Ayodele, 2011). This study aimed to

determine the profile of some basic secondary metabolites in the Nigerian species, which could be of chemotaxonomic significance.

MATERIALS AND METHODS

All reagents used were of analytical grade and were purchased from Zayo-Sigma Abuja, Nigeria. TLC plates used were also from the same source.

Plant collection and preparation

Twenty-seven specimens belonging to seven species were collected from the field through a field survey across host plant species and geographical location (Table 1). The specimens include Agelanthus dodoneifolius (4), Globimetula braunnii (4), Phragmanthera capitata (2), Phragmanthera nigritana (1), Tapinanthus bagwensis (4), Tapinanthus cordifolius (4) and Tapinanthus globiferus (8). Vouchers specimens were deposited at the University of Ibadan Herbarium (UIH)

The leaves of each specimen were air-dried for one week at ambient temperature, and then pulverized using a mortar and pestle. The powdered leaf samples were used for the phytochemical screening and thin layer chromatographic (TLC) profiling.

Phytochemical screening

The presence of basic secondary metabolites including saponins, alkaloids, tannins, flavonoids, sterols, phenols, glycosides, resins, balsam, volatile oil, phlobatannin, terpenes and anthraquinones were determined using standard methods (Evans, 2002; Sofowora, 1993; Brain and Turner, 1975; Segelman et al., 1971).

TLC profiling

Twenty-four specimens representing seven species were examined. The specimens are: *T. globiferus* (9), *T. bangwensis* (2), *T. cordifolius* (2), *P. capitata* (2), *P. nigritana* (1), *G. braunnii* (4) and *A. dodoneifolius* (4). A list of specimens and their corresponding numbers on the TLC plates are presented in Table 3.

Two grams of powdered leaf samples of each specimen were macerated in 20 ml of acetone for 24 h and filtered using filter papers. The extracts were spotted on three different pre-coated silica gel normal-phase TLC plates of dimension 12.5 by 8.5 cm. The dry spots were developed in a TLC tank of solvent system of ethylacetate : chloroform : methanol : water, in the ratio of 15:8:4:1. The developed spots were visualized by spraying the first plate with Vanillin in sulphuric acid reagent, the second plate with Gibbs reagent and the third plate with Dragendoff reagent for detection of terpenoids, phenolics and alkaloids, respectively. The retention factors (R_F values) were calculated for all the spots as distance moved by spot from the origin divided by distance moved by solvent front (Table 2).

RESULTS

Phytochemical screening

The result of the phytochemical screening for secondary

Table 1. Preliminary phytochemical screening of secondary metabolites from Loranthaceae species in Nigeria including taxa, hosts, localities, collection numbers and metabolites studied.

Таха	Host	Locality/No.	Gly	Rsn	Blm	Fla	Tnn	Akd	V.oil	Ptn	Spn	Тер	Str	Phn	Atq
Agelanthus dodoneifolius	Parkia biglobosa	Jos 65		-	+	-	+	-		-	+	-	-	+	++
Agelanthus dodoneifolius	Parkia biglobosa	Suleija 77	+	-	-	+	+	-	+	-	-	-	+	+	
Agelanthus dodoneifolius	<i>Casuarina</i> sp.	Yola 119		+	+	+	+	-		-	+	-	+		-
Agelanthus dodoneifolius	Vitellaria paradoxa	Yola 118	-		+	+	+	-	-	-	+	+	-	+	
Globimetula braunnii	Persea americana	Calabar 90		-	+	-	+	-		-	+	-	-	+	-
Globimetula braunnii	<i>Cola</i> sp.	Calabar 92		-	+	-	+	-		-	++	-	-	+	+
Globimetula braunnii	<i>Cola</i> sp.	Ibadan 97	+	-	+	+	-	-	+	-	+	+	-		-
Globimetula braunnii	Theobroma cacao	lbadan 102	-		+	+	-	-	-	-	+	+	-	-	
Phragmanthera capitata	Persea americana	Calabar 93		+	+	-	+	-		-	+	-	-	+	+
Phragmanthera capitata	Persea americana	Calabar 89		-	+	+	+	-	-	-	-	+	-		-
Phragmanthera nigritana	<i>Citrus</i> sp.	Chaza 78	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus bangwensis	Newboldia leavis	Ibadan 46		+	-	-	-	-		-	-	-	-		-
Tapinanthus bangwensis	Citrus medica	Ibadan 40	+	-	-	+	-	-	-	-	-	-	+	-	-
Tapinanthus bangwensis	Cola acuminata	Ibadan	-	-	+	+	-	-		-	+	+	+	+	-
Tapinanthus bangwensis	Theobroma cacao	Ibadan	-	-	+	+	-	-		-	+	-	-	+	-
Tapinanthus cordifolius	Citrus auranthifolia	Jos 63	+	+	+	+	+	-	+	-	-	-	-	+	
Tapinanthus cordifolius	<i>Cassia</i> sp.	Jos 86	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus.cordifolius	Syzygium eucalyptoide	Jos	+	+	+	+		-	+	-	+	+	+		-
Tapinanthus cordifolius	Ficus sp.	Jos	+	+		+		-	+	+	+	+	+		-
Tapinanthus globiferus	Piliostigma thoninngii	Kano 29		-	+	+	+	-		-	-	-	-	+	+
Tapinanthus globiferus	Azadirachta indica	Yola116		-	+	+	+	-		-	-	-	-	+	-
Tapinanthus globiferus	Tectona grandis	Kano 33		-	+	+	+	-		-	+	-	-	+	-
Tapinanthus globiferus	Parinari curattelifolia	Kano34	+	+	+	+	+	-	+	-	-	-	+	+	
Tapinanthus globiferus	<i>Zyzyphus</i> sp.	Yola 115		-	+	+	+	-		-	-	-	+		-
Tapinanthus globiferus	Unknown	Kano 27	+	-	+	+	+	-	+	-	-	-	-	+	
Tapinanthus globiferus	Vitex doniana	Suleija 73	-		+	+	+	-	-	-	+	+	-	+	
Tapinanthus globiferus	Gmelina arborea	Suleija 71		+	+	+	+	-		-	+	-	+		-

+ = Present; - = absent; ++ = abundant; Gly = glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.

metabolites of species of Loranthaceae on different hosts from different localities is presented in Table 1. Alkaloids were absent in all the species of Loranthaceae screened. Flavonoids were present in all except few specimens, *A. dodoneifolius* on *Parkia biglobosa* from Jos, the two specimens of *G. braunii* collected on an unidentified host from Calabar, *P. capitata* on

Persea americana from Calabar and Tapinanthus bangwensis on Citrus medica from Ibadan (Table 1). Balsams occurred in all the specimens of the Loranthaceae species except few species like A.

Таха	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
														0.06	0.06		0.04	0.04			0.06			0.04
						0.07	0.10							0.09	0.10	0.07	0.09							
				0.12				0.14			0.14					0.14		0.13	0.14			0.12	0.12	0.13
						0.16						0.16	0.16				0.17					0.16	0.16	
				0.20			0.19																	
															0.23	0.25	0.25	0.25	0.25			0.25	0.23	0.26
	0.29	0.30		0.27	0.29	0.30	0.30	0.30	0.29	0.27	0.29	0.27	0.29	0.29	0.29	0.29	0.29	0.29	0.29		0.29	0.30		0.30
				0.32								0.32											0.32	
	0.36			0.37							0.35	0.36	0.36	0.36	0.35	0.35	0.35	0.35					0.37	0.37
							0.39					0.40			0.40					0.40		0.39		
											0.46							0.43	0.43			0.43		
R _{F values} of spots	0.49		0.48	0.50		0.49	0.50	0.50	0.49			0.49	0.49			0.49		0.49	0.49	0.48	0.49	0.50	0.50	0.49
	0.53		0.53			0.52	0.53		0.52		0.53	0.52				0.53	0.52	0.53		0.53				
	0.56			0.55		0.56			0.55			0.56	0.56			0.56			0.55					
	0.59		0.59	0.58			0.59	0.59				0.59	0.58		0.58		0.59							
									0.63					0.63				0.63	0.63	0.63				
	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65				0.66	0.66			0.65	0.65				0.65			
												0.68						0.68	0.68	0.68		0.69	0.69	0.68
	0.73	0.73	0.73	0.72	0.72	0.72	0.72	0.72	0.72	0.71	0.71		0.71	0.71	0.71	0.71	0.71	0.72	0.71	0.71	0.71	0.73	073	
				-	-	-	-	-	-	-	-	0.78	-	-	-	0.76	-	-	-	-	-			0.75
								0.84									0.84	0.84	0.84	0.84		0.85	0.84	0.84
								0.91	0.91	0.91	0.92	0.91					0.91	0.91	0.91			2100		
No. of spots	8	3	5	10	3	8	9	8	7	3	7	13	8	6	8	11	12	13	11	7	5	10	9	9

Table 2. R_F values of phenolic spots from TLC profile of Loranthaceae species in Nigeria.

Key: See Table 3.

dodoneifolius on *P. biglobosa* from Suleija and *T. bangwensis* on *N. laevis* and *C. medica* (Table 1). Each of the four specimens of *T. bangwensis* on different host plants from Ibadan lacked tannins and also the two specimens of *G. braunii* from Ibadan lacked tannin (Table 1). Phenolics were also found to occur in most of the specimens screened except *G. braunii* on *Theobroma cacao* and *T. bangwensis* on *C. medica* (Table 1).

Figure 1 shows percentage response of the specimens to the metabolites screened. From this study, none of the metabolites occurred in all specimens or even all species (Figures 1 and 2).

Figure 2 present the percentage of species responding to metabolites in each location while Figure 3 shows the percentage response to metabolites by the species. Generally, about 90% of the species tested positive for balsam and phenols, while 76% tested positive for tannins, 63% for saponins and less than 5% for phlobatannin (Figure 1). All the samples of *G. braunnii* tested positive to balsam and saponins, all *P. capitata* tested positive to balsams, flavonoids and tannins, *P. nigritana* tested positive to balsams, terpenes and phenols, while samples of *T.*

bangwensis varied in their chemical profiles with no consistent positive indication for a particular metabolite. However, over 75% tested positive to balsams, flavonoids, and phenols, while about 75% also tested positive to glycosides, resins, balsams, volatile oils, saponins and terpenes. All samples of *T. globiferus* tested positive to balsam, flavonoids and tannins and 75% was positive to phenols.

TLC profiling

The TLC profiling of the specimens of

Specimen number on TLC plate	Name of Parasites	Name of Host	Host Family	Locality of collection no.			
1	Tapinanthus globiferus	Piliostigma thoninngii	Fabaceae-ceasalpinioideae	Kano 29			
2	T. globiferus	Azadirachta indica	Meliaceae	Yola116			
3	T. globiferus	Tectona grandis	Verbanaceae	Kano 33			
4	T. globiferus	Parinari curattelifolia	Chrysobalanaceae	Kano34			
5	T. globiferus	Zyzyphus sp	Rhamnaceae	Yola 115			
6	T. globiferus	Terminalia avicenoides	Combretaceae	Kano 35			
7	T. globiferus	-	-	Kano 27			
8	T. globiferus	Vitex doniana	Verbanaceae	Suleija 73			
9	T. globiferus	Gmelina arborea	5 5	Suleija 71			
10	Tapinanthus bangwensis	Newboldia leavis	Bignoniaceae	Ibadan 46			
11	T. bangwensis	Citrus medica	Rutaceae	Ibadan 40			
12	Tapinanthus cordifolius	Citrus auranthifolia	"	Jos 63			
13	T. cordifolius	<i>Cassia</i> sp.	Fabaceae-ceasalpinioideae	Jos 86			
14	Phragmanthera capitata	Persea americana	Lauraceae	Calabar 93			
15	P. capitata	Persea americana	"	Calabar 89			
16	Phragmanthera nigritana	<i>Citrus</i> sp.	Rutaceae	Chaza, Suleija 78			
17	Globimetula braunii	Persea americana	Lauraceae	Calabar 90			
18	G. braunnii	<i>Cola</i> sp.	Sterculiaceae	Calabar 92			
19	Globimetula braunnii	<i>Cola</i> sp.	3 3	Ibadan 97			
20	G. braunnii	Theobroma cacao	5 5	Ibadan 102			
21	Agelanthus dodoneifolius	Parkia biglobosa	Fabaceae-mimosoideae	Jos 65			
22	A. dodoneifolius	Parkia biglobosa	3.3	Suleija 77			
23	A. dodoneifolius	<i>Casuarina</i> sp.	Casuarinaceae	Yola 119			
24	A. dodoneifolius	Butryospermum parkii	Sapotaceae	Yola 118			

Table 3. List of specimens, hosts and their corresponding extract spot number on the TLC plates {(Figures 4-6) and Table 2}.



Figure 1. Percentage response of the total specimens of Loranthaceae species in Nigeria to presence of secondary metabolites. Gly = Glycoside; Rsn = Resins; Blm = Balsam; Fla = Flavonids; Tnn = Tannins; Akd = Alkaloids; V.oil = Volatile oil; Ptn = Phlobatannin; Spn = Saponin; Tep = Terpenes; Str = Sterols; Phn = Phenols; Atq = Anthraquinone



Figure 2. Percentage of species of Loranthaceae in Nigeria responding to secondary metabolites by location. Gly = Glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.



Figure 3. Percentage response of Loranthaceae species to secondary metabolites by species. Gly = Glycoside; Rsn = resins; Blm = balsam; Fla = flavonids; Tnn = tannins; Akd = alkaloids; V.oil = volatile oil; Ptn = phlobatannin; Spn = saponin; Tep = terpenes; Str = sterols; Phn = phenols; Atq = anthraquinone.



Figure 4. TLC profile of Loranthaceae specimens sprayed with Gibbs reagent.

Loranthaceae sp. using Gibbs, vanillin-sulphuric acid and Dragenddoff spray reagents for TLC are shown in Figures 4. 5 and 6, respectively. In Figures 4. 5 and 6, Gibbs reagent were used for visualizing phenolics, vanillin in sulphuric acid for terpenoids, while Dragendoff reagent was used to see if alkaloids were present on the TLC plate, respectively. Table 2 shows the R_f values of spots found on the TLC plate in Figure 4, which reveals that all the specimens had phenolics in them although to varying degree judging from the numbers of spots. T. cordifolius on Cassia sp. from Jos (spot 12), G. braunii on P. americana (spot17) and G. braunii (spot 18) from Calabar have the highest number of spots of 13, 13 and 12, respectively (Table 2). An intermediate number of spots were found in T. globiferus on P. curattelifolia from Kano (spot 4), P. nigritana on Citrus sp. from Suleija (spot 16) and G. braunii on Cola sp. from Ibadan (spot 19) with 10, 11 and 11 spots respectively (Table 2). The lowest spots are found in T. globiferus on A. indica (spot 2), T. globiferus on Tectona grandis (spot 3), T. globiferus on Zyzyphus sp. (spot 5), T. bangwensis on Newboldia laevis (spot 10) and Agelanthus dodoneifolius on P. biglobosa (spot 21) with 3, 5, 3, 3 and 5, respectively (Table 2). Spots with R_F values of 0.29 - 0.32 and 0.71 -0.73 are found to be present in over 90% of the specimens. In Figure 5, terpenoids were only observed in some of the specimens. Alkaloids were absent from all the specimens studied (Figure 6).

DISCUSSION

The phytochemical analysis and the TLC profiling showed variation in the constituent secondary metabolites among various species irrespective of their host and ecological location (Table 1; Figures 4 and 5). Variation in secondary metabolites among the same mistletoe species occurring on different host plants have been observed in earlier studies (Deeni and Sadig, 2002; Ibrahim et al., 2009). The only consistent pattern from this study was the lack of alkaloids from all the specimens analyzed (Table 1; Figures 1, 2 and 6). It is a known fact that quantitative and qualitative information on secondary metabolites is useful for taxonomic classification of plants (Harborne, 1968; Takhtajan, 1973). Hence absence of alkaloids, and the number of species testing positive for balsam and phenols appears to be of chemotaxonomic significance among the species in Nigeria. Alkaloids were not recorded for any of the Nigerian Loranthaceae specimens studied but Sanchez-Areola et al. (2004) recorded the presence of alkaloids in Psittacanthus calyculatus, a New World Loranthaceae endemic to Mexico (Kuijt, 2009).

The TLC R_f in Table 2 shows that there were similar phenolic compounds (R_f values of 0.29 - 0.32 and 0.71 -0.73) present in most of the specimens, over 90% of the species and this further reinforced the fact that phenolics could be a source of analytical marker compound(s) for



Figure 5. TLC profile of Loranthaceae specimens sprayed with Vanillin-sulphuric acid reagent.

standardization of herbal preparations from these species. High amounts of phenolics have long been known to be a phytochemical feature of parasitic flowering plants and they are said to occur at a level that is generally higher than the host plant (Khanna et al., 1968; Salatino et al., 1993). The study reveals that G. braunii specimens irrespective of their hosts or locations are rich in phenolic compounds as compared to other species while T. globiferus and T. bangwensis are depauperate in phenolics as compared to other species. Also of note is the absence of glycosides in the Phragmanthera species and total absence of tannins from all the specimens from Ibadan. These findings may be of chemotaxonomic importance. Thus, the presence of balsams and phenols could be used in specific combination with morphological characteristics and biogeographical distribution ranges for the delineation of genera and species in the family (Crockett and Robson, 2011).

Research on dwarf mistletoes (Viscaceae) in North America indicates that plant chemistry, particularly secondary metabolites, plays an important role in determining interactions between host and parasite (Snyder, 1996). This may not be applicable to Nigerian Loranthaceae because of the variation noted in the metabolites present in the same species on different hosts. Differences in chemical profiles of the various species studied underscore why the specific choice of species for the treatment of a particular ailment is very important. This study has shown that some species may not possess a particular metabolite that is common in other species. For instance, the absence of glycosides in Phragmanthera species or tannins and saponins in T. bangwensis may result in major pharmacological differences. Although the correlation between host and chemical profile of the species was not clearly defined in this study, it is believed that the host could play a role in the observed chemical profile of the plant or species. The influence of host chemistry on the chemical constituents of the parasite on different hosts might justify why the host is as important as the parasite in pharmacognosy, ethnopharmacology and ethnomedicine, and why the use of these Loranthaceae in the treatment of an ailment is often dependent on a particular or specific host (Burkill, 1995; Snyder 1996; Adodo, 2002; Olapade, 2002; Preston et al., 2010), for instance, in Brazil, where there is preference for Cladocolea micrantha growing on cashew tree (Anacardium occidentale) for the treatment of tumors



Figure 6. TLC profiling of Loranthaceae specimens sprayed with Dragendoff reagent.

and inflammatory diseases (Adodo, 2002; Olapade, 2002; Guimaraes et al., 2007).

Conclusion

From this investigation, species of Loranthaceae in Nigeria might not be delineated by scoring presence or absence of their secondary metabolites qualitatively or quantitatively due to variations which occur on same species form different hosts but the occurrence of similar metabolites like phenolics and balsam in most, if not all the species irrespective of the host and locality is useful taxonomically as a marker for the group. It is therefore our recommendation that caution should be exercised in the use of Loranthaceae as phytomedicine because of the chemical variations which exist in the same species found on different hosts. The same species collected hosts from two different might have different pharmacological effects in the body. The group is currently working on determining a phytochemical marker for the family Loranthaceae in Nigeria.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the following people who rendered assistance during field trips for specimen collection: Dr Theresa Omara-Achong, Oyepeju M.K.O, Baba Nafi of Keji village, Pastor Frank of University of Calabar, Dr. Colman Goji, Muazzam Ibrahim, Tanko Garba, Mrs. Sumbo Wahab and Mr. Owolabi; also Mr. John Apev, who assisted in the laboratory analysis.

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