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TLC phytochemical screening in some Nigerian Loranthaceae

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The phytochemical screening of specimens of the family Loranthaceae collected from the field was carried out with a view to ascertaining chemical constituents present and determining their importance in the taxonomic delimitation of the taxa. Thirty field collections from various localities were screened for secondary metabolites such as alkaloids, anthraquinones, terpenoids and ketones using thin layer chromatography (TLC). Most of the samples tested slightly positive for alkaloids, anthraquinone-related compounds, terpenoids and terpenoid-related compounds but ketonic compounds were of rare occurrence in all the samples. The chemical profile was useful in separating the collections of *Phragmanthera* from the other two genera while the collections of *Globimetula* were found embedded in *Tapinanthus*. The secondary metabolites obtained however showed the relative affinity of the Nigerian species of *Tapinanthus*. It is concluded that chemical characters may only be used as supporting evidence in the identification and delimitation of the taxa.

Key words: Loranthaceae, mistletoes, Nigeria, taxonomy, phytochemistry.

INTRODUCTION

The Loranthaceae (mistletoes) is a large family of about 75 genera and over 900 species (Judd et al., 2002). It is speculated to have originated in Eastern Asia (Barlow, 1983) or on the Gondwanan landmass with a further intrusive element spreading south into the Malesian and Australian regions (Barlow, 1990). The family has three terrestrial, root parasitic genera and 72 genera of aerial branch parasites (Wilson et al., 2006). The Loranthacean mistletoes are tropical and occur as parasites on both angiosperms and gymnosperms (Dembele et al., 1994). Six major genera are found in Nigeria namely: *Tapinanthus* [Blume] Reichb., *Agelanthus* Tieghem, *Loranthus* L., *Globimetula* Tieghem, *Phragmanthera* Tieghem and *Englerina* Tieghem. *Tapinanthus* is far more widespread in the Nigerian Savanna (Johri and Bhatnagar, 1972; Omolaja and Gamaye, 1998). The taxa infest many wild and domesticated tree and shrub species

of ethnobotanical and economic value, causing various degrees of structural and economic damage (Bako, 2001; Bako et al, 2001). Mistletoes are very important in curative medicine. They are known to be highly potent in curing circulatory problems and also as anticancer agents (Kafaru, 1994). Mistletoe extracts are widely used in complementary and alternative cancer therapy in Europe. The extracts possess cytotoxic as well as immunostimulatory effect (Delinassios, 2007). The activity principle of the mistletoe (*Viscum album* L.) phytotherapeutics could be considered as combined cytotoxic and 'biological response modifying' activities (increasing host defense against cancer) that result from the activities of the plant lectins and the other biologically relevant substances (Neven et al., 2001). In Nigeria, several herbal preparations from leaves and twigs of mistle-toes e.g. *T. bangwensis* [Engl. and K. Krause] Danser have become popular for the treatment of variety of diseases, such as diabetes and hypertension, which have been reported to be on the increase in the country (Olapade, 1995).

The taxonomy of Loranthaceae has been characterized by a lot of confusion especially in West Africa. In spite of the ecological and medicinal importance of the family, the

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hemi-parasitic nature of the plants appears to make them highly variable morphologically. This probably influenced the description of many species all of which may not be taxonomically distinct. For example in Nigeria, so many authors have referred to *Tapinanthus species* as *V. album* – a foreign plant that does not occur in this part of the world. Moreover, most collectors always claim to be using *Tapinanthus* for herbal treatment but they never specify which of the species despite the prevalence of other genera such as *Englerina* and *Globimetula* in the country. As part of the collaborative multidisciplinary research on Nigerian mistletoes between the Department of Botany and Microbiology, University of Ibadan, Ibadan and the Nigerian Institute of Pharmaceutical Research (NIPRID) Abuja, the present study aims at ascertaining the taxonomic importance of chemical characters in the identification and delimitation of some taxa of Loranthaceae in Nigeria. The study will also attempt to correlate the bioactive constituents obtained from the phytochemical screening to the medicinal uses of the taxa.

MATERIALS AND METHODS

Plant material and extraction

Thirty field samples from various localities were identified at the University of Ibadan herbarium and Forestry herbarium, Ibadan. Voucher specimens were deposited at the University of Ibadan herbarium. These samples were screened for secondary metabolites such as alkaloids, anthraquinones, terpenoids and ketones using thin layer chromatography. Table 1 shows the list of collections and their localities, host plants being parasitized by the mistletoes and the date of collection (day/month/year).

Thin layer chromatography (TLC) method

The thirty leaf samples were air-dried indoor for about four weeks and powdered with an electric blender. Extracts of the leaf samples, designated A – K are shown in Table 1. The leaf extract were then obtained by macerating 2 g each of the powdered samples separately in 5 ml of methanol and left to stand for 7 days, supernatant layer was used for the TLC analysis. Two types of TLC plates (stationary phase) were used; these are pre-coated plates of size 20 × 10 cm² and the glass micro-plates prepared in the laboratory. The TLC plates were made by mixing the adsorbent (silica gel) containing small amount of inert binder calcium sulphate (gypsum) with twice the amount of water. The mixture was spread as slurry on the previously cleaned glass micro- plates using the TLC spreader. The plates were allowed to set and then dried and activated at 110°C in an oven for 30 min.

The extracts were drawn with capillary tubes and applied as spots on a stationary phase (silica-gel coated plate) about 1 cm from the base. The plate was then dipped into a suitable solvent system (mobile phase) and placed in a well covered tank. The following solvent systems were used:

Solvent system I: Ethyl acetate: Toluene 8:4

Solvent system II: Ethyl acetate: Toluene: Acetic acid 8:4:1

Chromatographic tank was saturated with mobile phase at room temperature for 5 min prior to development.

At the end of the chromatographic development which lasted for about 20 min, the plate was removed from the chromatographic tank and the separated spots were visualized under daylight (visible light) and with ultra-violet light of two different wavelengths (UV₂₅₄ nm and UV₃₆₅ nm) after spraying with chromogenic reagents. Distances between the spots were measured and the retention factor (Rf) values were recorded, using the following:

$$R_f \text{ value} = (\text{Distance moved by the compound}) / (\text{Distance moved by the solvent front})$$

The presence of secondary metabolites in the leaf extracts were qualitatively determined by TLC using;

(a) Dragendorff's reagent and FeCl₃/HClO₄ spray reagent for the presence of alkaloids.

(b) 5% methanolic KOH for the presence of anthraquinones.

(c) 2, 4 – dinitrophenyl hydrazine for the presence of ketonic compounds.

(d) Anisaldehyde in H₂SO₄ for the presence of terpenoids.

RESULTS

Thirty field collections from various Nigeria localities screened for secondary metabolites such as alkaloids, anthraquinones, terpenoids and ketones are presented in Table 1.

Figure 1 shows the distribution of field collections in South Western part of Nigeria. These collections were made in the early hours of the day during the rainy season. Three genera, *Tapinanthus*, *Phragmanthera* and *Globimetula* were present in all the collections. Twenty-three of the 30 specimens were identified as *Tapinanthus species* using the different macromorphological characters. Eighteen of these were *Tapinanthus bangwensis* while five are yet to be identified to the species level. Six specimens belonged to *Phragmanthera* and one specimen to *Globimetula* (Table 1). The TLC screening of the bioactive compounds from mistletoe leaf extract are presented in Tables 2 - 4. Out of the 30 samples treated with Dragendorff's reagent to test for alkaloids, 21 tested slightly positive while the remaining 9 did not show any positive reaction.

Seventeen samples tested slightly positive for anthraquinone related compounds while 13 were negative. However, this number of samples increased to 24 when the solvent changed from I to II (Table 4). Twenty one samples out of 30 tested positive for the presence of terpenoids and terpenoid-related compounds while only 9 did not show any reaction with Anisaldehyde in H₂SO₄. No positive result was obtained with 2, 4– dinitrophenylhydrazine hence ketonic compound, is said to be of rare occurrence in all the samples examined.

DISCUSSION

The results obtained from the TLC phytochemical screening showed that the chemical profiles of the different species are variable. This may be as a result of their hemi-parasitic habit on the different hosts. The type of host

Table 1. List of the examined Nigerian Loranthaceae field collections.

Collection No.	Taxa	Collection code	Host plant	Locality	Date of collection
1.	<i>Phragmanthera</i> sp.	A ₁	<i>Leuceana leucocephala</i> [Lam.] De wit [Mimosaceae]	Faculty of Agriculture, U.I, Ibadan	03.05.07
2.	<i>Tapinanthus bangwensis</i>	A ₂	<i>Jatropha curcas</i> Linn. [Euphorbiaceae]	Ilaramokin via Akure	18.07.07
3.	<i>Phragmanthera</i> sp.	B ₁	<i>Tectona grandis</i> Linn. f. [Verbenaceae]	Oluwa forest Reserve, Ondo	18.07.07
4.	<i>T. bangwensis</i>	B ₂	<i>Terminalia</i> sp. Linn. [Combretaceae]	Oluwa forest Reserve, Ondo	18.07.07
5.	<i>Tapinanthus</i> sp.	B ₃	<i>Psidium guajava</i> Linn. [Myrtaceae]	Formecu quarters Ondo	18.07.07
6.	<i>Phragmanthera</i> sp.	B ₄	<i>Cola acuminata</i> [P.Beauv.] Schott [Sterculiaceae]	Adebayo, Ibadan	29.07.07
7.	<i>Phragmanthera</i> sp.	B ₅	<i>Cola</i> spp. Schott and Endl.[Sterculiaceae]	Adebayo, Ibadan	29.07.07
8.	<i>T. bangwensis</i>	B ₆	Unidentified host	Adebayo, Ibadan	14.08.07
9.	<i>T. bangwensis</i>	C ₁	<i>Tectona grandis</i> Linn. f. [Verbenaceae]	Aroro village, Ibadan	21.07.07
10.	<i>T. bangwensis</i>	C ₂	<i>Theobroma cacao</i> Linn. [Sterculiaceae]	Aroro village,Ibadan	21.07.07
11.	<i>Phragmanthera</i> sp.	C ₃	<i>Cola</i> spp. Schott and Endl. [Sterculiaceae]	Aroro village, Ibadan	21.07.07
12.	<i>T. bangwensis</i>	C ₄	<i>Theobroma cacao</i> Linn.[Sterculiaceae]	Aroro village,Ibadan	12.08.07
13.	<i>T. bangwensis</i>	C ₅	<i>Ficus</i> spp. Linn. [Moraceae]	Aroro village, Ibadan	12.08.07
14.	<i>T. bangwensis</i>	C ₆	Unidentified host	Aroro village, Ibadan	12.08.07
15.	<i>T. bangwensis</i>	C ₇	<i>Ficus exasperata</i> Vahl [Moraceae]	Aroro village, Ibadan	12.08.07
16.	<i>Tapinanthus</i> sp.	D ₁	<i>Dalbergia latifolia</i>	Botanical garden, U.I.	23.07.07
17.	<i>Globimetula</i> sp.	E ₁	<i>Theobroma cacao</i> Linn. [Sterculiaceae]	CRIN Ibadan	25.07.07
18.	<i>Phragmanthera</i> sp.	F ₁	<i>Ficus</i> sp. Linn. [Moraceae]	Papa Itoko, Abeokuta	01.08.07
19.	<i>T. bangwensis</i>	F ₂	<i>Citrus</i> sp. Linn. [Rutaceae]	Papa Itoko, Abeokuta	01.08.07
20.	<i>T. bangwensis</i>	F ₃	<i>Theobroma cacao</i> Linn. [Sterculiaceae]	Papa Itoko, Abeokuta	01.08.07
21.	<i>T. bangwensis</i>	F ₄	<i>Psidium guajava</i> . Linn. [Myrtaceae]	Lantoro, Abeokuta	01.08.07
22.	<i>Tapinanthus</i> sp.	G ₁	<i>Citrus</i> sp. Linn. [Rutaceae]	Adanbata otan, Ota Ogun state.	20.07.07
23.	<i>T. bangwensis</i>	H ₁	<i>Cola nitida</i> [Vent] Schott and Endl. [Sterculiaceae]	Shagamu, Ogun state.	02.08.07
24.	<i>T. bangwensis</i>	H ₂	<i>Ficus exasperata</i> Vahl [Moraceae]	Shagamu, Ogun state.	02.08.07
25.	<i>Tapinanthus</i> sp.	I ₁	<i>Cordia sebestena</i> Linn. [Boraginaceae]	O. A. U Ile-Ife, Osun state.	19.07.07
26.	<i>Tapinanthus</i> sp.	I ₂	<i>Citrus</i> sp. Linn. [Rutaceae]	O. A. U Ile-Ife, Osun state	19.07.07
27.	<i>T. bangwensis</i>	J ₁	<i>Calliandra heamatocephala</i> [Mimosaceae]	University of Ilorin, Kwara state.	03.09.07
28.	<i>T. bangwensis</i>	K ₁	<i>Theobroma cacao</i> Linn. [Sterculiaceae]	Abeokuta – Eruwa road	09.10.07
29.	<i>T. bangwensis</i>	K ₂	<i>Theobroma cacao</i> Linn. [Sterculiaceae]	Abeokuta – Eruwa road	09.10.07
30.	<i>T. bangwensis</i>	K ₃	<i>Tectona grandis</i> Linn. [Verbenaceae]	Olokemeji Forest reserve	09.10.07

The type of host tree seems to largely influence the chemical compounds (especially alkaloids) found in the respective mistletoe, and mineral content in mistletoe has been found to be much higher than that in the host especially relative to

the infected branch. These variations indicate that the same species occurring on different hosts in the same locality might have differences in their metabolites.

This variation in metabolites had been observed

by earlier workers (Deeni and Sadiq, 2002), where *A. dodoneifolius* on eleven different hosts were screened for metabolites. The differences noted in the chemical constituents of this parasite present on different hosts might justify why the host is



Figure 1. Collection sites of *Tapinanthus* and allied species in South-western Nigeria.

as important as the parasite in Pharmacognosy (Burkill, 1995) and why the use of this parasite in the treatment of an ailment is usually dependent on a particular or specific host (Adodo, 2002; Olapade, 2002; Ibrahim et al., 2009). Fourteen out of the 30 samples screened had similar compounds (Table 2) using solvent system I. However four other samples (A_1 , A_2 , B_1 and B_5) showed the presence of the same compounds with those of the 14 above but differed in the absence of anthraquinone-related compounds. In solvent system II, these anthraquinone-related compounds were found in traces (Table 2). The samples which tested slightly positive to Dragendorff for the presence of alkaloids did not show the actual orange colour expected but the colour obtained indicate the presence of alkaloid-related compounds in those samples. However, when the Dragendorff's reagent used was modified by the addition of a few drops of HCl to make it more sensitive, only 3 samples (A_1 , C_3 and F_1) showed the orange colour confirming the presence of alkaloids in these samples (Table 3).

These are the *Phragmanthera spp.* No positive result was obtained when 5% $FeCl_3$ in 35% $HClO_4$ was used to further

confirm the particular class of alkaloids (Table 3). This shows that these 3 alkaloid-positive samples do not contain indole-type of alkaloids but other classes of alkaloids which need further investigations in order to ascertain the particular type. Five other samples (C_1 , C_2 , C_4 , H_1 and I_1) appeared to be related in their chemical composition due to their lack of reactions towards the different chromogenic reagents used. However it is possible that they have other secondary metabolites which have not been detected in the present study. It is obvious from the foregoing that the chemical profile of the different samples may assist only in grouping them to the different genera but is not adequate for their identification to the species level. However the occurrence of some compounds may be related to the uses of mistletoes for the treatment of different ailments.

The occurrence of alkaloids may explain the usefulness of members of the family for reducing high blood pressure. Useful alkaloid such as rynchosphylline were reported by Jeffery and Harborne (2000) to help in improving to help in improving cardiac conditions by reducing blood pressure, increasing circulation and inhibiting the

Table 2. Summary of TLC phytochemical screening of some Nigerian Loranthaceae.

		<i>Phragmanthera</i>							<i>Tapinanthus</i>														<i>Globimetula</i>								
Secondary metabolites	Chromogenic reagents and mobile phase	A1	B1	B4	B5	C3	F1	A2	B2	B3	B6	C1	C2	C4	C5	C6	C7	D1	F2	F3	F4	G1	H1	H2	I1	I2	J1	K1	K2	K3	E1
Alkaloids	Dragendorff and solvent I	+++	+	-	+	+++	++	++	+	+	++	-	-	-	+++	-	-	+++	+++	+	+++	+++	-	+	-	+	++	+++	+++	+++	-
	Dragendorff and solvent II	+(0.11)				+(0.15)	+(0.08)																								
Anthraquinones	5% KOH and solvent I	-	-	-	-	+	+	-	+	+	+	-	-	-	+	-	+	++	++	+	++	+	-	++	-	-	+	++	++	++	-
	5% KOH and solvent II	+	+	+	+	++	+	++	++	+	++	-	-	-	++	-	+	+	+	+	++	+++	-	++	-	+	++	+++	+++	+++	+
Ketonic compounds	2,4-dinitrophenil hydrazine and solvent I																														
	2,4-dinitrophenil hydrazine and solvent II																														
Terpenoids	Anisaldehyde in acid medium and solvent I	+++	+++	-	++	+++	-	+	+	+	++	-	-	-	+++	++	+	++	++	-	+++	+++	-	+	-	+	++	+++	++	+++	-
	Anisaldehyde in acid medium and solvent II	++	+	++	++	++	+++	+	+	+	+	+++	++	+	+	+	+	++	++	+	++	++	+++	++	+++	+++	++	++	++	+	+++

+ = Presence; ++ = high; +++ = very high; - = absence.

Solvent I = Ethylacetate: Toluene 8: 4.

Solvent II = Ethylacetate: Toluene: Acetic acid 8: 4: 1.

Table 3. TLC profile of Dragendorff's positive samples (C₃, A₁, F₁) under UV and FeCl₃/HClO₄ spray reagent.

Spots	C ₃						A ₁						F ₁					
	Colour daylight	UV _{254nm}	UV _{365nm}	Colour with Dragendorff + Drops of HCl	Colour with 5% FeCl ₃ in 35% HClO ₄	Rf	Colour of day light	UV _{254nm}	UV _{365nm}	Colour with Dragendorff + drops of HCl	Colour with 5% FeCl ₃ in 35% HClO ₄	Rf	Colour of day light	UV _{254nm}	UV _{365nm}	Colour with Dragendorff + drops of HCl	Colour with 5% FeCl ₃ in 35% HClO ₄	Rf
1	Light brown	Purple	Yellow	-	No colour change	0	Light brown	Purple	Yellow	-	No colour change	0	Light brown	Purple	Yellow	-	No colour change	0
2	Light yellow	Purple	-	-	"	0.05	Light yellow	Light purple	Pink	Orange	"	0.06	Light yellow	Light purple	Pink	Orange	"	0.04
3	-	-	-	Orange	"	0.09	-	-	Pink	Orange	"	0.12	-	Light purple	Pink	Orange	"	0.10
4	-	Light purple	-	Orange	"	0.25	-	Light purple	-	-	"	0.26	-	Light purple	-	-	"	0.29
5	-	-	-	-	"	0.79	-	-	-	-	"	0.80	-	-	-	-	"	0.65
6	-	-	-	-	"	0.88	-	-	-	-	"	0.86	-	-	-	-	"	0.81
7	Light green	Greenish yellow	Pink	-	"	0.98	-	Light purple	Pink	-	"	0.94	-	-	-	-	"	0.92
8							Greenish yellow	Greenish yellow	Pink	-	"	0.97	Greenish yellow	Light purple	Pink	-	"	0.95
9													Light green	Greenish yellow	Pink	-	"	0.98

Table 4. Influence of mobile phase (solvent system) on the identification of anthraquinones using 5% methanolic KOH as chromogenic reagent.

Extracts	Chromogenic reagent 5% KOH MeOH		Extracts	Chromogenic reagent 5% KOH MeOH		Extracts	Chromogenic reagent 5% KOH MeOH	
	Solvent I	Solvent II		Solvent I	Solvent II		Solvent I	Solvent II
A1	-	+	C3	+	++	F4	++	++
A2	-	++	C4	-	-	G1	+	+
B1	-	+	C5	+	++	H1	-	-
B2	+	++	C6	-	-	H2	++	++
B3	+	+	C7	+	+	I1	-	-
B4	-	+	D1	++	+++	I2	-	+
B5	-	+	E1	-	+	J1	+	++
B6	+	++	F1	+	+	K1	++	+++
C1	-	-	F2	++	+++	K2	++	+++
C2	-	-	F3	+	+	K3	++	+++

accumulation of arteriosclerosis plague and blood clots. Terpenoids have antimicrobial activities, they exhibit antiamebic activity. Antibacterial and antifungal properties have been due to terpenoids (Amaral et al., 1998). African mistletoe, *Agelanthus dodoneifolius* is used ethno medicinally by the Hausa and Fulani tribes of Northern Nigeria as a remedy for several human and animal ailments that include stomach ache, diarrhea, dysentery, wound and cancer (Deeni and Sadiq, 2002). The antimicrobial activities of terpenoids may possibly be through membrane disruption of the micro-organisms by their lipophilic compound. The occurrence of anthraquinone in the plants is an indication that it can be used as a mild laxative.

According to Evans (1989), anthraquinones act on gastro intestinal tract to increase the peristalsis action. The presence of the identified chemical compounds shows the usefulness of the plants in the treatment of various diseases.

It is evident from the work that *Tapinanthus* is the most prevalent genus in the south western part of the country. Further work continues to identify the specimens of species level.

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