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Phytolacca dodecandra (Phytolaccaceae) in Africa: Geographical Variation in Leaf Chemistry

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Key Word Index—*Phytolacca dodecandra*; Phytolaccaceae; endod; hexane extractables; geographic variation.

Abstract—The non-polar hexane extractables from leaves of *Phytolacca dodecandra* L'Hert. were found to yield considerable amounts of hentriacontane, oleic acid, myristic acid, sterols, α-tocopherol and phytol-palmitate, -oleate and -linoleate. The major patterns of geographic variation were the divergence of *P. dodecandra* from Madagascar, Zambia, Zimbabwe and Nigeria. The results are compared with a previous study using morphological characters.

Introduction

The African soapberry plant, *Phytolacca dodecandra* (Phytolaccaceae), locally called endod, produces a series of triterpenoid saponins which possess very potent and useful biological properties including antifungal, antiprotozoan, spermicidal, insecticidal and molluscicidal activities [1]. The yields of crude triterpene saponins can exceed 25% dry berry weight [2]. The molluscicidal compounds of endod have been identified as oleanolic acid saponin glycosides [3–5]. The compound which has been shown to have the greatest molluscicidal activity was named lemmatoxin after Dr Aklilu Lemma, the Ethiopian scientist who made the initial field observations and was largely responsible for its discovery.

Previously, we have reported on geographical variation in the morphology of *P. dodecandra* throughout its range in Africa, where it is endemic [6]. Although there was considerable infraspecific variation in morphology, no evidence was found to support the recognition of varieties previously named (vars *apiculata* Engl. and *brevipedicellata* H. Walt.). The major trends in the morphology were the separation of a pubescent form in Ethiopia, the divergence of the population on Madagascar, and divergence between populations from East and West Africa [6].

The purposes of this paper are to analyse geographic variation in the non-polar hexane extractables of *P. dodecandra* and compare these results with variation in morphology. The taxonomic and chemical literature on *P. dodecandra* has recently been reviewed [6].

Results and Discussion

The hexane extracts of the leaves varied in yields from 3.3% (AA, Type 44, in Addis Ababa, Table 1) to 2.1%, but not significantly. However, the extract proved to be a complex mixture of alkanes, fatty acids, sterols and phytol esters (Table 1). The hexane extract is dominated by hentriacontane (C_{31}), and oleic and myristic acids. Moderate amounts of eicosane (C_{20}), palmitic acid, nonacosane (C_{29}), α-tocopherol, sterols, tritriacontane (C_{33}), pentatriacontane (C_{35}), and phytol-palmitate, -oleate and -linoleate (Table 1). Analysis of variance (ANOVA) revealed numerous significantly different components (Table 1).

Previous analysis of variation in morphology [6] showed that the major trend (coordinate 1, 27%, Fig. 1) was the separation of the pubescent plants (E2 in Fig. 1) from other populations, and the separation of the East and West African populations. The second coordinate (16%, Fig. 1)

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TABLE 1. AVERAGE VALUES FOR EACH POPULATION OF *PHYTOLACCA DODECANDRA* FOR THE THIRTY-SEVEN COMPONENTS USED IN PRINCIPAL COORDINATE ANALYSIS

Constituent	AA	GE	EE	KE	ZI	ZB	GH	NI	MA	*F* ratio
Yield (%)	3.3	2.6	2.1	2.8	2.2	2.1	2.9	3.0	2.4	1.68
Myristic acid	10.1	12.7	9.3	7.4	6.7	4.0	7.7	5.4	10.1	3.19**
PD70	1.1	1.1	0.6	0.7	T	T	T	1.2	1.1	3.32**
Eicosane (C_{20})	1.3	1.4	1.0	0.9	2.2	0.9	T	1.6	2.2	2.66*
PD2	T	T	T	T	T	T	T	0.7	1.0	3.03**
PD3	0.8	0.8	0.6	0.6	0.5	T	—	0.6	1.1	5.48**
Henicosane (C_{21})	T	T	T	T	0.9	T	—	—	1.0	1.91
Palmitic acid	1.4	1.8	1.0	2.1	2.5	2.0	6.0	3.9	4.0	6.90**
Oleic acid	15.8	18.5	17.4	22.8	6.6	1.8	12.4	3.3	11.5	4.97**
PD93	T	T	0.5	T	T	—	T	—	1.1	2.06
PD7	1.0	1.2	0.7	1.4	0.7	T	1.1	T	1.8	1.31
PD92	T	T	—	—	T	—	—	—	1.5	34.36**
Tricosane (C_{23})	0.6	T	T	T	T	T	T	T	1.4	4.67**
PD11	—	—	0.8	1.5	T	—	T	0.6	T	3.76**
PD13	—	—	—	—	T	—	T	1.0	T	1.60
PD17	T	T	T	0.6	T	—	T	T	1.3	2.37*
PD24	0.6	1.5	T	T	1.0	1.9	1.1	1.2	T	1.45
Nonacosane (C_{29})	1.7	1.2	1.0	1.1	1.5	1.7	0.6	1.2	1.2	4.42**
PD91	—	T	—	—	T	—	—	—	1.0	2.25*
PD27	T	0.6	T	T	1.1	0.5	T	—	T	8.81**
α-Tocopherol	2.4	3.1	1.4	1.5	5.8	4.1	4.0	5.6	3.7	2.18
Hentriacontane (C_{31})	30.3	21.4	36.0	30.1	36.1	52.3	19.9	29.2	11.3	11.87**
STL1	6.8	6.5	4.7	3.8	4.5	5.8	5.7	11.0	6.7	5.07**
PD38	T	T	T	T	T	T	T	1.2	T	6.79**
PD39	1.4	0.6	5.9	0.8	0.6	0.8	T	T	0.6	10.51**
PD40	0.7	T	0.6	T	0.7	T	T	1.4	0.8	6.83**
PD41	2.8	2.8	0.9	0.7	T	2.9	1.0	3.8	1.2	6.78**
Tritriacontane (C_{33})	1.2	0.8	0.9	0.7	2.7	1.9	1.6	4.6	0.9	11.55**
PD42	1.2	1.3	T	T	0.8	1.1	1.2	2.4	0.7	7.44**
PD43	1.5	1.4	0.5	0.9	2.0	1.3	0.9	2.7	1.8	6.22**
Pentatriacontane (C_{35})	T	T	—	T	0.9	1.3	0.9	1.8	T	9.38**
Phytol palmitate	1.8	2.5	0.9	2.2	2.0	0.8	6.3	1.7	1.7	10.32**
Phytol oleate	2.4	3.4	1.3	4.5	1.7	0.8	6.4	1.3	1.5	10.30**
Phytol linoleate	1.8	2.4	0.9	4.4	1.4	T	9.7	T	1.2	11.16**
PD61	1.3	1.0	1.7	1.1	0.7	T	1.0	0.6	2.6	10.63**
PD63	T	0.5	T	T	T	T	T	1.1	1.3	8.40**
PD64	T	0.6	T	T	1.3	0.5	T	1.2	1.0	8.44**

T=trace (less than 0.5%), —=not detected. *, $P_{0.05}$; **$P_{0.01}$ in the Student-Newman-Keuls (SNK) multiple range test among populations. Population acronyms are given in the Experimental.

separates the Madagascan, Kenyan and Tanzanian populations (MA, KE and TZ, respectively) from the other populations. The third trend in the morphology (12%, Fig. 1) separates the Madagascan population from the mainland populations.

Principal coordinate (PCOORD) analysis of the chemical data accounted for 83.40% of the variation among populations by the first five eigenroots (24.6, 21.4, 15.5, 11.8 and 10.1%). A three-dimensional plot of the population coordinate scores for the first three coordinates is shown in Fig. 2 along with a minimum spanning network (dashed lines). The minimum spanning network is based on overall distances using coordinate scores on all eight coordinates extracted. The major trends are the separation of the Madagascan population (MA, Fig. 2), the tight clustering of the northeast African populations (AA, EE, GE, KE, Fig. 2), the divergence of the southern populations (Zambian, ZB; Zimbabwean, ZI), and Nigerian population (NI, Fig. 2) from the other East African populations. The loose clustering of the Ghanaian population (GH, Fig. 2) with the northeastern populations (AA, EE and GE all in Ethiopia; KE, Kenyan) does not

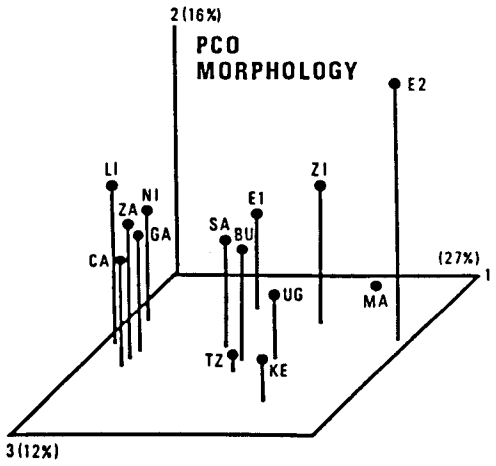

FIG. 1. PRINCIPAL COORDINATE ANALYSIS BASED ON MORPHOLOGICAL CHARACTERS OF *PHYTOLACCA DODECANDRA* FROM ADAMS *ET AL.* [6]. OTU E2 are pubescent plants from near E1 (Ethiopia).

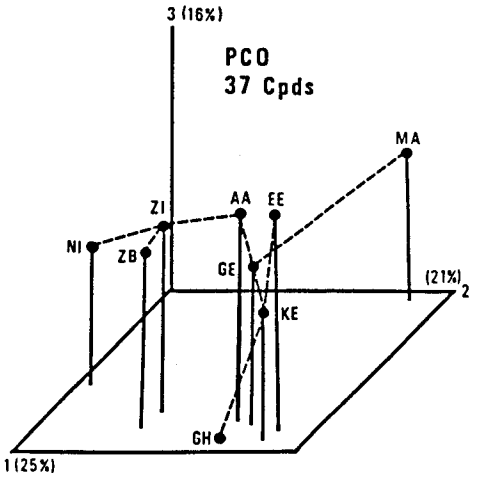

FIG. 2. PRINCIPAL COORDINATE ANALYSIS BASED ON THIRTY-SEVEN LEAF CHEMICALS FOR NINE POPULATIONS OF *PHYTOLACCA DODECANDRA* WITH A MINIMUM SPANNING NETWORK SUPERIMPOSED.

agree with the morphology (Fig. 1). However, one should note that the previous study [6] on the morphology utilized extensive herbarium specimens collected over the past century. Since collecting the chemical data would destroy part of a specimen, new collections had to be made for this study. Unfortunately, only a few plants were found in Ghana and Nigeria where the species appears to be rare and endangered. It is likely that the small sample sizes led to this unusual result. Another factor that must be considered is that *P. dodecandra* is used for washing clothes and traditional medicine in East Africa and is widely cultivated in Ethiopia. The seeds may have been taken to Ghana from Ethiopia or Kenya and likewise, seeds may have been carried to Nigeria from Zambia in the past.

It is interesting to note that only three pubescent plants were found in the previous study [6]: type 44 (AA in this study); one plant from Entoto Mountain (EE in this study); and a specimen from Ethiopia collected in 1955. The leaf chemistry does not correlate with pubescence as AA (pubescent) and EE (glabrous) cluster well with GE (glabrous, Guder, Ethiopia) whereas the pubescent plants (E2, Fig. 1) were very distinct in the previous morphological study [6]. Of course, pubescence was a highly significant character and likely accounted for almost all the morphological differences between the two Ethiopian samples (E1, E2, Fig. 1) in the previous study.

Examination of the contour maps of each principal coordinate shows some differences that are not apparent in the three-dimensional diagram. Figure 3 (principal coordinate 1, 24%) clearly shows the divergence of the

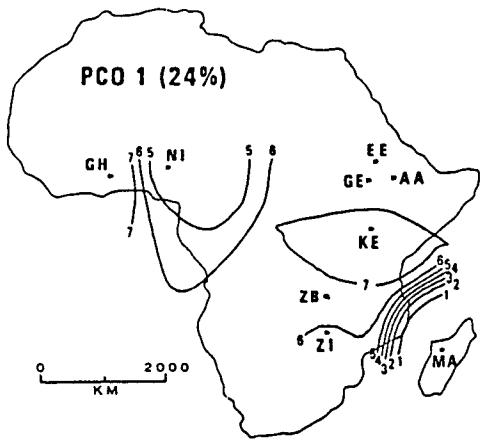

FIG. 3. CONTOUR MAP OF COMPONENT 1 SHOWING THE MAJOR TREND IN DIVERGENCE OF THE MADAGASCAN POPULATION (MA) OF *PHYTOLACCA DODECANDRA*.

Madagascan population and some divergence of the Nigerian population. The second coordinate (Fig. 4) depicts more divergence of the Nigerian population and separation of the southern populations of Zambia (ZB) and Zimbabwe (ZI). The third trend is due to the divergence of the Ghanaian population (GH) and minor differences among the East African populations (Fig. 5). The fourth coordinate (principal coordinate 4, 12%, Fig. 6) reveals a very strong differentiation of the Zambian (ZB) and Zimbabwean (ZI) populations from Nigerian, Kenyan and Madagascan populations (NI, KE and MA, respectively). In addition, some differentiation is seen between the Entoto Mountain (EE) and other Ethiopian populations (AA, GE).

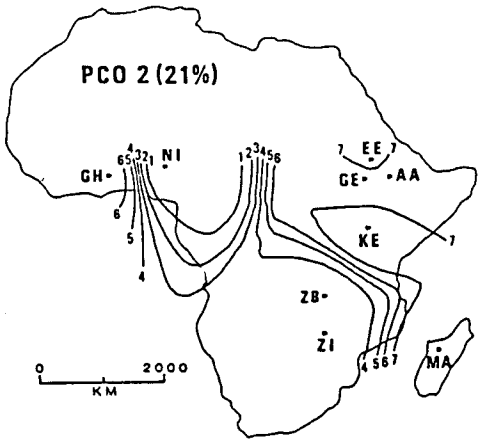

FIG. 4. THE SECOND MAJOR TREND IS THE DIVERGENCE OF THE NIGERIAN POPULATION (NI) FROM THE GHANAIAN (GH) AND EAST AFRICAN POPULATIONS.

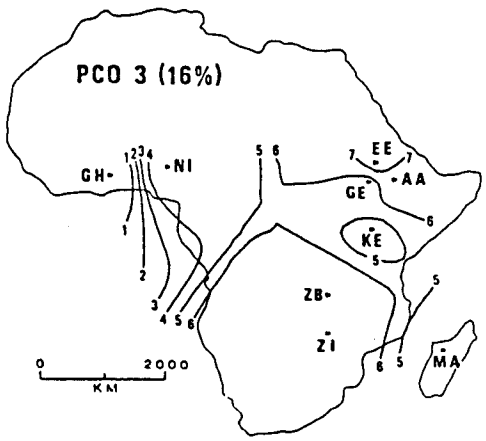

FIG. 5. COMPONENT SHOWING THE EAST–WEST CHANGES FROM ETHIOPIA TO GHANA WITH SLIGHT DIFFERENTIATION OF THE KENYAN POPULATION (KE).

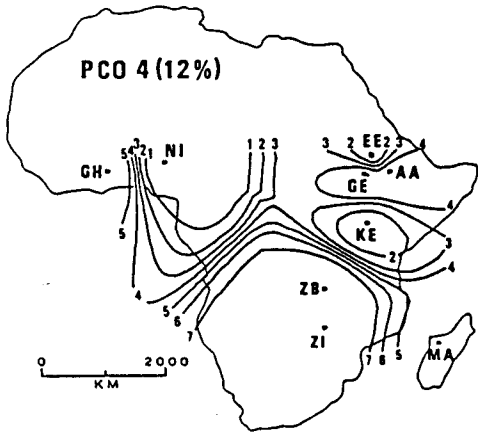

FIG. 6. THE DIVERGENCE OF THE SOUTHERN POPULATIONS (ZAMBIA, ZB; ZIMBABWE, ZI) IS SHOWN BY THE FOURTH PRINCIPAL COMPONENT, ALONG WITH MINOR DIFFERENCES BETWEEN OTHER POPULATIONS.

Overall *P. dodecandra* is quite variable in its leaf chemistry. The northeast African populations yielded 15.8–22.8% oleic acid compared to 1.8% in Zambia (ZB, Table 1). The C_{31} alkane, hentriacontane, varied from 52.3% in Zambia to 11.3% in Madagascar (MG, Table 1). α-Tocopherol ranged from 5.8% in Zimbabwe (ZI) to 1.4% in Kenya (KE, Table 1). The phytol esters showed large differences with the Ghanaian population yielding 6.3–9.7% of each ester (Table 1). All this points to considerable infraspecific variation within the taxon.

One of our reasons for examining infraspecific variation in *P. dodecandra* was to aid future germplasm collection for domestication trials [6]. It now appears, based on chemical and morphological data, that germplasm collections should focus on four areas: northeast Africa (Ethiopia, Kenya); Madagascar; southern Africa (Zambia, Zimbabwe); and West Africa, with close attention to differences between Nigeria and Ghana.

Experimental

Voucher specimens of *P. dodecandra* were collected from: (AA) Addis Ababa, Ethiopia, Type 44, Adams 5289, 5304–5306,

5308; (EE) Entoto Mtn, Ethiopia, Adams 5313–5317, (GE) Guder, Ethiopia, Adams 5294, 5296–5299; (KE) Kenya, Adams 5328–5332; (NI) Nigeria, Adams 5388, 5389, 5394; (GH) Ghana, Adams 5559–5564, 5566; (ZI) Zimbabwe, Adams 5586–5590; (MA) Madagascar, Adams 5598, 5623, 5624; (ZB) Zambia, Adams 5675–5678. Vouchers are deposited at the herbarium (BAYLU).

The air-dried leaves were ground in a Udy Cyclone mill and Soxhlet extracted (8 h) with hexane. The marc was dried (48 h, 100°C) after extraction. The hexane was removed from the extracts and percentage yields were calculated as: hexane extract weight divided by the sum of the extract weight plus the dry extracted residue (marc) weight.

The hexane extracts were analysed by gas chromatography on a Varian 6500 gas chromatograph equipped with a flame ionization detector (350°C), using a J & W fused quartz capillary column (DB1, 0.1 μm coating, 30 m × 0.32 mm i.d.) with helium as a carrier gas (30 cm s^{-1}). All GLC analyses were performed in the split mode (20:1 split ratio) with the injector temperature at 275°C. The oven temperature was programmed from 160–340°C as follows: 8°C min^{-1} for 12 min; 4°C min^{-1} for 21 min; then isothermal at 340°C for 9 min. Peak areas were quantitated using a Columbia Scientific Industries Supergrator-2 electronic digital integrator. Mass spectral data were collected with a Ribemarg R10–10C mass spectrometer and component identifications were based on computer searches of the EPA/NIH Mass Spectral data base [7] and comparisons with authentic materials. Several smaller components were unidentified and are under further analysis.

The chemical data were coded and analysed by one-way ANOVA with nine populations (8 *df* and 33 *edf*). PCOORD followed the formulation of Gower [8] using the Manhattan metric, scaled by the range (= Gower metric) [9] and weighted by F-1 (from ANOVA) as formulated by Adams [10–11]. The scores for each population were contoured onto a base map of Africa to examine geographic trends [12, 13].

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