Asian Journal of Chemistry Vol. 19, No. 3 (2007), 1809-1818

Assessment of Variation in *Cryptolepis sanguinolenta* (Lindl.) Schter., An Antimalaria Plant of The Family Periploceae Using Principal Component Analysis

Y. AMEYAW*, H.K. AKOTOYE†, A.N.M. PAPPOE†, Y. OPOKU BOAHEN‡ and F. EYIAH-BEDIAKO**
Centre for Scientific Research into Plant Medicine, P.O. Box 73, Mampong-Akuapem, E/R, Ghana, West Africa
E-mail: y61ameyaw@yahoo.com

A survey research was conducted on *Cryptolepis sanguinolenta* (Lindl.) Schtr., an antimalarial plant harvested from three sampling sites, namely; Pepease, Mamfe and Abonse in the eastern region of Ghana. The aim was to determine whether there is an intraspecific relationship within the plant species, *C. sanguinolenta* using a secondary chemical constituent, that is; the total alkaloid content, morphological and anatomical characters. To this end, Principal Component Analysis (PCA) was applied to the data from the three sites to determine the existence of variations. The results showed the existence of variation and some of the variables were more influential or weighted more than others

Key Words: Principal component analysis, *Cryptolepis sanguinolenta*.

INTRODUCTION

Plant species are often highly variable in morphological and other characteristics; such intraspecific variations are usually linked to many crieria or factors. Several authors[1-5] suggested that in a phenetic study all characters from any part of the plant body must be considered and this could be morphological, anatomical, cytological, physiological, biochemical, ecological, geographical, *etc.* and should be employed throughout a species range of distribution. Davis and Heywood[1] and Sneath and Sokal[3], who suggested the phenetic method postulated that in delimitation of taxa in a natural classification, correlation of characters supported by ecological or geographical distributions is the basis of creating taxonomic groups.

†Environmental Science Department, University of Cape Coast, Cape Coast, Ghana.
‡Organic Chemistry Department, School of Physical Science, University of Cape Coast, Cape Coast, Ghana.
**Mathematics and Statistics Department, University of Cape Coast, Cape Coast, Ghana.

According to them, weighting of characters is also very important because some characters are given greater importance or weight than another, *e.g.*, they may show the highest correlation with others in a natural group. Hatheway[5], who constructed the Weighted Hybrid Index, also considered the weight given each character being proportional to its usefulness in demonstrating a known or suspected relationship.

The Species, *Cryptolepis sanguinolenta* (Lindl.) Schtr.

Taxonomy of *C. sanguinolenta* (Lindl.) Schtr.: The plant now belongs to the family Periploaceae, initially it belonged to Ascelpiadeceace[6]. According to Hutchinson and Dalziel[6], the new family to which the plant belongs can be distinguished from the Ascelpiadeceace by the presence of granular pollen carried on spathulate glandular carriers. *Cryptolepis* belongs to the order Apocyales. The genus, *Cryptolepis* is made up of several species, which include *Cryptolepis brazzei*, *C. deciduas*, *C. oblongifolia*, *C. nigritana*, *C. triangularis* and *C. sanguinolenta* are common in West Africa.

It is also known as Pergularia sanguinolenta Lindl[6]. Another common name given to the plant is "Ghana quinine". It has different local names in Ghana; Nurubima (Guans), Kadze (Ewe) and Nibima (Twi).

Ecology of *C. sanguinolenta* (Lindl.) Schtr.: The plant is a tropical shrub indigenous to West Africa, from Senegal to Nigeria and found where rainfall is not very high. It avoids the wet rain forest where there is abundant shade. It does well in areas where there is adequate supply of sunshine and water; hence, it is totally absent from the marshy and salty swamps of coastal regions.

In Ghana, the plant is commonly found in certain districts in certain months of the year. On the Aburi hills, in the Akuapim area, it found in June when there is enough rainfall for optimum growth. In the Ejura District of Ashanti region, the plant thrives well in the woody savanna vegetation and flourishes well in June. Around Lake Bosomotwe in the Kuntanase-Bosomtwe District also in the Ashanti Region, the plant grows well in April and sometimes the dominant vegetation cover on deserted farmlands[6].

Botany of *C. sanguinolenta* (Lindl.) Schtr.: *C. sanguinolenta* (Lindl.) Schtr is a twining and scrambling thin-stemmed shrub with blood-red sap. It is indigenous to tropical Africa, and is use locally as a medicinal plant. The leaves of *C. sanguinolenta* are glabrous, oblano-elliptic or ovate. The leaf apex is acute to shortly acuminate. The leaf base is symmetrical, petiolate, and up to 2.2-7 cm long. The plant has a cymose inflorescence. There may be yellow flowers on the shoot and seeds 10 - 12 mm long with a tuft of silky hairs at the end.

The roots are rather tortuous and branches with little or no rootlets. The outer surface is yellowish brown and when dry, show longitudinal ridges with occasionally cracks. The roots break easily with fractures leaving a smooth transverse surface, which is yellow in colour. The sap is extremely bitter and is characterized by the rapidity with which it turns deep red on exposure to air[7,8].

Ethnobotany of *C. sanguinolenta* (Lindl.) Schtr.: A concoction of the stem and roots serve as herbal medicine in Ghana, West Africa to treat malaria, venereal disease and rheumatism[9,10]. The root decoction of *C. sanguinolenta*, has been used clinically by Oku Ampofo and Boye[10] at the Centre for Scientific Research into Plant Medicine, Mampong-Akuapim since 1974 for the treatment of malaria fever, urinary and upper respiratory tract infections[11].

In folklore tradition, the roots of *C. sanguinolenta* have been used as a bitter stomachic. It is has been used in the dyeing of textiles and leather[12]. The whole root water extract has been used by Ghanaian Traditional Healers for the treatment of fevers. In the Peoples' Democratic Republic of Congo and in Cesamace, Senegal, infusions of the root serve for the treatment of stomach and intestinal disorders and rheumatism[13]. The root is also sold and used as a yellow dyestuff amongst the Hausa of Northern Nigeria and other parts of West Africa and South Angola[8].

At the Centre for Scientific Research into Plant Medicine's (CSRPM's) Clinic however, Mist Nibima prepared from the roots of *Cryptolepis sanguinolenta* is prescribed mainly for the management of malarial fever and urinary tract infections. In a clinical trial by the CSRPM under the auspices of the Ministry of Health and sponsored by the World Health Organisation (WHO), "Mist Nibima" has proved to be very effective and efficacious, hence, recommendation for it's mass production for onward distribution to other hospitals. From the foregone background, the plant, *C. sanguinolenta* is or will be a great potential in the quest by researchers, para-medicals and herbalists for an antimalaria drug, to tackle the malaria menace. Therefore, the need to unearthed everything about this particular plant from variation in it's anti-malaria constituent (alkaloid content) through morphological and anatomical characters to the assessment of the nature of soil supporting it from the harvesting sources.

Principal component analysis is a data reduction technique, which enables us to interpret variation in data. It trials to explain the variance-covariance structure of a data through a few linear combinations of the original variables. For instance, in a data of *P* variables, it is expected that *P* principal components can be obtained to reproduce the total system variability; however, it is possible that the first few principal components can account for a very high percentage of the total variability. There are a

number of computer programmes available for performing principal component analysis[14,15].

This research is aimed at establishing the infraspecific variations within the plant species, *C. sanguinolenta* using morphological characters, anatomical characters and the total alkaloid content of the plant organs of the plant species obtained from the locations through the application of the Principal Component Analysis.

EXPERIMENTAL

Specimens of the *C. sanguinolenta* were collected from three populations namely, Pepease in the Kwahu district, Mamfe and Abonse in the Akuapem north district of Ghana. Fifty individuals from each of the three populations were collected for morphometric (morphological studies), anatometric (histological studies) and chemometric (chemical analyses).

Time of collection: The time and method of harvesting medicinal plants are very important. Plants contain numerous active constituents, chemical compounds responsible for the therapeutic activity, which are affected by environmental factors such as temperature, humidity, light and manner of handling during harvest. Collection of sample materials was on monthly basis, from April, 2002 to March, 2003.

Processing of plant materials for analyses: Materials for analyses were properly handled to avoid the growth of molds and other micro-organisms, since, their presence cause destruction of the active principles and the deterioration of the plant drug. Air-drying and sun-drying were the methods employed, and drying on top of concrete pavements or roof-tops were avoided since excessive heat could destroy some of the plant constituents.The dried roots, stems and leaves were grounded using the Manesty disintegrator to coarse powders. Samples were put in sample-bags, labelled both inside and outside; including the date/month of collection and placed in air-tight containers (dessicators) to prevent the growth of molds and other micro-organisms, as well as, the infestation by insects and rodents.

Chemical analysis: The root, stem and leaf of *C. sanguinolenta* (Lindl.) Schtr. were air dried and ground to provide fine powders. The powders were defatted with hexane or petroleum ether for 12 h and extracted by percolation with 500 mL of ethanol. After the solvent the syrup material was mixed with aqueous acetic acid (AcOH) (10 %, 200 mL) and allowed to stand overnight. The mixture was filtered, alkalined with ammonia (NH_4OH) to pH 10 and extracted with chloroform (CH_2Cl_2) (2 × 200 mL). The combined CH_2Cl_2 was washed with distilled water, dried over anhydrous sodium sulphate (Na_2SO_4) filtered and evaporated to afford a dark alkaloid residue. The weight of the evaporating dish was deducted from

the weight of the evaporating dish plus the alkaloid residue to give the weight of the alkaloid content, and the percentage of alkaloid calculated.

Morphohistological studies: Morphological and histological studies were carried out in the plant species with the view to determine those characters, which could be diagnostic for the delimitation of the plant species.

Morphological studies: Morphological characters were scored for the morphometric analysis. They were measured using a caliper and hand lens. Of these characters, leaf blade length (LfL) and width (LfW) were measured from fifty leaves in order of size among those attached at the base; petiole lengths (LfPtL) and leaf area (LfAr) were also taken alongside; length and width of pod (PodL and PodW) were measured on ten randomly selected plant from each ecotype; length and width of the seed (SeedL and SeedW) were measured on ten replicates from each population; length of the seed plus hair (SedhirL) as well as seed hair length (HairL) were measured on five mature specimen from each of the three populations.

Anatomical studies: The anatomical studies of 20 of the plant species from each of the samples sites carried out include: maceration and transverse sections of the root and stem.

Maceration of the root and stem: The root and stem of the plant were harvested from 5 different trees from each of the 3 sample locations (Pepease, Mamfe and Abonse). The root and stem each of about 10 cm long were cut into small pieces to the thickness of match-sticks and put into beakers. A mixture of 2:1 (v/v) H_2O_2 and CH_3COOH was added to the contents of the beakers and heated gently to boiling point on a water-bath in a fume chamber, until the tissues were softened and turned whitish. The beakers were sealed mesh of 212 µm and contents were then thoroughly washed with distilled water.

Drops of the macerated samples were then placed on glass slides and covered with cover slips and examined under high power magnification of a light microscope. The length and width of the following: vessel element, fibres and prismatic crystals were measured with the eyepiece at a magnification of X40. Slides, which were found to be satisfactory, were photographed. In all, a total of 30 slides were prepared, 5 each for the root and stem from each of the sample locations.

RESULTS AND DISCUSSION

The results focus on the application of morphological characters, anatomical characters and the total alkaloid content of the plant organs of the plant species, *C. sanguinolenta* obtained from the populations in Principal Component Analysis (PCA) to bring out variability among the characters.

Principal Component Analysis (PCA) was applied to the mean values of the morphological characters (Table-1); namely, the leaf characters and the fruit characters of the plant, *C. sanguinolenta* of the populations in 15 iterations of varimax rotation, which led to the extraction of 9 principal components (PCs), which accounted for 96.3 % of the total 100 % variance. Furthermore, these components had eigenvalues > 1 (Table-1). This renders the remaining principal components insignificant

In Table-1 the cumulated % of variance under the Principal Component 1 is 25.9. It implies that 25.9 % of the variations have been extracted leaving a further 74.1 %. This calls for further extraction of the variations leading finally to 96.3 % of cumulated % of variances under the PC 9. According to Table-1, the weights of the pod width at Pepease (P-PodW at -0.982), the seed hair length at Pepease (P- SedhirL at 0.947), the hair length at Pepease (P-HiryL at 0.913), as well as the petiole length obtained from Pepease (0.791) were the most influential variables comparatively within their respective populations at a cut-off point of 0.5 in the 1 principal component (PC1). In the second principal component (PC2), petiole length (A-PtL at 0.831), the ratio of seed length to width (P-SedL/W at 0.816), the leaf length at Abonse (A-LfL at 0.621) and the ratio of leaf length to width at Pepease (P - LfL/W at 0.582) were also the highly influential variables comparatively within their respective populations at a cut-off point of 0.5, *etc.*

Again, the Principal Component Analysis (PCA) was applied to the mean values of the anatomical characters of the plant, *C. sanguinolenta* in 17 iterations of varimax rotation, which led to the extraction of 6 principal components that accounted for 86.4 % of the total 100 % variance. Furthermore, these components had eigenvalues > 1 (Table-2). This renders the remaining principal components insignificant.

The cumulated percentage of variance for the first component was 22.2 leaving out 77.8 % not accounted for. This prompted further rotation of the original variables leading to the establishment of 5 additional principal components to produce a cumulative percentage (%) of variance of 86.4, which is really an accepted proportion of the 100 % variance.

Finally, applying the Principal Component Analysis (PCA) to the mean values of the total alkaloid content of the various plant organs of *C. sanguinolenta* in 5 iterations of varimax rotation, 3 principal components were extracted, where a maximum extraction of 79.96% of the variations was recorded (Table 3). This accounts for a significant amount of the original data, although, there are 9 principal components (PCs). Therefore, rendering the remaining components insignificant.

TABLE-1
PRINCIPAL COMPONENT ANALYSIS (PCA) ROTATED USING VARIMAX METHOD
BASED ON THE MORPHOLOGICAL CHARACTERS CONVERGED IN 15 ITERATIONS

	Component								
	1	2	3	4	5	6	7	8	9
P - LfL	0.128	-0.309	-0.331	-0.211	0.775	0.251	0.208	-0.033	-0.089
P - LfW	0.137	-0.430	-0.228	-0.306	0.635	0.188	0.235	-0.202	-0.187
P - PtL	0.791	-0.168	0.206	0.278	0.220	0.116	0.212	-0.308	-0.020
M - LfL	-0.091	-0.409	-0.278	-0.146	0.396	0.338	0.063	-0.166	-0.611
M - LfW	0.240	0.112	-0.556	0.075	0.185	0.699	-0.049	-0.023	0.263
M - PtL	0.250	-0.093	-0.012	0.080	-0.260	-0.789	0.219	0.311	0.068
A - LfL	-0.311	0.621	-0.394	-0.486	-0.159	0.217	-0.112	-0.029	-0.018
A - LfW	-0.076	0.760	-0.272	-0.108	-0.099	0.118	-0.311	0.392	0.210
A - PtL	0.247	0.831	0.039	-0.099	0.047	-0.034	-0.363	-0.134	-0.105
P - PodL	-0.590	0.049	0.635	-0.344	-0.235	-0.124	0.028	-0.107	-0.196
P - PodW	0.982	-0.108	0.078	-0.004	0.070	-0.017	-0.081	0.050	0.046
P - PdL/W	-0.900	0.097	0.290	-0.227	-0.139	-0.042	0.016	-0.093	-0.093
P - SedL	0.542	-0.024	0.568	0.147	0.365	0.032	0.226	-0.116	-0.102
P - SedW	0.126	-0.920	-0.114	-0.207	-0.025	0.029	0.117	0.072	-0.189
P -SedL/W	0.219	0.816	0.407	0.208	-0.074	-0.224	0.045	0.092	0.081
P - SedhiL	0.947	0.241	0.056	0.145	-0.052	-0.051	-0.073	-0.014	0.038
P - HiryL	0.913	0.263	-0.065	0.106	-0.175	-0.071	-0.113	-0.001	0.050
M - PodL	-0.024	-0.131	0.130	0.273	-0.084	0.873	0.097	0.117	0.025
M - PodW	0.017	0.008	0.921	0.143	-0.175	0.247	-0.078	0.062	0.052
M - PdL/W	0.038	0.039	-0.939	0.020	0.233	0.146	0.193	0.029	0.023
M - SedL	-0.082	-0.599	0.227	-0.105	-0.026	-0.432	-0.330	0.046	0.422
M - SedW	-0.554	-0.100	-0.291	-0.051	0.102	0.291	-0.282	0.303	0.553
M -SdL/W	0.541	0.266	0.636	0.028	0.184	-0.286	-0.316	-0.002	-0.090
M - SdhirL	0.248	0.048	0.092	0.925	-0.054	0.041	0.017	0.185	0.052
M - HiryL	0.257	0.203	0.027	0.904	-0.044	0.153	0.103	0.163	-0.062
A - PodL	0.439	0.323	-0.088	0.391	-0.116	0.026	-0.234	0.499	0.461
A - PodW	0.166	0.006	0.006	0.116	-0.812	0.033	-0.210	0.361	0.222
A - PdL/W	0.177	0.325	-0.122	0.218	0.828	-0.039	0.091	0.026	0.204
A - SedL	0.206	-0.139	-0.196	0.629	-0.090	0.392	-0.380	-0.111	0.384
A - SedW	0.254	0.053	-0.052	-0.211	0.336	0.135	0.035	-0.846	0.035
A -SdL/W	-0.140	-0.129	0.096	0.550	-0.326	0.076	-0.177	0.617	0.244
A - SdhirL	-0.030	-0.217	-0.150	0.036	0.215	0.004	0.927	-0.117	-0.059
M - HiryL	-0.067	-0.180	-0.096	-0.065	0.223	-0.066	0.929	-0.103	-0.108
P - LfL/W	0.301	0.582	-0.311	-0.086	0.160	-0.051	-0.026	0.513	0.375
M - LfL/W	-0.259	-0.460	0.232	-0.208	0.140	-0.329	0.082	-0.169	-0.670
A - LfL/W	-0.286	-0.326	-0.059	-0.448	-0.107	0.065	0.298	-0.603	-0.301
Eigenvalue	9.317	6.712	5.481	4.181	2.735	2.392	1.753	1.083	1.002
% of variance	25.882	18.645	15.225	11.614	7.596	6.644	4.868	3.009	2.785
Cumu. % of Variance	25.882	44.527	59.751	71.365	78.961	85.605	90.474	93.482	96.267

TABLE-2
PRINCIPAL COMPONENT ANALYSIS (PCA) ROTATED USING VARIMAX METHOD OF THE ANATOMICAL CHARACTERS OF THE *C.SANGUINOLENTA* CONVERGING IN 8 ITERATIONS

	Components					
Variable	PC1	PC2	PC3	PC4	PC5	PC6
P - FiRL	0.121	-0.187	0.142	0.229	-0.067	-0.628
P - FiRW	-0.055	-0.217	-0.238	0.302	0.284	0.246
P - VeRL	-0.067	0.356	0.221	0.227	-0.392	-0.910
P - VeRW	-0.117	0.108	-0.484	0.137	0.088	-0.221
P - PcRL	-0.112	0.366	0.280	-0.256	0.167	-0.099
P - PcRW	-0.397	0.202	0.088	-0.157	-0.008	-0.111
M - FiRL	-0.370	-0.122	-0.216	-0.107	-0.248	-0.123
M - FiRW	0.320	-0.018	-0.276	-0.102	-0.315	-0.109
M - VeRL	0.223	0.046	0.095	0.286	0.413	-0.422
M - VeRW	0.148	0.195	-0.224	0.324	-0.384	-0.104
M - PcRL	0.237	0.356	-0.231	-0.180	0.081	0.202
M - PcRW	-0.084	-0.162	-0.376	-0.271	0.259	-0.244
A - FiRL	-0.355	-0.132	-0.182	0.103	-0.312	0.046
A - FiRW	0.361	0.044	-0.179	0.145	-0.017	0.242
A - VeRL	-0.266	-0.299	0.040	0.178	0.075	0.083
A - VeRW	-0.132	0.041	0.167	0.505	0.106	0.280
A - PcRL	0.272	-0.342	0.170	-0.246	-0.155	0.067
A - PcRW	-0.120	0.411	-0232	0.056	0.187	-0.017
Eigenvalue	4.0029	3.4036	2.8032	2.0385	1.8923	1.4117
% of Variance	0.2220	0.1890	0.1560	0.1130	0.1050	0.0780
Cumulative % of variance	0.2220	0.4110	0.5670	0.6800	0.7860	0.8640

The results in Table-3, shows the cumulative (%) of variance under the first principal component (PC1) to be 39.432 Therefore, 60.568 % of variation has not been accounted for, hence, the need to carry on further extractions under the principal components 2 and 3, respectively. From the Table-3, the final cumulative percentage (%) of variance is 79.958 of the variation, which is a substantial extraction, out of the 100% of variance. Therefore, the application of the first three principal components (PC3) extracted in this PCA is acceptable; eigenvalues are > 1.

As shown in the first principal component (PC1) in Table-3, the weights of the root total alkaloid at Pepease (PR-Alk) of 0.953, the root total alkaloid at Abonse (AR-Alk) of 0.919 and the root total alkaloid of Mamfe (MR-Alk) of 0.860 were more influential comparatively at a cut-off point of 0.5. In the second principal component (PC2), the leaf total alkaloid at Mamfe (ML-Alk) of 0.888, the leaf total alkaloid at Pepease (PL-Alk) of 0.845, the stem total alkaloid at Abonse (AS-Alk) of 0.831 and the stem total alkaloid at Pepease (PS-Alk) of 0.550 were the also most influential

variables comparatively at a cut-off point of 0.5. Lastly, the stem total alkaloid at Mamfe (MS-Alk) of 0.901 was the only influential variable comparatively in the third principal component (PC3) at a cut-off point of 0.5.

Results presented in Tables 1-3 on morphological characters, anatomical characters and total alkaloid content of the plant species, *C. sanguinolenta*, respectively, indicate that some of the characters can be rated as highly variable, more influential or weighted more than others. Similar, sentiments on the correlation weighting of characters have earlier been expressed by several authors[1-5].

TABLE-3
PRINCIPAL COMPONENT ANALYSIS (PCA) ROTATED USING VARIMAX METHOD OF THE TOTAL ALKALOID CONTENT OF THE PLANT ORGANS, *C. SANGUINOLENTA* CONVERGING IN 5 ITERATIONS

	Component		
	1	2	3
PR-Alk	0.953	0.150	0.132
AR-Alk	0.919	-0.013	0.067
MR-Alk	0.860	-0.244	0.262
AL-Alk	-0.595	0.060	-0.495
ML-Alk	0.045	0.888	-0.304
PL-Alk	-0.090	0.845	-0.105
AS-Alk	-0.194	0.831	0.371
PS-Alk	0.307	0.550	0.419
MS-Alk	0.245	-0.046	0.901
Eigenvalue	3.549	2.581	1.066
% of variance	39.432	28.682	11.844
Cumu.% of variance	39.432	68.114	79.958

Morphological characters, anatomical characters and the total alkaloid content of the plant organs obtained from the populations were generally, unrelated, some of them were more influential or weighted more than others, although, all the characters were statistically, treated equally (Tables 1-3).

Harvesting of a medicinal plant need to be collected from the right ecological zone, since variation do exist, morphologically, anatomically and phytochemically. Therefore, harvesting of the plant from different habitats for herbal preparations should be examined critically, since, the phytochemical constituents may be highly variable.

Conclusion

Collectors of plant materials should always have pre-knowledge of certain characters, which will really aid them in the harvesting of plant parts from the wild, so as to avoid fatalities as a result of wrong identification of plants.

Some of the characters (morphological, histological or total alkaloid content) were more influential or weighted more than others when it comes to use of characters as tools in taxonomic studies using the Principal Component Analysis (PCA).

ACKNOWLEDGEMENT

The authors acknowledge Professor B.K. Gordor, Mathematics and Statistics Department, University of Cape Coast for his immense support and encouragement.

REFERENCES

1. P.H. Davis and V.H. Heywood, Principles of Angiosperm Taxonomy, Oliver and Boyd, Edinburgh (1963).
2. P.H.A. Sneath, *Bio. J. Linn. Soc.*, **3**, 147 (1973).
3. P.H.A. Sneath and R.R. Sokal, Numerical Taxonomy: The Principles and Practice of Numerical Classification, Freeman and Company, San Francisco (1973).
4. N. Jardine and R. Sibson, Mathematical Taxonomy, John Wiley, London (1971).
5. W.H. Hatheway, *Evol.*, **16**, 1 (1962).
6. J. Hutchinson and J.M. Dalziel, Flora of Tropical West Africa, The Whitefrairs Press Ltd., London, pp. 102-223 (1963).
7. G.L. Boye, Ghana Herbal Pharmacopoeia, The Advert Press, Osu-Accra, p. 37, edn. 1 (1992).
8. A. Sofowora, Medicinal Plants and Traditional Medicine in Africa, The Pitman Press Limited; Bath, Avon. 1, edn. 2, p. 6 (1984).
9. K.B.-Yiadom, *J. Pharm. Sci.*, **68**, 1510 (1979).
10. O. Ampofo and G.L. Boye, Proceedings of the First International Symposium on Cryptolepine, Kumasi, Ghana, University of Science and Technology, pp. 37-40 (1983).
11. D.D.-Badu, J.S.K. Ayim, F.N.I.Y. Fiagbe, J.E. Knapp, P.L. Schiff and D.J. Slatkin, *J. Pharm. Sci.*, **67**, 433 (1978).
12. J.M. Dalziel, Useful Plants of West Tropical Africa, Crown Agents for Overseas Government, London (1956).
13. A. Sofowora, Medicinal Plants and Traditional Medicine in Africa, John Wiley and Sons, Chichester (1982).
14. R.A. Johnson and D.W. Wichern, Applied Multivariate Statistical Analysis, Prentice - Hall International, Inc. London, U.K., edn. 3 (1992).
15. S. Sharma, Applied multivariate techniques, John Wiley, New York, USA, edn. 1 (1983).

(*Received*: 22 February 2006; *Accepted*: 13 October 2006) AJC-5185