



THE ALKALOID CONTENTS OF THE ETHNO-PLANT ORGANS OF THREE ANTIMALARIAL MEDICINAL PLANT SPECIES IN THE EASTERN REGION OF GHANA

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ABSTRACT

A survey research was conducted on *Cryptolepis sanguinolenta* (Lindl.) Schtr., *Morinda lucida* Benth and *Voacanga africana* Stapf antimalarial plant species harvested from Mampong-Akuapem in the Eastern Region of Ghana. Comparative studies were performed using the total alkaloid content of the plant organs of three plant species used ethnobotanically for the preparation of antimalarial decoctions. Statistically, a series of linear models (SPSS 10.0, Minitab 13.32 and MStats for WINDOWS, Version 6.1) was fitted to the dataset to determine whether variations do exist. Results showed that: (i) Phytochemical screening of the plant confirmed the presence of alkaloid in the plant organs (ii) The alkaloid contents of the plant organs of the plant species were significantly different from one another and (iii) The total alkaloid content of *V. Africana* was the highest among the three plant species.

Key words: Ethno-plant organs, Cluster Analysis, Dendrogram, Alkaloid.

INTRODUCTION

After millions of years of use of herbs, the isolation of active principles like alkaloids, morphine and quinine, in the 19th Century, marked a new era in the use of herbs, and the start of plant medicine research in modern disease.

The importance of natural molecules in medicine lies not only in their pharmaceutical or chemotherapeutic effects but also in their role as template molecules for the production of synthetic drugs. Most antimalarial drugs currently in use are quinoline derivatives modeled on the quinine molecule isolated from the bark of *Cinchona* species native to high altitudes of South America. This genus is said to have been named after

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Spanish Countess of Cinchona who was successfully treated with the powdered bark¹.

The quinine molecule inspired the synthesis of chloroquine, and this drug became the chief replacement for quinine, during the Second World War². Chloroquine was close to the ideal antimalarial drug and was used for decades owing to its high efficacy against all species of malaria parasites, low toxicity, low cost and high tolerance. It is still widely used to treat malaria in areas, where notable drug resistance has not yet appeared.

There have been numerous attempts to test plants extracts for antimalarial activity^{3, 4}. Over 600 plants from 125 families were screened for *in vitro* activity against *P. gallinaceum* in chicks and against *P. cathemerium* and *P. lophurae* in ducklings⁴. The species of some 33 genera gave positive results; the most significant levels of activity were found in extracts of species from Amaryllidaceae and from the Simaroubaceae.

Antimalarial plants in Africa

Many medicinal plants of Africa have been shown to possess interesting biological activity. Gbeassor *et al.*⁵ investigated the antimalarial properties of several African plants. Some of these plants include *Cassia siamea* lam., *Jatropha gossypifolia* Linn., *Nauclea latifolia* Benth, *Azadirachta indica* A. Juss., and *Cryptolepis sanguinolenta* (Lindl.) Schtr. In Sierra Leone, out of 18 plant species screened against fevers and malaria, *Trichlisia patens* proved to be the most effective against the malaria parasite *P. falciparum*. *Cochlospermum tinctorium* A. Rich, a plant commonly used in Burkina Faso for the treatment of malaria has been investigated⁶.

In Ghana, the bark decoction of *Nauclea latifolia* of the family Rubiaceae, is used as an antimalarial remedy. In fact, it was exported from West Africa as a possible substitute for cinchona⁷. The aqueous extract of *Cryptolepis sanguinolenta* has been used by herbalists in Ghana for centuries for the treatment of various conditions notably malaria, fever, rheumatism and urinary tract infections⁸.

Alkaloidal significance in plant species

The significance of medicinal plants is directly linked to the wide range of chemical compounds synthesised by the various biochemical pathways. These compounds are classified as secondary plant products, because they are not much related to the plant's survival. Previously, researchers took many of these compounds to be simply waste

products of metabolism, but they are now known to possess important functions. One major category of such compounds is alkaloids. Although they vary greatly in their chemical structures, alkaloids have several common characteristics: they possess nitrogen (most are derived from a few common amino acids), and are alkaline (basic), but have non-basic forms such as quaternary compounds and *N*-oxides.

Multiple uses of alkaloids in medicinal plant species

The alkaloid extracts obtained from medicinal plant species have multiplicity of host-mediated biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory and pharmacological effects^{9, 10}.

Taxonomic review of *Cryptolepis sanguinolenta* (Lindl.) Schtr.

The plant now belongs to the family Periploaceae, although it had earlier been classified as a member of the family Asclepiadeceae¹¹.



Plate 1: Photograph of the morphological features of *C. sanguinolenta*

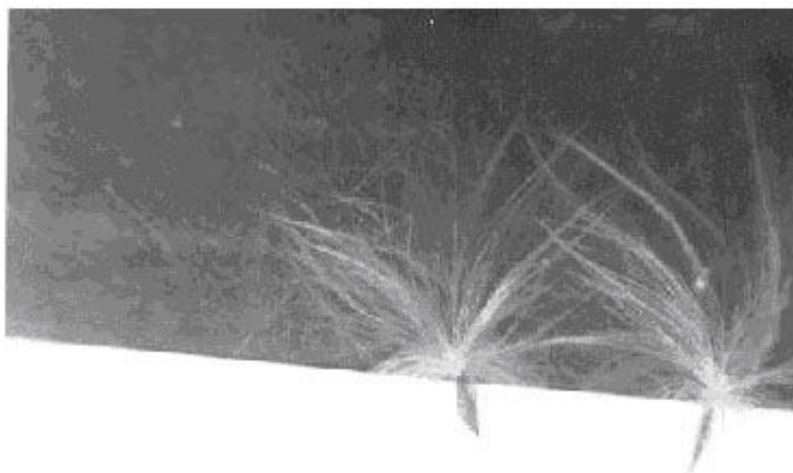


Plate 2: Brown seeds of *C. sanguinolenta* (x1) with hairy appendages.

According to Hutchinson and Dalziel¹¹, the new family to which the plant belongs can be distinguished from the Asclepiadeceae by the presence of granular pollen borne on spathulate glandular carriers. *Cryptolepis* belongs to the order Apocyaes. The genus, *Cryptolepis* includes *Cryptolepis brazzei*, *C. deciduas*, *C. oblongifolia*, *C. nigritana*, *C. triangularis* and *C. sanguinolenta*, are common in West Africa. *Cryptolepis sanguinolenta* is also known as *Pergularia sanguinolenta* Lindl.¹¹. In Ghana, the plant is also commonly known as “Ghana quinine”, although different localities have different local names for it; such as: nurubima (Guans), kadze (Ewe) and nibima (Twi).

Taxonomic review of *Morinda lucida* Benth

M. lucida Benth is of the family Rubiaceae and its common name is Brimstone tree. It is a medium-sized tree up to 15 m, not buttressed and rarely cylindrical. The plant is very common in Africa and mostly found in the secondary forest. The bark is grey to yellowing, very scaly whilst bark slash is very yellow to orange brown. The genus, *Morinda* includes *Morinda geminate*, *Morinda longiflora*, *Morinda morindioides* and *Morinda lucida*.



Plate 3: Grey bark with slash bark yellow to orange brown of *M. lucida*



Plate 4: Fruits of *M. lucida* in ball-shaped measuring 1.5cm in diameter. Taxonomic review of *Voacanga africana* Stapf

V. africana Stapf belongs to the family Apocynaceae. The genus, *Voacanga*

includes *Voacanga bracteata*, *Voacanga obtusa* and *Voacanga Africana*, which are common in West Africa. In Ghana, the plant species is locally known as “Kakapempe” in the Ashanti Region and “Ofuruma” in Nzema in the Western corner of Ghana.



Plate 5: Photograph of an aerial part of *V. africana* (x1) showing some fruits

The medicinal plant species are used in Ghana and Benin, all along the West Coast of Africa in the management of malaria, which may be attributed to the presence of alkaloid in the plant species. Therefore, this research is to comparatively assess the alkaloid content levels in the ethno-plant organs of *Cryptolepis sanguinolenta* (Lindl.) Schtr., *Morinda lucida* Benth and *Voacanga Africana* Stapl. harvested from Mampong-Akuapem in the Eastern Region of Ghana.

EXPEREMENTAL

Materials and methods

The ethno-plant raw materials of the plant species were collected from the environs of Mampong-Akuapem in the Eastern Region of Ghana and dried under the shade until the ethno-plant organs snaps at the finger tips. Ethno-plant organs dried were pulverised into powder.

Phytochemical studies

Phytochemical studies carried out include qualitative and quantitative analyses of

the ethno-plant materials of *C. sanguinolenta* (Lindl.) Schtr., *Morinda lucida* Benth and *Voacanga Africana* Stapl obtained from the sample location.

Qualitative analysis

Qualitative test for alkaloid was carried out on the plant materials that were obtained from the sample locations. An alcoholic extract of each material was prepared separately by adding 50 mL of 70 % ethanol to 1g of each powdered material of the various plant organs collected. To 2 mL portions of each alcoholic extract of the various organs in separate test tubes was added the following test reagents:

- (a) Mayer's reagent (b) Dragendorff's reagent (c) N/10 Iodine solution

Quantitative analysis

Quantitative determination of the alkaloid content in the ethno-plant materials of *C. sanguinolenta* (Lindl.) Schtr., *M. lucida* Benth and *V. Africana* Stapl obtained from the sample location were carried out. Three replicates were prepared for each plant sample and the mean values computed. The plant materials were air-dried for 30 days and pulverised into powder using the Manesty disintegrator. 50 g of the powdered material obtained in each case were Soxhlet extracted with hexane for 12 hours in order to defat it. The defatted powder in each case was taken and the alkaloid extracted with 500 mL of ethanol. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The residue was mixed with 200 mL of 10 % aqueous acetic acid and allowed to stand overnight. The mixture was filtered and the pH of the resulting filtrate adjusted to 10 using drops of dilute ammonium solution. The alkaloids were extracted with two equal volumes of 200 mL of chloroform. The chloroform extract was dried with anhydrous sodium sulphate, the solvent evaporated and the residue weighed. The percentage of alkaloid was calculated using the following formula:

$$\text{Total Alkaloid (\%)} = \frac{W}{Y} \times 100$$

Where W = Weight of alkaloid content extracted and

Y = Weight of powdered plant material.

Statistical analysis

The SPSS 10.0, Minitab 13.32 and MSstats were fitted to the dataset to determine whether there was any variation in *C. sanguinolenta* (Lindl.) Schtr., *Morinda lucida* Benth

and *Voacanga africana* Stapl collected from the sample locations. The variable used in the analyses was the total alkaloid content of the ethno-plant organs of the plant species. The Duncan's Multiple Range Test and T-test were applied to assess the level of significant differences between the variables. A cluster analysis using average linkage (Ward's method) was performed on the modified data to evaluate the degree of similarity, association, closeness or resemblance among the ethno-plant organs of the plant species. Significance differences were determined at a probability level of 0.05.

RESULTS AND DISCUSSION

The results of phytochemical test for alkaloid in the different ethno-plant organs of *C. sanguinolenta* (Lindl.) Schtr., *M. lucida* Benth and *V. africana* Stapl obtained from the sample location confirmed the presence of alkaloid using Mayer's reagent, Dragendorff's reagent and N/10 iodine solution.

Qualitative assessment for the presence of alkaloid

Plant species	Part used	Result
<i>Morinda lucida</i>	Dried roots	Positive
<i>Voacanga Africana</i>	Dried leaves	Positive
<i>Cryptolepis sanguinolenta</i>	Dried roots	Positive

Variations of the alkaloid content of the plant materials from the locations.

Tables 1, 2 and 3 give the percentage mean of the alkaloid content of the ethno-plant materials obtained from the sample location.

Quantitative assessment of alkaloid content in the plant species

Table 1. *Morinda lucida*

Samples	1	2	3
Volume of samples (mL)	100	100	100
Mass of dish + residue (g)	28.07	53.25	33.65
Mass of dish (g)	27.65	52.79	33.21
Mass of residue (g)	0.42	0.46	0.44

$$\text{Average mass of residue} = (0.42 + 0.46 + 0.44) \div 3 = 0.44(c) \pm 0.13g$$

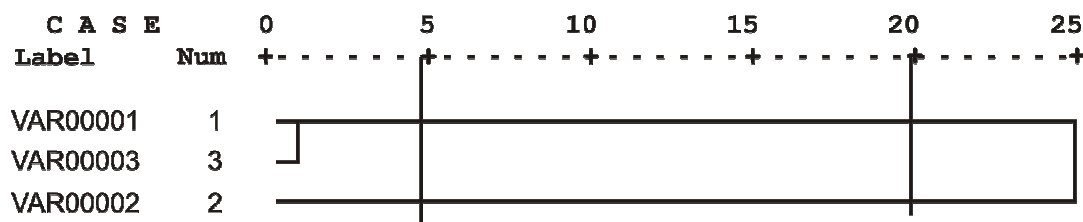
Table 2: Voacanga africana

Samples	1	2	3
Volume of samples (mL)	100	100	100
Mass of dish + residue (g)	33.73	28.20	33.79
Mass of dish (g)	33.22	27.65	33.21
Mass of residue (g)	0.51	0.55	0.58
Average mass of residue = $(0.51 + 0.55 + 0.58) \div 3 = 0.55(a) \pm 0.13g$			

Table 3: Cryptolepis sanguinolenta

Samples	1	2	3
Volume of samples (mL)	100	100	100
Mass of dish + residue (g)	27.93	33.49	73.80
Mass of dish (g)	27.65	33.21	73.53
Mass of residue (g)	0.28	0.28	0.27
Average mass of residue = $(0.28 + 0.28 + 0.27) \div 3 = 0.28(b) \pm 0.10g$			

The average mass of alkaloid content do differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test.



Rescaled distance cluster combine

Fig. 1: Dendrogram showing Ward's method using alkaloid content levels in the ethno-plant organs of the plant species.

Var00001 – *Morinda lucida*

Var00002 – *Voacanga africana*

Var00003 – *Cryptolepis sanguinolenta*

The degree of similarity, closeness or resemblance in terms of the alkaloid content levels in the ethno-plant organs of the plant species, dendrogramatically presented using Ward's Method.

The biodynamics of the ethno-plant organs of the plant species revealed the presence of alkaloid, which was unevenly distributed in the plant species, *C. sanguinolenta* (Lindl.) Schtr., *Morinda lucida* Benth and *Voacanga africana* Stapl obtained from the sample location (Tables 1, 2 and 3). Within the plant species the levels of alkaloid content were significantly different among the ethno-plant organs using Duncan's Multiple Range Test as specified in the average mass of alkaloids in the plant species, shown in Tables 1, 2 and 3. The ethno-plant organ alkaloid content levels obtained from *V. Africana* and *M. lucida* showed close resemblance or similarity at rescaled distances of 5 and 20 as compared to that of *C. sanguinolenta*.

CONCLUSION

From the foregone discussions, *V. Africana* and *M. lucida* may be more effective as compared to *C. sanguinolenta* in the management of malaria in terms of their alkaloid content levels. Therefore, there is the need to pharmacologically ascertain the parasitaemia levels in blood samples in patients on applying the ethnomedicines of the plant species aforementioned.

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