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COMPARATIVE PHARMACOGNOSTIC AND ANTIMICROBIAL STUDIES ON LEAVES OF TWO VARIETIES OF *HEINSIA CRINITA*

K. AJIBESIN KOLA, J. EKPO BENJAMIN and N. BALA DANLADI

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ABSTRACT

Heinsia crinita (Rubiaceae) is classified as dark and white varieties in Akwa Ibom State, though the two varieties appear macroscopically indistinguishable. However, microscopical examination of the leaves of the two varieties was executed by adopting standard microscopic methods like surface preparation, transverse section and powder clearing to reveal some discrepancies in stomatal types, occurrence of vascular bundles and presence and absence of sclereids. The stomatal number, stomatal index and palisade ratio showed disparate values for both varieties. The total ash, sulphated ash and acid insoluble ash values also gave disparity to further distinguish the two varieties and ostensibly justify their classification as dark and white varieties. This finding will aid proper identification and collection. *In vitro* antimicrobical assay using agar gel-diffusion method was also carried out on the leaves of the two varieties to determine differences in their antimicrobial activities: Dark variety showed both antibacterial and anti fungal activities while the white variety yielded only the antibacterial activity.

KEY WORDS: *Heinsia crinita*, dark and white varieties, antimicrobial activities, Pharmacognostic studies.

INTRODUCTION

Heinsia crinita (Rubiaceae) is a shrub with woody stems and branches (Hutchinson and Dalziel, 1954). It is indigenous to West Africa, especially the Eastern part of Nigeria, but it is now well cultivated in Central Africa, South of Sahara and Francophone Africa (Babady-Bila *et al.*, 1994). *Heinsia crinita* is casually classified as white and dark by indigenes of Akwa Ibom State in Southern Nigeria. The white variety is cultivated by Annang locals of the State while the Ibibio Community (Akwa Ibom) cultivate the dark variety. Nontheless, both varieties are readily available in the market.

Both varieties are cultivated for their nutritious values. However, the locals use the leaf juice, to treat various skin diseases and wounds. Two triterpenoid saponins have been isolated from the plant (Babady-Bila *et al.*, 1994).

The two varieties show striking morphological resemblance, and distinction between them is made only on the basis of taste. The dark variety leaf is bitter while that of the white variety is only slightly bitter. A nutritional study of *Heinsia crinita* leaves has been reported by Etuk *et al.*, (1998) and Etuk *et al.*, (2002).

This work attempts differentiating the

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leaves of the two varieties on the basis of microscopy, pharmacognostical evaluations and antimicrobial assay to facilitate their proper identification and collection.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Heinsia crinta* varieties (dark and white were collected in March, 2000, at Abia Okpo Ikot Essien in Ikot Ekpene Local Government Area of Akwa Ibom State, Southern Nigeria, and authenticated by a taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State.

Macroscopy

Leaves of the white and dark varieties were visually examined.

Microscopy

Specimens were prepared for microscopical examination by adopting standard methods (African Pharmacopoeia, 1986; Trease and Evans, 1989) for sections and powders. All observations were made using binocular microscope (ultra SWIFT Lite, M3500D) at x 200 and x 400.

Physical Constant Determinations

Stomatal number, stomatal index, palisade ratio, total ash, acid insoluble ash and sulphated ash were determined by standard methods (African Pharmacopoeia, 1986, Trease and Evans, 1989).

Leaf Extraction

The leaves of the two varieties were separately extracted cold with ethanol to give ethanol extracts. These were concentrated *in vacuo* at 40°C to dryness. Phytochemical screening was carried out on the dry ethanol extract of each variety to reveal the presence of secondary metabolites in them. (Sofowora, 1993, Trease & Evans 1989).

The dry crude ethanol extract of each variety was successively partitioned with n-hexane, chloroform, ethylacetate and butanol to give their respective fractions. The fractions were individually concentrated *in vacuo* at 40°C to afford dry residues.

Antimicrobial assay: Micro-organisms

The fungus and the bacteria employed were clinical isolates sourced from St. Luke's hospital Anua Akwa Ibom stock culture units. The fungus *Epidermophyton gametophyte* was maintained on Sabouraud's dextrose agar (Oxoid) slants at 4°C prior to use; while the bacterial species: *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* were sustained on blood agar slant at 4°C before use for antimicrobial susceptibility test.

Antimicrobial susceptibility testing

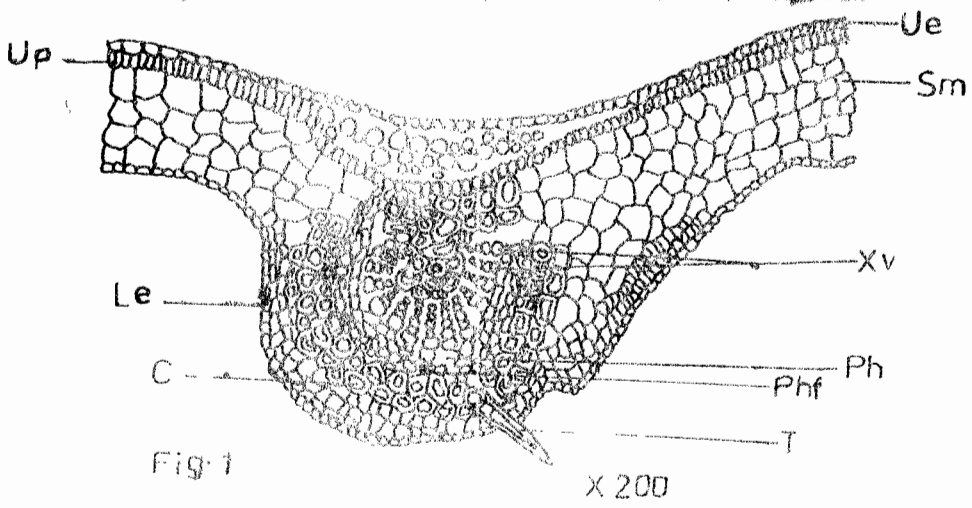
The dry ethanol extract and the dry fractions were reconstituted in DimethylsulphoxideDMSO. The ethanol extract was tested at varying concentrations of 40mg/ml, 80mg/ml and 100mg/ml, while the fractions were tested at 15mg/m, 10mg/ml and 5mg/ml. The solutions (150ul) of ethanol extract, fractions and DMSO were separately introduced into equidistant wells borne by the surface of the agar and Sabouraud's plates, which had been individually inoculated, with one of the test organisms. A well containing a standard, Gentamicin was placed in each of the plates seeded with bacteria, while the plate seeded with fungus bore hole containing Nystatin as standard. The bacteria were incubated at 37°C for 24 hours, while the plate bearing the fungal assay was incubated at 25°C for 7 days. The presence of zones of inhibition encompassing the wells was taken as an indication of antimicrobial activity.

RESULTS

Macroscopy

Leaf

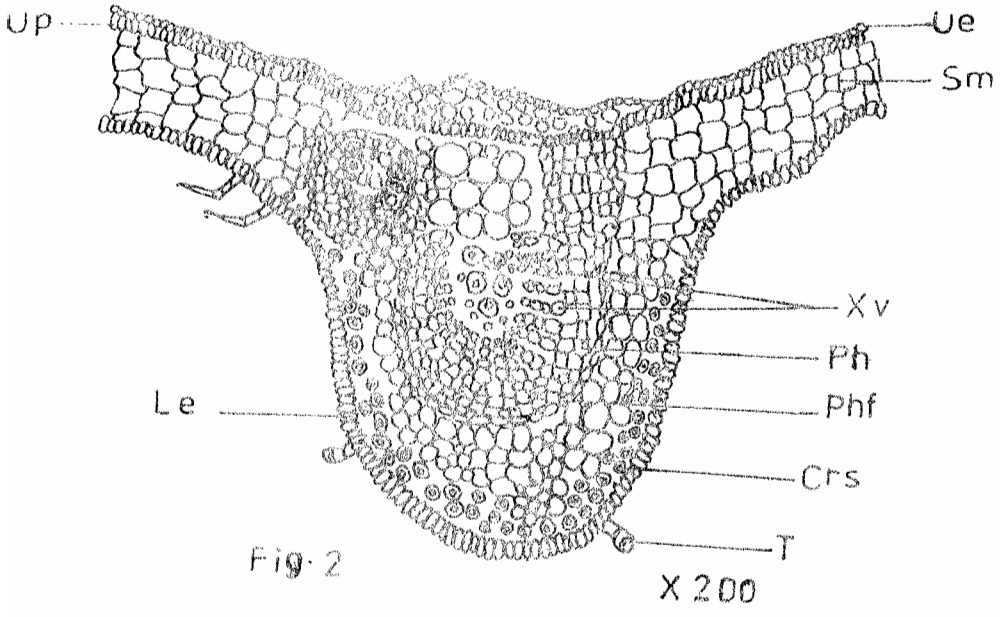
The leaves of both varieties are simple



Key

Ue	:	Upper epidermis
Up	:	Upper palisade
Sm	:	Spongy mesophyll
Le	:	Lower epidermis
Xv	:	Xylem vessels
Ph	:	Phloem
Phf	:	Phloem fibre
C	:	Collenchyma
T	:	Trichome

Fig. 1 Transverse Section of the leaf of dark variety of *H. crinita* x 200



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Xv	:	Xylem vessels
Ph	:	Phloem
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Le	:	Lower epidermis
Crs	:	Crystal sheath
T	:	Trichome

Fig. 2 Transverse Section of the leaf of white variety of *H. crinita* x 200

and opposite; shape elliptic, margin entire, venation reticulate, apex acuminate and base acute. The leaves are usually 5 - 10 cm long; 2 - 4cm wide with short stipules.

Organolepty

The powder of the dark variety is deep green, the taste is very bitter but the odour is mild. Though the white variety powder shares the same colour and odour as that of the dark

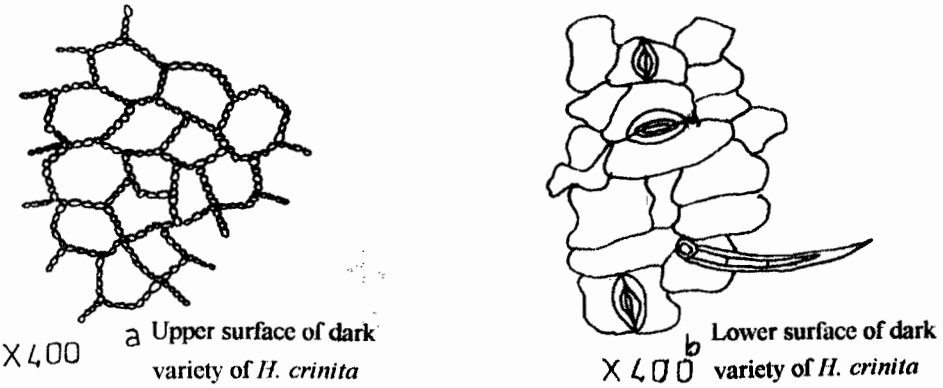


Fig. 3 Leaf surfaces of dark variety of *H. crinita* x 400

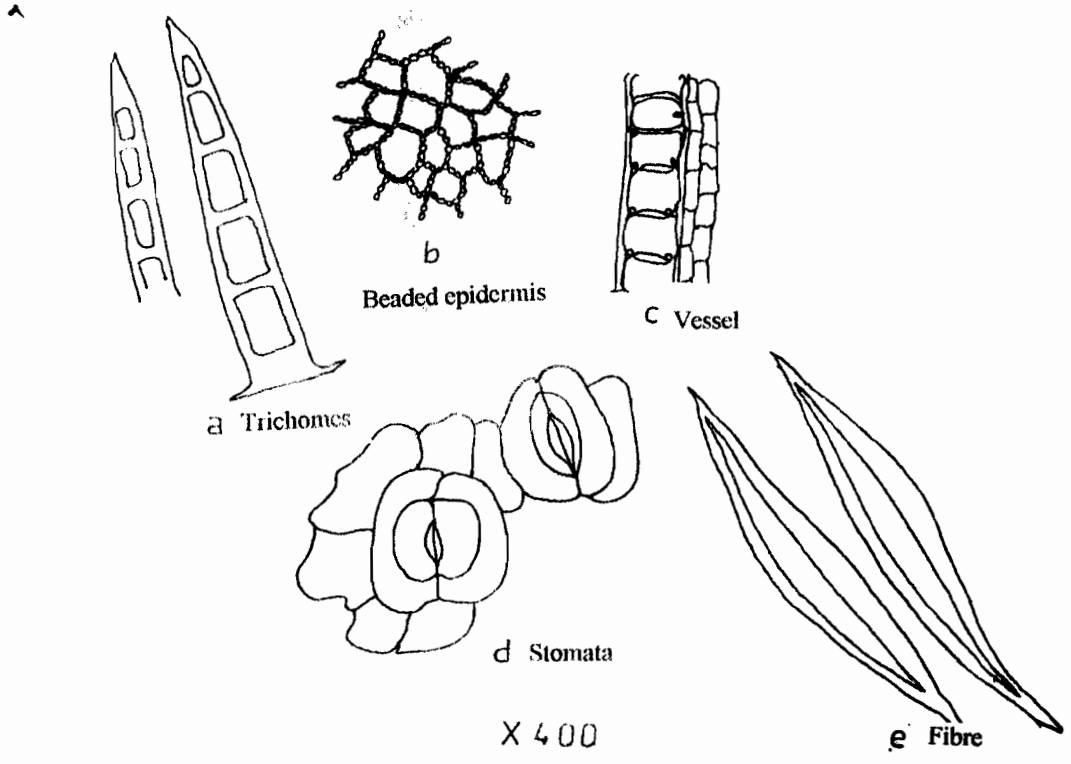


Fig. 4 Leaf powder of dark variety of *H. crinita* x 400

variety, the taste of the dark variety powder is slightly bitter.

Microscopy

Surface Morphology

The leaves of both varieties are dorsiventral. The epidermal cells are similarly polygonal, but variation is found in their anticlinal walls occurring as beaded walls in dark variety, and as slightly sinuous walls in the white varicty (Fig. 3 and 5). Stomata are present only on the lower surface of both varieties. However, the dark variety shows only paracytic stomatal type while the white

TABLE 1: PHYTOCHEMICAL SCREENING OF THE LEAF ETHANOL EXTRACTS OF *HEINSIA CRINITA* VARIETIES

METABOLITES	WHITE VARIETY	DARK VARIETY
Saponins	+++	+
Tannins	++	-
Anthraquinones	-	-
Cardiac glycosides	++	++
Terpenes	+++	+++
Flavonoids	++	+
Alkaloids	+	++

+++ : High concentration; ++: moderate concentration;

+ : trace; - : absent.

TABLE 2: ANTIMICROBIAL ACTIVITY OF THE LEAF ETHANOL EXTRACTS OF *HEINSIA CRINITA VARIETIES.*

MICROORGANISMS	MEAN ZONE OF INHIBITION IN MM[a] CONCENTRATION IN mg/ml								
	WHITE VARIETY			DARK VARIETY					
BACTERIA	40	80	100	40	80	100	GEN 2 g/ml	NYS 2 g/ml	DMSO
S. aureus	-	-	-	-	-	-	30	NA	-
B. subtilis	-	-	-	-	-	-	24	NA	-
P. aeruginosa	-	-	-	-	-	-	29	NA	-
E. coli	-	-	-	-	-	-	30	NA	-
FUNGUS	-	-	-	-	-	-			-
E. gametophyte	-	-	-	-	-	-	NA	20	-

GEN: Gentamicin; NYS: Nystatin; NA: NOT applicable;

a: Mean of four plates.

variety gives two different types: anisocytic and paracytic (Fig. 3 and 5).

Transverse section

The two varieties possess striking similar features: the epidermal cells are polygonal; glandular and non-glandular hairs are present. The glandular hairs are pedunculate with unicellular head, while the covering hairs are appressed, uniseriate and unicellular (Figs. 1 and 2).

Organolepty

The powder of the two varieties yields similar components such as fibers, trichomes, tracheids, epidermal cells and stomata. However, sclereids present in the white variety are lacking in the dark variety and stomatal types and anticlinal walls are similar to those of the lower and upper surfaces (Figs. 4 and 6).

Physical Constant Determinations

The palisade ratio of the white variety is 6.6, while that of the dark variety is 5.5. The white variety also yields stomatal number and stomatal index of 32.60 - 35.74 - 40.25 and 6.32 - 6.84 - 7.48, respectively, as opposed to dark variety stomatal number and stomatal index values of 29.02 - 30.96 - 33.59 and 3.61 - 4.14 - 4.80, respectively.

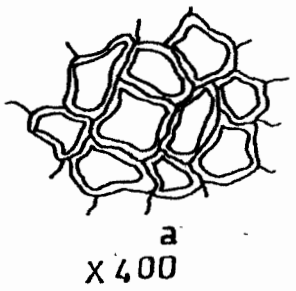
The total ash value of 25.6g per 100g is recorded for the dark variety leaf as against 24.1g per 100g of the white variety leaf. The sulphated ash and the acid insoluble ash values of the dark variety leaf are 41.20g and 2.01g per 100g respectively, while variety leaf shows 32.01 and 2.05g per 100g respectively.

Phytochemical Screening

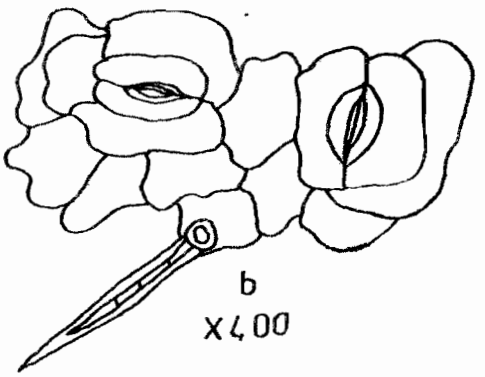
The dark and white varieties revealed the presence of saponins, tannins, cardiac glycosides, terpenes and alkaloids (Table 1). However, anthraquinones were absent in both varieties. Greater concentration of alkaloids was shown by the dark variety, while the white variety yielded more saponins concentration.

Antimicrobial Screening

The various concentrations of the ethanolic extract of the leaves of both varieties were microbially inactive (Table 2). Conversely, activities were educed by the partitioned fractions of the ethanolic extract of both varieties (Table 3). Only n-hexane and ethylacetate fractions showed activity among the fractions of the white variety, while only butanol and aqueous fractions showed activity among the dark variety fractions. Only butanol fraction of the dark variety elicited activity



Upper surface of white variety of *H. crinita*



Lower surface of White variety of *H. crinita.*

Fig. 5 Leaf surfaces of white variety of *H. crinita* x 400

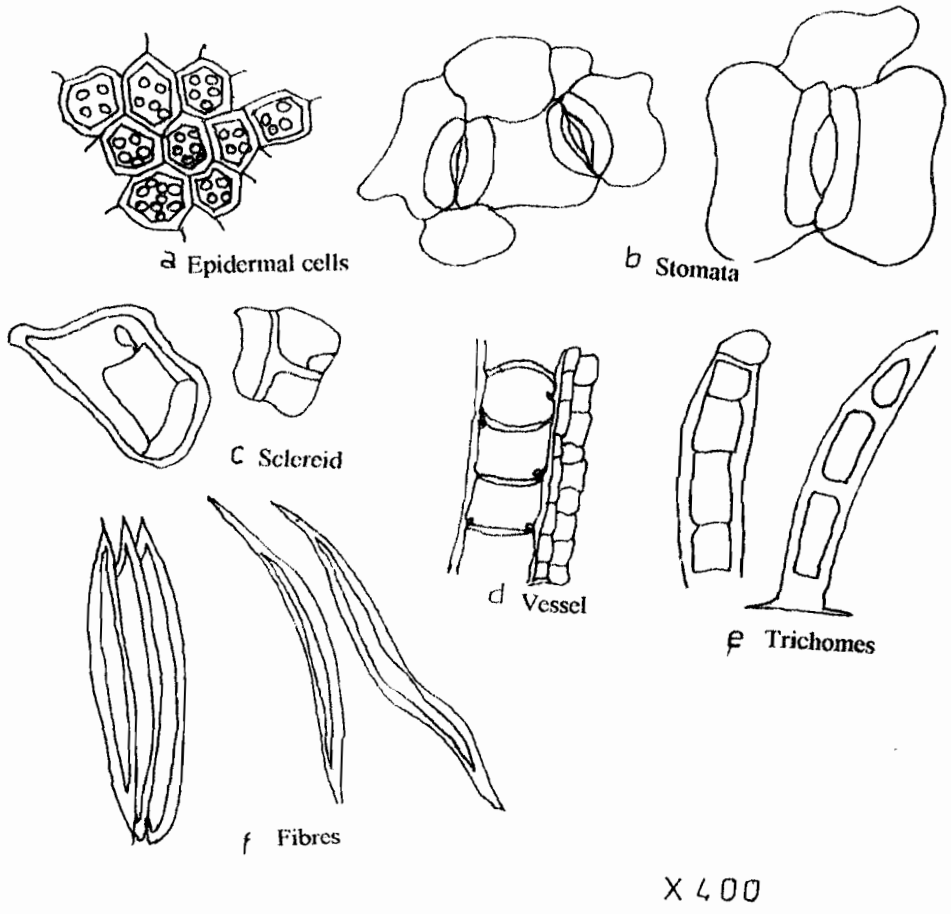


Fig. 6 Leaf powder of white variety of *H.crinita* x 400

against *Epidermophyton gametophyte, the only fungus used.* However, n-hexane fraction of the white variety gave the most antibacterial activity against *Staphylococcus aureus* (Table 3).

Although no apparent distinction can be drawn from the macroscopical features of the leaves of both varieties except on the grounds of taste (deep bitter taste for dark variety and slight bitter taste for white variety), gross microscopical discrepancies exist between the two.

Sufficient distinctions can be drawn among the two varieties on the pedestal of their variation in the epidermal wall which is beaded in dark variety and slightly wavy in white variety (Fig. 3 and 5); stomatal type (Figs. 3 - 6); larger number of vascular bundles in white variety (Figs. 1 and 2); and presence of sclereids in the white variety powder (Fig. 6).

The two varieties are further distinguished by their varied palisade ratio, stomatal number and stomatal index, since these values are supposed to be constant for the same species or same varieties.

The disparate values of the total ash, sulphated ash and acid insoluble ash for the leaves of both varieties lend further credence to their varietal differentiation. The amount of

TABLE 3: ANTIMICROBIAL ACTIVITY OF THE PARTITIONED FRACTIONS OF *HEINSIA CRINITA* WHITE AND DARK VARIETIES

ZONE OF INHIBITION IN MM[a], CONCENTRATION IN mg/ml											
		WHITE VARIETY					DARK VARIETY				
MICROORGANISMS	CONC	HEX	CHL	ETA	BUT	AQ	HEX	CHL	ETA	BUT	AQ
BACTERIA											
S. aureus	5	4	0	2	0	0	0	0	0	2	8
	10	6	0	4	0	0	0	0	0	4	0
	15	10	0	6	0	0	0	0	0	6	0
B. subtilis	5	0	0	0	0	0	0	0	0	0	0
	10	2	0	0	0	0	0	0	0	0	0
	15	4	0	0	0	0	0	0	0	0	0
P. aeruginosa	5	0	0	0	0	0	0	0	0	3	0
	10	3	0	0	0	0	0	0	0	5	0
	15	8	0	0	0	0	0	0	0	7	0
E. coli	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	3	0
	15	0	0	0	0	0	0	0	0	8	0
FUNGUS											
E. gametophyte											
	5	0	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0	4	0
	15	0	0	0	0	0	0	0	0	7	0

a: mean of four plates; HEX: n-hexane fraction;
CHL: chloroform fraction; ETA: ethylacetate fraction;
BUT: butanol fraction; AQ: aqueous fraction; Conc:concentration.

residual substances not volatilized is slightly higher in the dark variety (25.6g) than in the white variety (24.1g). The sulphated ash also shows higher value for dark variety (41.20g) than for white variety (32.01g), indicating availability of higher inorganic substances in the dark variety. However, the white variety contains a little more silica (2.05g) than does the dark variety (2.01g).

Although the two varieties gave similar classes of chemical constituents, higher concentration of alkaloids is produced by the

dark variety, while the white variety shows higher concentration of saponins (Table 1). This corroborates the work of Etuk *et al.*,(1998) which reported similar concentration levels for alkaloids in both varieties.

The inactivity of the ethanol extract (Table 2) against microorganisms tested necessitated the purification of the extract for same purpose The substances shrouding the activity might have been slightly eliminated through purification. It is hoped that further purification will elicit enhanced activity. The n-hexane fraction of the white variety showed the most significant antibacterial activity against *Staphylococcus aureus* at 15mg/ml, though it lacked anti-fungal activity. The broadest spectrum of activity was evoked by butanol fraction of the dark variety, since it also showed activity against the only fungus used. However, the butanol fraction of the dark variety did not give any activity against *Bacillus subtilis* (Table 3). Thus, dark variety showed both the antibacterial and antifungal activities, while the white variety showed only the antibacterial activity. The locals of Akwa Ibom State should be taught simple purification processes to afford effective use of the two varieties for the treatment of diseases of microbial origin. Ekpa and Ebana, (1991) reported antimicrobial activities of unspecified variety of *H crinita.* The unspecified varietv may not include either of the two varieties in this study, since they did not show activity at extract level.

CONCLUSION

The dark and white varieties of *Heinsia crinita* have been distinguished on the bases of microscopy, pharmacognostic, evaluation, phytochemical screening and antimicrobial activities. These bases provide some justification for the classification of the plant into the two varieties: dark and white.

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