

THE ECOLOGICAL DYNAMICS AND TRAJECTORIES OF BIOACTIVE COMPOUNDS IN PLANTS OF THE GENUS - *ANTHOCLIESTA* FOUND IN PARTS OF NIGER DELTA ECOLOGICAL ZONE, NIGERIA

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ABSTRACT

Several studies have revealed the botany of *Anthocleista* species found in parts of Niger Delta, which include the species ecodiversity, various lines of taxonomic studies, ethnomedicine, ethnobotany, etc. However, records have not shown the dynamic trajectories of bioactive phytochemicals in relation to ecological niche adaptation and geographical location among the species. This informed the present research with the aim of assessing in qualitative terms the bioactive presence and dynamics in various parts of the species under their ecological preference. Determination of secondary metabolites in leaves, stem and root of four species of *Anthocleista* from four States in the Niger Delta ecological region of Nigeria was carried out using standard methods. Result revealed seven bioactive compounds with anthraquinone totally absent from all the species in the four locations. The seven bioactive compounds were apparently more in the leaves than other parts of the plants. Among the four locations alkaloid, triterpene, glycoside, carbohydrate, flavonoid and tannin were high in foliar parts with saponin in moderate level in the stem part of the plants in Akwa-Ibom State. Similarly a high bioactive level was recorded in foliar parts in Cross River and Rivers States despite the moderate level of flavonoid and tannin in Cross River and alkaloid, carbohydrate and flavonoid in Rivers State. The Bayelsa State study location recorded moderate level in all bioactive compounds in various parts of the plants with exception of high level saponin in leaf and stem of all the plants. This study reveals that distribution of natural products (secondary metabolites) varies greatly among plant species, individual plants, organs and tissues, during development and maturation, seasonal fluctuations and variation in geographical location.

KEYWORDS: Bioactive compound, trajectories, *Anthocleista*, species ecodiversity, Niger Delta.

INTRODUCTION

Bioactive compounds are often regarded as secondary metabolites of phytochemical composition. They have a fundamental role in the maintenance of life processes in plants and industries by products such as pharmaceuticals, food additives, flavours and important biochemicals; they are important bioindicators of plant species response to ecological environmental factors. Plant secondary metabolites are a group of naturally occurring compound classes biosynthesized by different biochemical pathways whose plant content and regulation is strongly susceptible to environmental influences of abiotic and biotic factors, which might be specifically induced by means of various mechanisms. Thus creating variation in the accumulation or biogenesis (Daniel, *et al.*, 2012). Such factors among others include moisture, temperature, light, soil, humidity, water, pH, drought, salt, UV β , etc. Plants species have developed a variety of strategies and mechanisms to react to any changes in their environment. Many hypotheses assume that the

synthesis of secondary metabolites is a plant response to environmental factors and part of an adaptative strategy leading to tolerance of abiotic stresses. However, fluctuations of its content resulting from temporal changes in environmental circumstances might be expected due to differences in geographical location (Anna *et al.*, 2011).

Several studies have recorded evidence that secondary metabolites are means by which plants species communicate or respond to external stimuli (Bouwmeester *et al.*, 2007; Rasmann and Turlings, 2008; Frost *et al.*, 2008; Maffei, 2010). Such bioactive compounds accumulate in plant tissues through biochemistry mechanisms and other mechanisms of regulation such as variation in agro-climatic condition and geographical location (Dinchev, *et al.*, 2008; Ma *et al.*, 2009).

The production of bioactive compounds is often low (less than 1% dry weight) and depends greatly on the physiology and developmental stages of the plant (Rao and Ravishankar, 2002). Some of the plant derived natural products are flavonoid, alkaloid,

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glycosides, tannins etc, whose concentrations are strongly dependent on the prevalence of growing conditions and its impact on the accumulation of related natural products. The accumulation of osmolytes is a common phenomenon in plants exposed to abiotic stress (Daniel, *et al.*, 2012). Accumulation of such metabolites often occurs in plants subjected to stress including various biotic and abiotic signal molecules which has been shown to induce a broad range of phytochemicals which absorb harmful radiations and protect plants against damaging reactive oxygen species (Bieza and Lois, 2003; Burritt and Mackenzie, 2003; Gitz *et al.*, 2004). Similarly, high temperature has been shown to induce phytochemical accumulation in several plant species as a defense strategy (Lefsrud *et al.*, 2005).

Similar studies have been recorded on the production and dynamic distribution of bioactive compound in different species and with such dynamism not only regulated by genetic background, growth and development process but induced also by geographical location, ecological and environmental factors (Zhang *et al.*, 2000; Salmore and Hunter, 2001; He *et al.*, 2002; Dong *et al.*, 2005 and Wang, *et al.*, 2007). Phytochemical investigations have shown that different therapeutic practices and applications may be explained by the large variations in bioactive composition and content of plant depending on the geographical regions (Joshi and Uniyal 2008). Therefore, different preparations from plants harvested from separate locations in the world may yield different results. Plant secondary metabolism has been investigated for decades as a dynamic phenomenon with real and apparent result established (Schultz, 2002; Daniel *et al.*, 2012). The secondary metabolism of plants, and the expressed metabolite levels, may change considerably due to the influence of several biotic and abiotic stress signals. Generally, knowing these factors and the potential of plant metabolism variations in a specific area may actually inform the bases of this research.

Several research works has been recorded on the diverse nature of Phytochemical attributes of *Anthocleista* spp. in a tropical rainforest environment (Jensen and Schripsema, 2002; Rank *et al.*, 2004; de Ruijter, 2007). However, no report has revealed the dynamic trajectories of these bioactive compounds in relation to geographical location and ecological niche adaptation of species found in parts of Niger Delta, Nigeria and that was the aim of this research.

MATERIALS AND METHODS

Description of the study area (overview).

The Niger Delta region of Nigeria is situated in the humid-wet tropics about 5°N latitude. The Niger Delta area is the Southern segment and coastline parts of Nigeria approximately 853 kilometers facing the Atlantic ocean and lying between latitude 4° 10' to 6° 20'N and longitude 2° 45' to 8° 35'E. The terrain is endowed with myriads of Islands segmented by lagoons and channels, which empty into the Bight of Biafra in the East Atlantic Ocean. Similarly, it is a center of endemism for Africa and is the most extensive and lowland forest / aquatic ecosystem in West Africa. The Niger Delta in its ecological characteristics and situate may not be over emphasized, hence several studies have been carried out based on its climatic condition (Kuruk, 2004), agro-ecological zones and type of forest ecosystem (Teme, 2001; Ogbe, 2003; Michael, 2013), geographical location and dimension (Afolabi, 1998; Alagoa, 1999) and altitudinal range (Dubli-Green, *et al.*, 1999). The core Niger Delta area includes Rivers, Bayelsa, and Delta states. Others included are Akwa Ibom, Cross River, Edo, Abia, Imo and Ondo States. However, the areas covered in this study include parts of Rivers, Bayelsa, Akwa-Ibom and Cross River States (Fig.1).

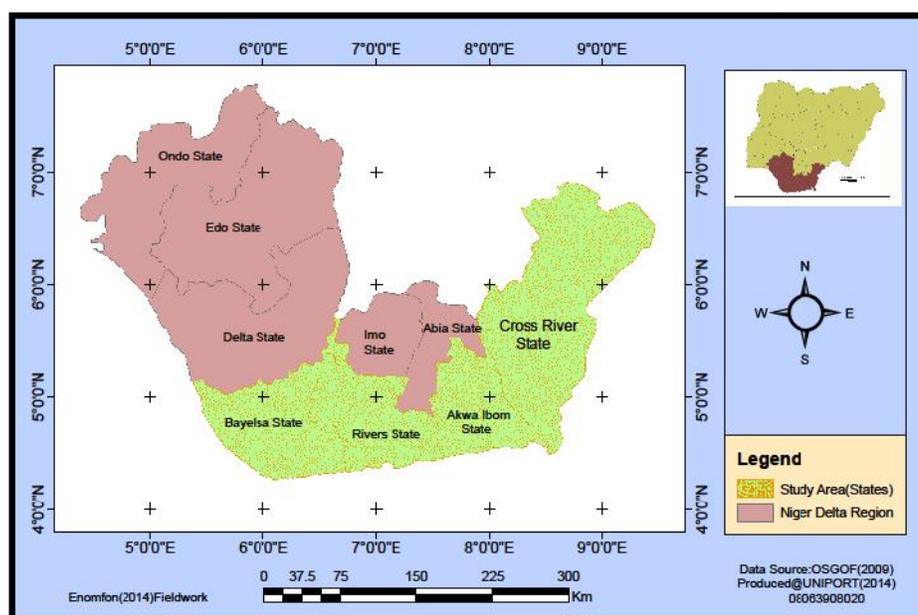


Fig. 1: Niger Delta study areas (States)

SPECIES ASSESSMENT

The spatial distribution of plant and location, their degree of habitat niche adaptation and the spatial distribution of sensitive habitats (Edwin-Wosu and Omara-Achong, 2010; Edwin-Wosu *et al.*, 2015) were some of the major aspects considered during field sampling. The Species of the genus -*Anthocleista* used for this Study were observed and collected from parts of the ecozone mentioned above in Figure 1. Despite the various hot spot in Niger Delta the areas under study were chosen for the reason of accessibility, availability and prevalence of the species. A systematic random sampling based on simple ecological procedure was carried out with various sampling points of different geographical precision coordinates noted in parts of the States under consideration. A hand-held geographic positioning system (GPS- *etrex* 12 channel model) was used to record the location coordinates of each sample point for the distribution status of the *Anthocleista* species in the area under observation. The sampled areas covered include:

- i. Rivers State: Comprising areas such as Alakahia UPTH and UNIPORT botanic garden axis in Obio /Akpor Local Government Area, Abalama community in Asari-toru Local Government Area. (Figs. 2a – e).
- ii. Bayelsa State: Comprising the Okutukutu axis of the forest in Yenagoa Local Government Area (Fig.3a – b).
- iii. Akwa Ibom State comprising parts of Ikot Offiong and Oku Iboku towns in Itu Local Government Area (Fig 4a-b.).
- iv. Cross River State, comprising parts of the adjoining forest around the New Calabar Airport proposed site in Calabar South Local Government Area and AWI forest in Akamkpa Local Government Area (Fig.5a-c).

Materials collected were identified and authenticated using relevant Flora. Confirmation of identification was done by matching the specimens with authenticated specimens available at the University of Port Harcourt Herbarium by the Curator. Voucher samples of *Anthocleista nobilis*; *A. liebrechtsiana*; *A. vogelii* and *A. djalonesis* were deposited at the herbarium.

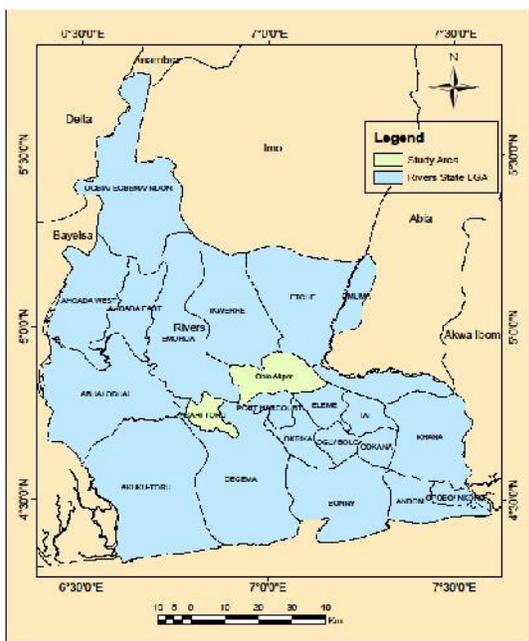


Fig.2a: Asari-Toru and Obio / Akpor LGA Study Area in Rivers State, Nigeria

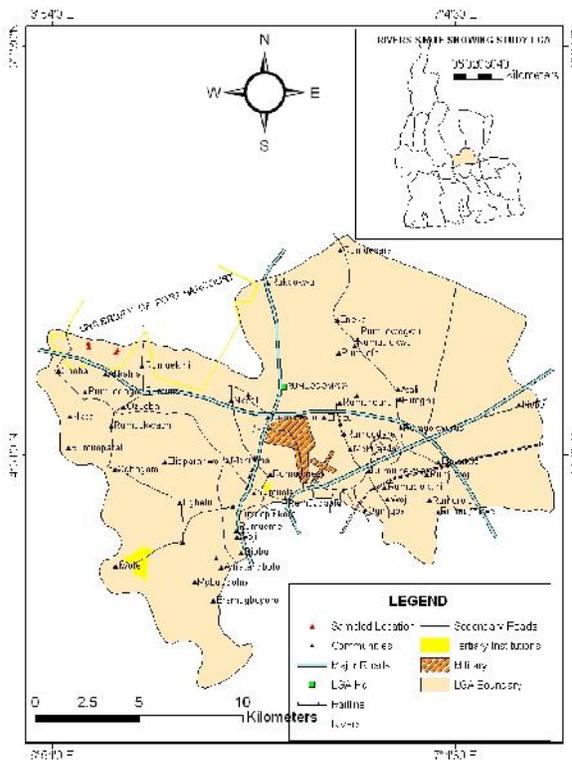


Fig.2b : Obio / Akpor Local Government Area study location

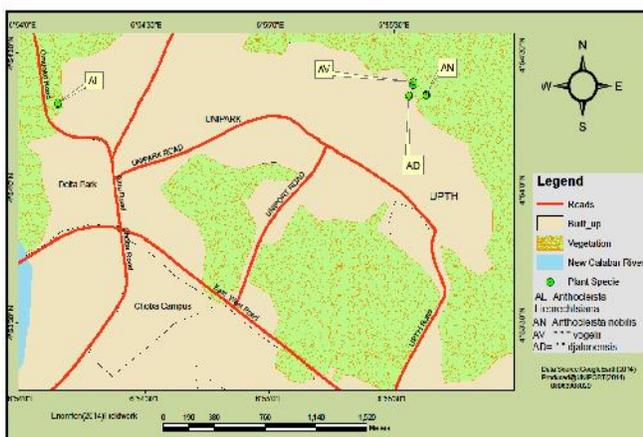


Fig.2c: University of Port Harcourt species sampled site

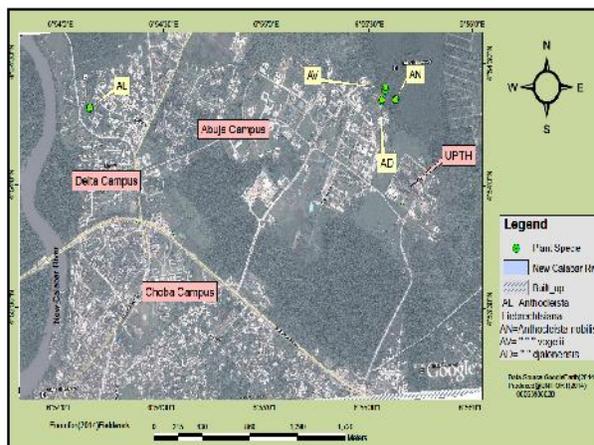


Fig. 2d: Satellite imagery of UNIPORT species sampled site

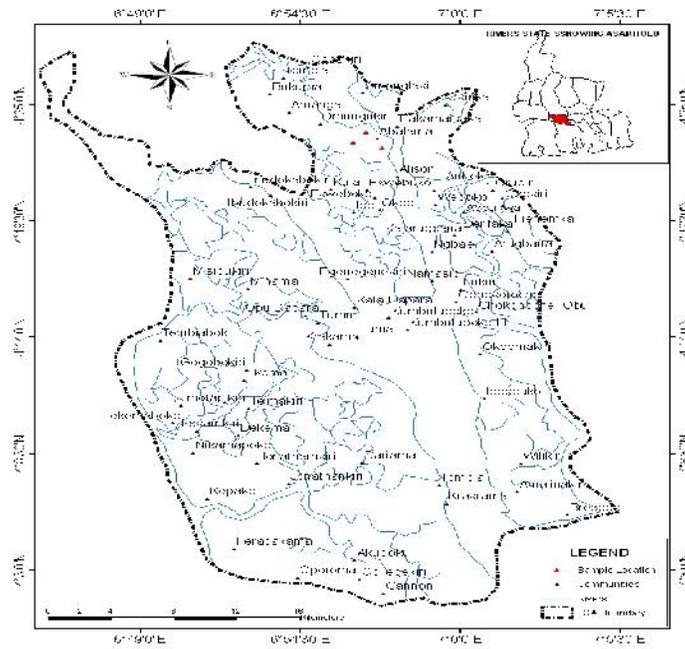


Fig.2e: Asaritoru LGA study location and sampled site

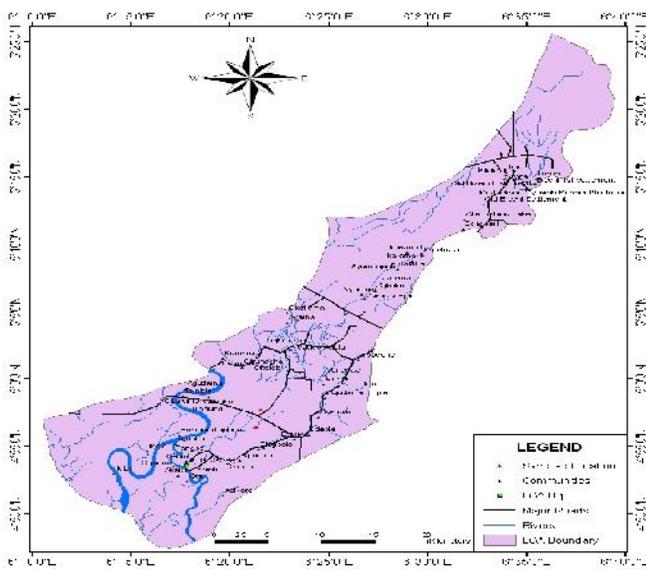


Fig. 3a: Bayelsa showing study location Yenagoa

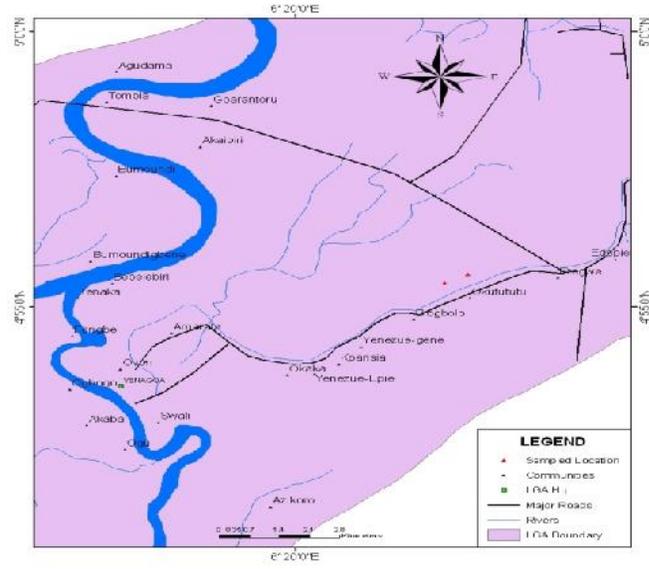


Fig. 3b: Yenagoa showing sample location

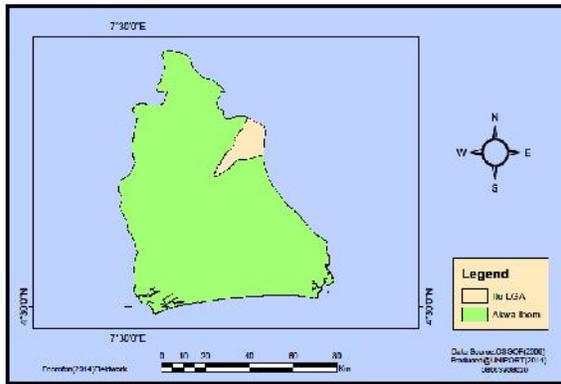


Fig.4a: Akwa-Ibom State showing Itu LGA (study location))

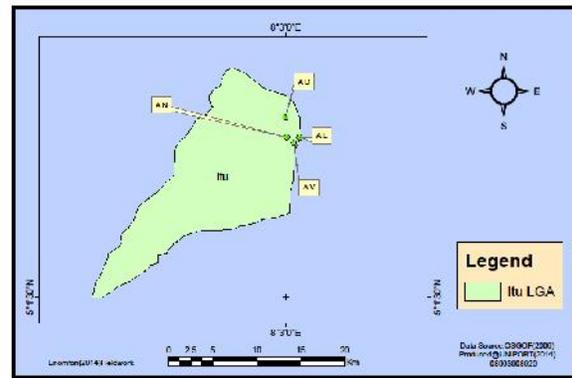


Fig. 4b: Itu LGA showing sampled species location

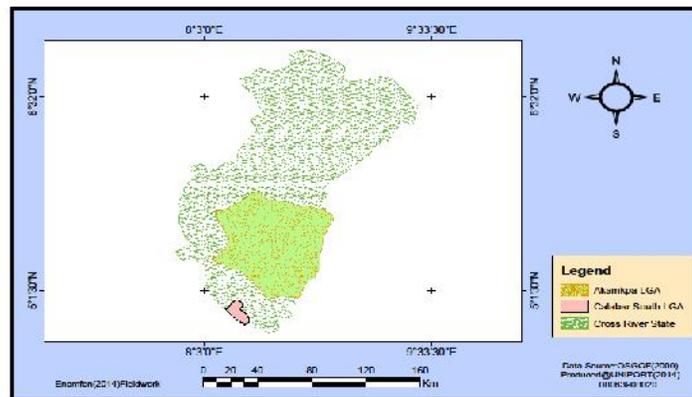


Fig. 5a: Cross River State showing Akamkpa & Calabar South LGA's

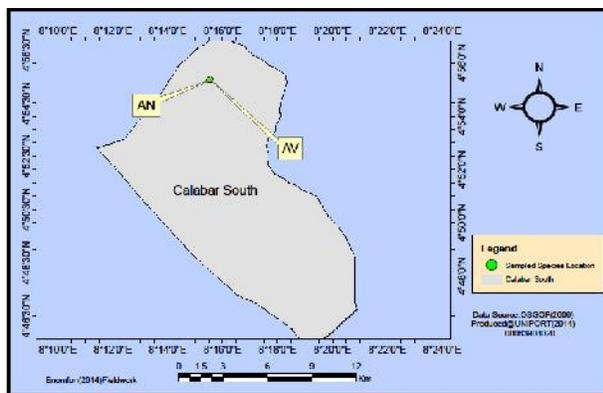


Fig.5b : Calabar South LGA sampled species location

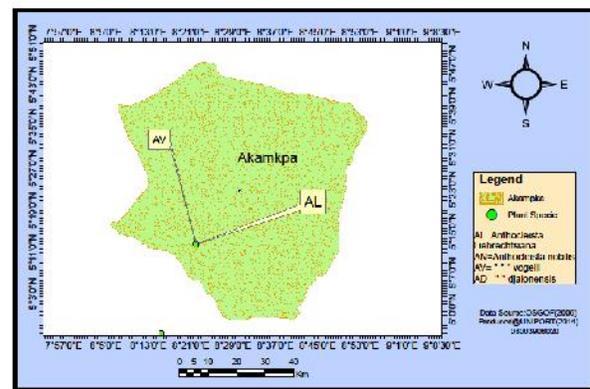


Fig.5c : Akamkpa LGA sampled species location

Phytochemical determination

The four tree (*Anthocliesta nobilis*; *A. liebrechtsiana*; *A. vogelii* and *A. djalonesis*) species commonly found in different parts of Niger Delta were randomly selected from the study area and tagged. Leaves stem barks and roots were obtained from the tagged trees and used for phytochemical screening.

The samples collected were stored and transported to the laboratory in black polyethylene bags to prevent contamination. The samples were rinsed with deionized water in the laboratory and then used for preparation of aqueous crude extracts. The aqueous extract was prepared by homogenizing with distilled water (50ml / 2g fresh weight) on a water bath for 5 mins in a homogenizer. The homogenate was left to stand for 24h

at room temperature. The aqueous mixture was then filtered using Whatman No.1 filter paper while hot. The filtrate was used for the bioactive assays. Qualitative analysis of the bioactive components in plant extracts were carried out using classical methods and in their diverse presence indicated as either slightly present (+), moderately present (++) or highly present (+++).

The saponin test was carried out using the emulsion method of Olanipekun *et al.* (2013) where 3- 4 drops of olive oil was added to the filtrate and shaken vigorously for five minutes, then allowed to stand undisturbed recorded a soluble 1cm thick layer of emulsion indicating the presence of Saponins.

The flavonoid test was carried out by adopting the lead acetate method in which 2mls of 10% Pb (CH₃COO)₂ added to the filtrate recorded a yellow precipitate indicating the presence of Flavonoids (Trease and Evans, 1983).

The terpenoid / steroid test, by Trease and Evans (1983) involved the Salkowski's method in which 50mls of concentrated H₂SO₄ was carefully added to the filtrate and observed. A reddish brown colouration at the interface indicates the presence of steroids / Triterpenoids.

For carbohydrate test with the Molisch method adopted 1ml of Molisch's reagent added to the filtrate followed by 50mls of conc. H₂SO₄ carefully added. A deep violet ring was observed at the interphase of the 2 liquids, which indicates the presence of Carbohydrates (Trease and Evans, 1989).

The Dragendoofs', Meyers' and Hagers' methods of Sofowora (1993) were adopted for Alkaloid test, in which 2g of the plant sample was pulverised and boiled with 50mls of 10% H₂SO₄ for 5mins on a water bath, then filtered while hot. 3- 4 drops of the reagents were added to the filtrate (1ml) and observed. In Dregendroff's test (solution of potassium Bismuth iodide) formation of red precipitate indicate, the presence of alkaloid.

For Anthraquinone determination, Borntreger's test for free Hydroxyl Anthraquinone was adopted according to the method of Trease and Evans, (1983).

2g of the plant sample was pulverised and macerated with 50mls of Chloroform (CHCl₃) then filtered. 50mls of 10% NH₃ was added to the filtrate and shaken vigorously, then allowed to stand for a few minutes. A decolouration from pink ammoniacal layer to colourless indicates the absence of free hydroxyl Anthraquinone (Sofowora, 1993).

Ferric chloride test was adopted according to the method of Sofowora, (1993) for Tannin determination in which 2g of the plant sample was pulverised and boiled with 50mls of distilled water for about 5mins on a water bath, then filtered while hot. 1ml of 5% FeCl₃ was added to the filtrate and observed. The presence of a blue-black, green or blue green colouration indicates the presence of tannins.

Cardiac Glycosides determination involved 2g of the plant sample been pulverised and macerated in 50mls of Chloroform and filtered. Using the Kedde's Test for Lactone Ring, 1ml of 2% 3, 5-dinitrobenzoic acid was added to the filtrate followed by 2mls of 10% NaOH. An immediate purple or violet colouration indicates the presence of Cardiac glycosides (Sofowora, 1993).

RESULT

The result in Table 1 shows the coordinates of sampled species of *Anthocleista* in their diverse and narrow niche geographical location and Table 2 as voucher accessions deposited at the University of Port Harcourt reference herbarium. The dynamic of phytochemical or bioactive composition within and among species in their various niche adaptations under the prevailing variation of local environmental condition has been recorded in Tables 3-6.

A total of eight bioactive compounds were tested. However anthraquinone was not recorded in any of the species in the various niche adaptations. At the Akwa Ibom State ecological zone (Table 3), the dynamics of alkaloid within and among species showed a moderate foliar presence than the stem and root which has slight presence; however *A. vogelii* had moderate alkaloid in the three parts of the plant.

Table 1: GPS coordinates of species sampled location

Study location	Species	Georeference point	
		Latitude (N)	Longitude (E)
Akwa Ibom State			
Itu Local Government area	<i>A. liebrechtsiana</i>	5 ^o 10' 79"	8 ^o 03' 51"
	<i>A. djalonesis</i>	5 ^o 12' 34"	8 ^o 21' 59"
	<i>A. nobilis</i>	5 ^o 10' 79.5"	8 ^o 03' 51.1"
	<i>A. vogelii</i>	5 ^o 10' 59"	8 ^o 2' 91"
Bayelsa State			
Yenagoa Local Government Area	<i>A. nobilis</i>	4 ^o 56' 29"	6 ^o 21' 19"
	<i>A. vogelii</i>	4 ^o 56' 29"	6 ^o 21' 19"
	<i>A. liebrechtsiana</i>	4 ^o 57' 43"	6 ^o 21' 35"
		4 ^o 53' 52.17"	6 ^o 54' 56.79"
Cross River State			
Calabar South / Akamkpa	<i>A. liebrechtsiana</i>	5 ^o 12' 30"	8 ^o 21' 60"
	<i>A. djalonesis</i>	5 ^o 12' 30"	8 ^o 21' 65"
	<i>A. nobilis</i>	4 ^o 55' 59"	8 ^o 19' 25"
	<i>A. vogelii</i>	4 ^o 55' 59"	8 ^o 19' 21"
River State			
Asari - Toru	<i>A. vogelii</i>	4 ^o 46' 29"	6 ^o 49' 43"
	<i>A. nobilis</i>	4 ^o 46' 50"	6 ^o 50' 28"
	<i>A. djalonesis</i>	4 ^o 46' 28"	6 ^o 49' 43"
	<i>A. nobilis</i>	4 ^o 53' 39"	6 ^o 55' 40"
Obio / Akpor		4 ^o 53' 81.1"	6 ^o 54' 96.7"
	<i>A. vogelii</i>	4 ^o 53' 39"	6 ^o 55' 40"
		4 ^o 53' 84"	6 ^o 54' 95"
	<i>A. djalonesis</i>	4 ^o 53' 41.97"	6 ^o 55' 41.73"
		4 ^o 53' 81"	6 ^o 54' 96"
	<i>A. liebrechtsiana</i>	4 ^o 53' 00"	6 ^o 54' 90"
	<i>A. liebrechtsiana</i>	4 ^o 53' 52.17"	6 ^o 54' 56.79"

Table 2: Herbarium Voucher Samples

S/NO	SPECIES	ACCESSION NO	UPH NO
1	<i>Anthocleista djalonesis</i>	006	F- 611
2	<i>Anthocleista nobilis</i>	007	F- 612
3	<i>Anthocleista vogelii</i>	008	F-613
4	<i>Anthocleista liebrechtsiana</i>	009	F- 614

Table 3: The phytochemical composition of *Anthocleista* species in parts of Akwa Ibom State

Species / part of sample		<i>Anthocleista nobilis</i>			<i>Anthocleista liebrechtsiana</i>			<i>Anthocleista vogelii</i>			<i>Anthocleista djalonesis</i>		
		Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar
S/n	Metabolites / test performed												
1	ALKALOID												
	Dragendorff	+	+	++	+	+	++	++	++	++	+	+	++
2	ANTHRAQUINONE												
	Free hydroxyl	-	-	-	-	-	-	-	-	-	-	-	-
3	TRITERPENE/STERIOD												
	Salkowski	+	+	++	+	+	+	+	+	+++	+	+	++
4	GLYCOSIDE												
	Kedde	+	+	+	+	+	+	+	+	++	+	+	+++
5	CARBOHYDRATE												
	Molisch	+	+	++	+	+	+	+	+	+++	+	+	+++
6	FLAVONNOID												
	Lead acetate	+	+	+	+	+	++	++	+	+	+	+	+
7	TANNIN												
	FECL ₃	+	+	+++	+	+	+++	++	+	+++	+	+	++
8	SAPONIN												
	Emulsion	+	+	+	+	+	+	++	+	+	+	+	+

Key: + = slightly present; ++ = moderately present; +++ = highly present; -- = absent

Table 4: The phytochemical composition of *Anthocleista* species in parts of Bayelsa State

Species / part of sample		<i>Anthocleista nobilis</i>			<i>Anthocleista liebrechtsiana</i>			<i>Anthocleista vogelii</i>			<i>Anthocleista djalonesis</i>		
		Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar
S/n	Metabolites / test performed												
1	ALKALOID												
	Dragendorff	+++	++	+	+	+	++	+	++	++	++	+++	++
2	ANTHRAQUINONE												
	Free hydroxyl	-	-	-	-	-	-	-	-	-	-	-	-
3	TRITERPENE/STERIOD												
	Salkowski	+	+	+	+	++	++	+	++	++	++	+	++
4	GLYCOSIDE												
	Kedde	+++	+	++	++	++	+	++	+	+	++	+	+
5	CARBOHYDRATE												
	Molisch	+	+	++	++	+	+	++	+	+	++	++	++
6	FLAVONNOID												
	Lead acetate	+	+	++	++	+	++	++	+	+	++	+	++
7	TANNIN												
	FECL ₃	+	+	++	+	+	++	+	+	+	+	+	++
8	SAPONIN												
	Emulsion	+	+	++	++	+	+	++	+	+	+	+	++

Key: + = slightly present; ++ = moderately present; +++ = highly present; -- = absent

Table 5: The phytochemical composition of *Anthocleista* species in parts of Cross River State

Species / part of sample		<i>Anthocleista nobilis</i>			<i>Anthocleista liebrechtsiana</i>			<i>Anthocleista vogelii</i>			<i>Anthocleista djalonesis</i>		
		Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar
S/n	Metabolites / test performed												
1	ALKALOID												
	Dragendorff	+	+	++	+	+	++	+	+	+++	+	+	+++
2	ANTHRAQUINONE												
	Free hydroxyl	-	-	-	-	-	-	-	-	-	-	-	-
3	TRITERPENE/STERIOD												
	Salkowski	+	+	++	+	+	++	+	+	++	+	+	+++
4	GLYCOSIDE												
	Kedde	+	+	+	+	+	+	+	+	++	+	+	+++
5	CARBOHYDRATE												
	Molisch	+	+	++	+	+	+	+	+	+++	+	+	+++
6	FLAVONNOID												
	Lead acetate	+	+	++	+	+	++	+	+	+	+	+	++
7	TANNIN												
	FECL ₃	+	+	++	+	+	++	+	+	++	+	+	++
8	SAPONIN												
	Emulsion	+	+	+++	+	+	+	+	+	+	+	+	++

Key: + = slightly present; ++ = moderately present; +++ = highly present; -- = absent

Table 6: The phytochemical composition of *Anthocleista* species in parts of River State

Species / part of sample		<i>Anthocleista nobilis</i>			<i>Anthocleista liebrechtsiana</i>			<i>Anthocleista vogelii</i>			<i>Anthocleista djalonesis</i>		
		Stem	Root	foliar	Stem	Root	foliar	Stem	Root	foliar	Stem	Root	Foliar
S/n	Metabolites / test performed												
1	ALKALOID												
	Dragendorff	+	+	++	+	+	++	+	+	+++	+	+	+++
2	ANTHRAQUINONE												
	Free hydroxyl	-	-	-	-	-	-	-	-	-	-	-	-
3	TRITERPENE/STERIOD												
	Salkowski	+	+	++	+	+	++	+	+	++	+	+	+++
4	GLYCOSIDE												
	Kedde	+	+	+	+	+	+	+	+	++	+	+	+++
5	CARBOHYDRATE												
	Molisch	+	+	++	+	+	+	+	+	+++	+	+	+++
6	FLAVONNOID												
	Lead acetate	+	+	++	+	+	++	+	+	+	+	+	++
7	TANNIN												
	FECL ₃	+	+	++	+	+	++	+	+	++	+	+	++
8	SAPONIN												
	Emulsion	+	+	+++	+	+	+	+	+	+	+	+	++

Key: + = slightly present; ++ = moderately present; +++ = highly present; -- = absent

Triterpenoid / steroid recorded some level of variation in which the foliar component of the *A. vogelii* had the highest, with *A. nobilis* and *A. djalonesis* respectively recording moderate foliar presence while other part had slight presence. In the same location the presence of glycoside was highly recorded in foliar part of the *A. djalonesis*, followed by a moderate presence in *A. vogelii* foliar and a slight presence in other parts among the species.

The presence of carbohydrate also varied with the foliar part of *A. vogelii* and *A. djalonesis* recording a high presence, while *A. nobilis* had a moderate presence of the compound. *Anthocleista liebrechtsiana* had moderate presence of flavonoid in foliar and stem parts respectively with a slight presence in other parts among the species, while the presence of tannin was highly recorded in foliar part of *A. nobilis*, *A. liebrechtsiana* and *A. vogelii* with *A. djalonesis* moderately recorded. However, *A. vogelii* had moderate

presence at stem. The presence of saponin was slightly recorded in all parts among the species except the stem part of *A. vogelii* with moderate presence.

In a similar ecological zone with various local conditions in Bayelsa study area, the bioactive component also had difference in their dynamics in various parts of the species (Table 4). The alkaloid compounds were highly present in the stem and root part of *Anthocleista nobilis* and *Anthocleista djalonesis* respectively, while the triterpenoid / steroid were moderately present in stem and leaf part of *A. djalonesis* and root and leaf part of *A. liebrechtsiana* and *A. vogelii*. The dynamics of glycoside was also recorded in different part of the species with the stem of *Anthocleista nobilis* indicating the highest presence of the compound while the foliar was noted for moderate presence. In a similar dynamic *A. liebrechtsiana* stem and root had moderate amount while the stems of *A. vogelii* and *A. djalonesis* also had moderate amount glycoside.

A moderate presence of carbohydrate compound was also observed among the species with all the parts of *A. djalonesis* noted for such compound which was only present in the stem and root of *A. vogelii* and foliar and stem of *A. nobilis* and *A. liebrechtsiana* respectively. The presence of flavonoid was noted at a moderate level in the stems of *A. liebrechtsiana* and *A. vogelii* and foliar of *A. djalonesis* and *A. nobilis* respectively. Tannin was moderately present in the foliar of *A. nobilis*, *A. liebrechtsiana* and *A. djalonesis*, while saponin was highly present in the foliar of *A. nobilis* and *A. djalonesis* and stems of *A. liebrechtsiana* and *A. vogelii* respectively.

In parts of Cross River sampled area some level of phytochemical dynamics (Table 5) were observed with a high presence of alkaloid in the foliar of *A. vogelii* and *A. djalonesis* respectively but *A. nobilis* and *A. liebrechtsiana* had moderate level. Triterpenoid / steroid and glycoside recorded high presence in the foliar of *A. djalonesis* while other three species were moderate in their foliar content of triterpenoid / steroid and also moderate occurrence in the foliar content of glycoside in *A. vogelii*.

The carbohydrate was high in the foliar part of *A. vogelii* and *A. djalonesis* and moderate in *A. nobilis* foliar content. The dynamics of flavonoid recorded moderate level in the foliar part of *A. nobilis*, *A. liebrechtsiana* and *A. djalonesis* respectively, while the tannin content was moderate in foliar part of all the species but saponin recorded a high presence in the foliar part of *A. nobilis*.

In Rivers State there was also some level of variation in the phytochemical dynamic (Table 6) of alkaloid among the species, despite their moderate presence in about 66.67% of the parts of species except in the stem and root of *A. liebrechtsiana* and *A. djalonesis* respectively. Triterpenoid / steroid was highly present in the foliar part of *A. nobilis*, *A. vogelii* and *A. djalonesis* while glycoside and carbohydrate were slightly present in all part among the species except the root of *A. nobilis* and *A. djalonesis* with moderate content of carbohydrate. Flavonoids were moderately present in the foliar and stem parts of *A. nobilis* and *A. vogelii* respectively. Tannin content was high in foliar part of *A. nobilis*, *A. liebrechtsiana* and *A. vogelii* while saponin was high in foliar part of *A. nobilis*, *A. vogelii*

and *A. djalonesis* which recorded moderate content in the stems and roots.

DISCUSSION

Among the studied localities of species under the local environmental conditions, the dynamics of the bioactive compound varied. The alkaloid content in the foliar part of *A. vogelii* in Akwa Ibom study area was moderate, but high in the stem and root of *A. nobilis* and *A. djalonesis* in Bayelsa State, and also high in foliar part of *A. vogelii* and *A. djalonesis* in Cross River. Plants belonging to the same species but occurring in different geographical zones may significantly differ in qualitative and quantitative content of their particular secondary metabolites (Anna *et al.*, 2011). Moderate content in all parts of *A. nobilis* and *A. vogelii* and foliar part of *A. liebrechtsiana* and *A. djalonesis* were recorded in Rivers State. Similar studies have recorded the production and dynamic distribution of alkaloid in different species and genera, organs and tissues at different period of growth, and with such dynamism not only regulated by genetic background, growth and development process but induced also by geographical location, ecological and environmental factors (Zhang *et al.*, 2000; Salmore and Hunter, 2001; He *et al.*, 2002; Dong *et al.*, 2005 and Wang, *et al.*, 2007).

Triterpene had high foliar content in *A. vogelii* in River State, *A. djalonesis* in Cross River and moderate content in the root and leaves of *A. liebrechtsiana* and *A. vogelii* respectively, stem / foliar of *A. djalonesis* in Bayelsa and leaf of *A. djalonesis* in Rivers. The glycoside content was recorded high in three area of species location, with Akwa Ibom and Cross River recording high foliar content in *A. djalonesis* while *A. nobilis* was high in stem content in Bayelsa. All species in Rivers had high content in all part of localization. Record has shown that Plants obtained from habitats in various agro-climatic zones showed variation in triterpene content besides wide morphological variation among species (Ma *et al.* 2009). Also Geographical localization has shown a significant impact on the quality of many medicinal plants. For example, quantitative variations in the content of seven triterpenoid were identified in 22 samples of *Clematis chinensis* Osbeck and *Clematis hexapetala* Pall. (Ranunculaceae) from different habitats in China (Ma *et al.* 2009).

The carbohydrate content was high in foliar part of *A. vogelii* and *A. djalonesis* in both Akwa Ibom and Cross River but in Bayelsa was moderate in all part of *A. djalonesis*, stem/root of *A. vogelii*, foliar part in *A. nobilis* and stem of *A. liebrechtsiana* while in Rivers, root of *A. djalonesis* was moderate in content. Similar studies have recorded the dynamics of carbohydrate content in leaves, stems and flowers of plant species in different locations (Larcher, 2000 and Viera *et al.*, 2010).

The inability of plant species to move has modified species to develop mechanisms to survive unfavourable environmental conditions. Among such mechanisms is the synthesis of secondary metabolites such as flavonoid. Flavonoid content recorded a moderate level with the foliar part of *A. liebrechtsiana*, in Akwa Ibom, Bayelsa, and Cross River States, and the stem of *A. vogelii* in Akwa Ibom, Bayelsa and Rivers States, while *A. nobilis* in part of Bayelsa, Cross River and River States had similar content in their foliar parts.

Anthocleista djalonesis had moderate content in the stem/foliar in Bayelsa and only foliar part in Cross River. Similar synthesis and localization has been recorded in cell walls and epidermal tissues of stems, foliar tissues and fruit of plant species under varied geographical location, habitat niche ecology and environmental stimuli (Steyn *et al.*, 2002; Koes *et al.*, 2005; Ogata *et al.*, 2005 and Bakhshi and Arakawa, 2006).

The present work has revealed the occurrence and distribution of tannins in all the 4 species of *Anthocleista* studied. The presence or frequency of abundance and pattern of distribution of the tannins vary apparently among the species. The tannin content was high in foliar of *A. nobilis*, *A. liebrechtsiana* and *A. vogelii* in sample areas of Akwa Ibom and Rivers State, while a moderate content was recorded in *A. nobilis*, *A. liebrechtsiana* including *A. djalonesis* foliar in Bayelsa and Cross River; all the species had some content level in their foliar parts. The results obtained from the study carried out on four species of *Anthocleista* in the family Loganiaceae showed that tannins are of universal occurrence in this taxon. The increased presence of tannins in the foliar tissues as against those of the stem and root observed in this study is noteworthy. This observation tends to suggest that tannins are metabolically produced by the leaves and are probably mobilized to the stem or other tissues for excretion or storage. The high level of tanniferous cells observed in the phloem tissues further strengthened this suggestion since the phloem is the route of translocation of organic compounds (food) in vascular plant. This corroborates the earlier find by Edwin-Wosu and Ndukwu, (2012).

In this present research saponin was high in foliar part of *A. nobilis* in Cross River and Rivers State, which also has *A. vogelii* and *A. djalonesis* with high content in foliar parts. *Anthocleista vogelii* stems has recorded moderate content in Akwa Ibom and Bayelsa, which still has recorded moderate level of saponin in foliar part of *A. nobilis*, *A. djalonesis* and stem of *A. liebrechtsiana*. Different types of saponins have been isolated from roots, stem, bark, leaves, seeds, fruits and flowers, and from whole plants (Vincken *et al.*, 2007). In many plants, saponins are primarily synthesized and stored in underground organs.

The relationship between saponin content and habitat has been demonstrated for many wild plants as well as cultivated crops. Variations in saponin level have been described for wild legumes harvested in different regions of Korea during an evaluation of selected plants as a material for foodstuffs (Shim *et al.*, 2003). A remarkable correlation between the saponin content and the region of cultivation was demonstrated for cultivars of the sweet lupin *Lupinus angustifolius* L. (Fabaceae) grown over 2 years in 4 different areas of Australia (Shim *et al.*, 2003).

Similarly, the relationship between habitat and the variation in saponin content has been examined, indicating higher saponin production by trees grown under harsh conditions. Analysis of aqueous extracts from bark and woody part of plants collected at three different locations in central Chile has shown saponin contents variation in branches and in bark of trunk of dried material, depending on the part of the plant and the site of collection. The highest levels of saponins in the bark of the trunk were found in plants growing in

region with poor soil quality, water deficit in the summer and frosts in winter, while the lowest levels of saponins in this plant organ were detected in plants from agricultural zones with a milder climate, good soil and available water. These results suggest that the synthesis of saponins is up-regulated in response to stress and these compounds could be involved in the adaptation of plants to survive in adverse soil and climate conditions. It may also be suggested that commercial exploitation of saponin should take into account the environmental characteristics of the location of plant collection (Anna *et al.*, 2011). Also studies has reported saponins as widely distributed in various geographical and climatic zones around the world, and in various life forms, habit, parts (roots, leaves, stems, bulbs, flowers and fruit) and lifecycle of plants involving trees, shrubs, herbs, perennial evergreen and deciduous species, biennial and annual species, wild and cultivated species. (Anna *et al.*, 2011).

Studies have also revealed the combined influence of agro-climatic conditions on the qualitative and quantitative variation of saponins occurring in the puncture vine *Tribulus terrestris* L. (Zygophyllaceae) grown in 4 different localities in south Slovakia. The highest levels of saponins (3.7% in dried whole plants, 1.22% in roots, 0.7% in seeds) were found in plants growing in temperate climatic region with fertile neutral soil, whereas lower levels (2.9, 1.04, 0.48% in whole plants, roots and seeds, respectively) were found in plants from warm regions with rather poor neutral or alkaline soils (Salamon *et al.*, 2006). These results, showing significant changes in saponin content in plants growing within a small region, is an instance of a plant having numerous chemotypes and changing its composition in response to the local geoclimate (Kostova and Dinchev 2005; Dinchev *et al.*, 2008). Determination and evaluation of saponin content of herbs was found to vary greatly in plants from different habitats. (MacDonald *et al.*, 2005). The local geoclimate, seasonal changes, external conditions such as light, temperature, humidity and soil fertility, as well as cultivation techniques, are known to affect the quantitative and qualitative composition of saponins (Hanus *et al.*, 2003; Wink 2003; Henry 2005; Kalinin *et al.*, 2005). Generally, this study have shown that secondary metabolites accumulation in organs and tissues under the influence of developmental factors, and with their isolation from roots, stem, bark, leaves, seeds, fruits and flowers and whole plant is indicative of differential synthesis and regulation.

CONCLUSION

Secondary metabolism of plants, and the expressed metabolite levels, may change considerably due to the influence of several biotic and abiotic stress signals. The *Anthocleista* plant contained highly polar bioactive compounds (Alkaloid, triterpene, glycoside, carbohydrate, flavonoid, tannin, and saponin). The prevalence concentration of these compounds varied in the different parts of localization due to geographical location among other stress inducing factors. The presence and recognition of these metabolites is an indication of their potentials as medicinal plants and can also enhance conservation strategies for the

Anthocleista species in Nigeria. Knowing the factors which induce variations of plant secondary metabolite contents should further encourage studies to define conditions and periods during which cultivation and / or harvest can occur to achieve desirably high concentrations of bioactive compounds. Identification and understanding natural variation in metabolite concentration may extend the knowledge of ecologic interactions of plants with their environment and allow alternative strategies to increase productivity of the plant.

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