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METABOLIC PROFILE ASSESSMENT OF PHYTOCHEMICALS IN *CEIBA PENTANDRA* (L.) GAERTN, FROM ZARIA CITY, NIGERIA

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ABSTRACT

Ceiba pentandra (Silk cotton) is a multipurpose tree in the province of Zaria city, Kaduna State, Nigeria, which is being exposed to uncontrolled exploitation by the natives. Samples of the plant were collected from standing trees located at Zaria city: Kofar Gayan, Rimin Doko, Kwarbai, Kofar Doka and Kofar Kibo. The study was aimed at assessing the secondary metabolites in *C. pentandra* plant extracts of leaves, stem barks and roots This was achieved through subjecting the extracts to phytochemical screening and gas chromatography/mass spectrometry fingerprinting (metabolic profiling) of phytochemical markers. The results of the screening revealed flavonoids, saponins, steroids, tannins, triterpenoids and reducing sugars to be present in moderate variation, across the extracts. The metabolic fingerprinting indicated high variation among the phytochemicals obtained from the plant extracts. Where, twenty-seven (27) secondary metabolites were predicted from the methanol leaf extracts, twenty-five (25) from the methanol stem bark extracts and twenty-seven (27) from the methanol root extracts, out of which only one of the metabolites (2-hydroxypropyl ether) was predicted in all the extracts. Therefore, for a clearer and broader identification of these phytochemical markers, Nuclear Magnetic Resonance spectrometry (NMR) should be employed to determine the detailed chemical structures of the identified metabolites, which will help in determining their functions and how they can be used to improve the biodiversity of these tree species.

Keywords: Metabolic profile, GC-MS, Secondary metabolites, *Ceiba pentandra*.

INTRODUCTION

Over the centuries humans have relied on plants for basic needs such as food, clothing, and shelter, all produced or manufactured from plant matrices (leaves, woods, fibers) and storage parts (fruits, tubers) (Saurabh *et al*., 2015). Plants have also been utilized for additional purposes, namely as arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines. The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. (Anurag *et al.,* 2014) Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.,* 2006).

A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), (Anurag *et al.,* 2014) and secondary metabolites which are substances produced by plants as defense chemicals. Their absence does not cause bad effects to the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannins etc.
Secondary metabolites are compounds biosynthetically derived from primary metabolites and are compounds that are not required for normal growth and development, and are not made through metabolic pathways common to all plants (Bourgaud *et al*., 2001). Secondary metabolites are accumulated by plant cells in smaller quantities than primary metabolites (Anurag *et al.,* 2013).

Secondary metabolites from plants, which are distinguished from primary metabolites such as nucleic acids, amino acids, carbohydrate, fat, etc. (Justin *et al.,* 2014) are extremely diverse; thousands of them have been identified in several classes. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants (Thrane, 2001). Many scientific sources state that their role is not crucial for living cells in normal growth, development, and reproduction (Justin *et al.,* 2014), but they act in defense purposes to protect a plant from any possible harm in the ecological environment (Stamp, 2003), and other interspecies protection (Samuni-Blank *et al.,* 2012). Therefore, they are usually synthesized in plants for particular needs, while the primary metabolites have generally the shared biological purposes across all species. Plants have been evolving to adapt to the environment with genetic encoding of useful and diverse syntheses for secondary metabolites (Waterman, 1992).

Silk cotton (*Ceiba pentandra*) tree was first introduced into the study area, Zaria (named as 'Rimi' in Hausa language) from Indo-Arabian sub-continent by the slave merchants who exchanged its products (mattresses and horse saddles) for slaves (Al-Amin, 2013). The tree's seed was brought through the trade, dispersed into the area and eventually germinated and grown as a tree through such trade. Its uses in the area helped in the initial propagation, planting and protection when sooner, the people of Zaria realized several uses of the tree products for

animal forage, medicinal uses and several other utility uses. This made the tree species a "Hotcake" for the natives (Al-Amin, 2013).

The silk cotton tree colony is relentlessly being depleted by several human activities like; deforestation, the use of the timber as domestic fuel and construction processes among others. As the destruction is going unrestricted, the effect is obtrusively manifesting at least in the last two decades (Al-Amin, 2013). Unfortunately, no or less effort is being made to study this phenomenon more or less to stop it. The resulting negligence on the tree colony has incited this present research to find and provide possible solutions for the amplification of conservational (both *Ex situ* and *In situ*) actions of the plant in order to protect, conserve and beautify the environment.

MATERIALS AND METHODS

Study Area

Zaria city is located at 11° 5’ 7.9476’’N and 7° 43' 11.8020’’ E in Kaduna State on the central high plains of Hausa-land in northern part of Nigeria (Al-Amin, 2013). The major soil type in the area is the tropical ferruginous; while along the wide gentle sloping valley bottom lands are the dark vertisol (Fadama soils). Zaria falls within the northern guinea savanna vegetation. The climax vegetation of the area was thought to be tropical deciduous, however, because nearly all the vegetation of the area had been degraded due to intense urban agriculture, fuel wood harvesting and urbanization processes, tree climax vegetation is absent except in the remnant silk-cotton tree colony around the southern suburb (Al-Amin, 2013).

Sampling Points

Sampling points were purposely selected, and includes; the Kofar Doka, Rimin Doko, Kofar Gayan, Kwarbai and Kofar Kibo (Figure 1).

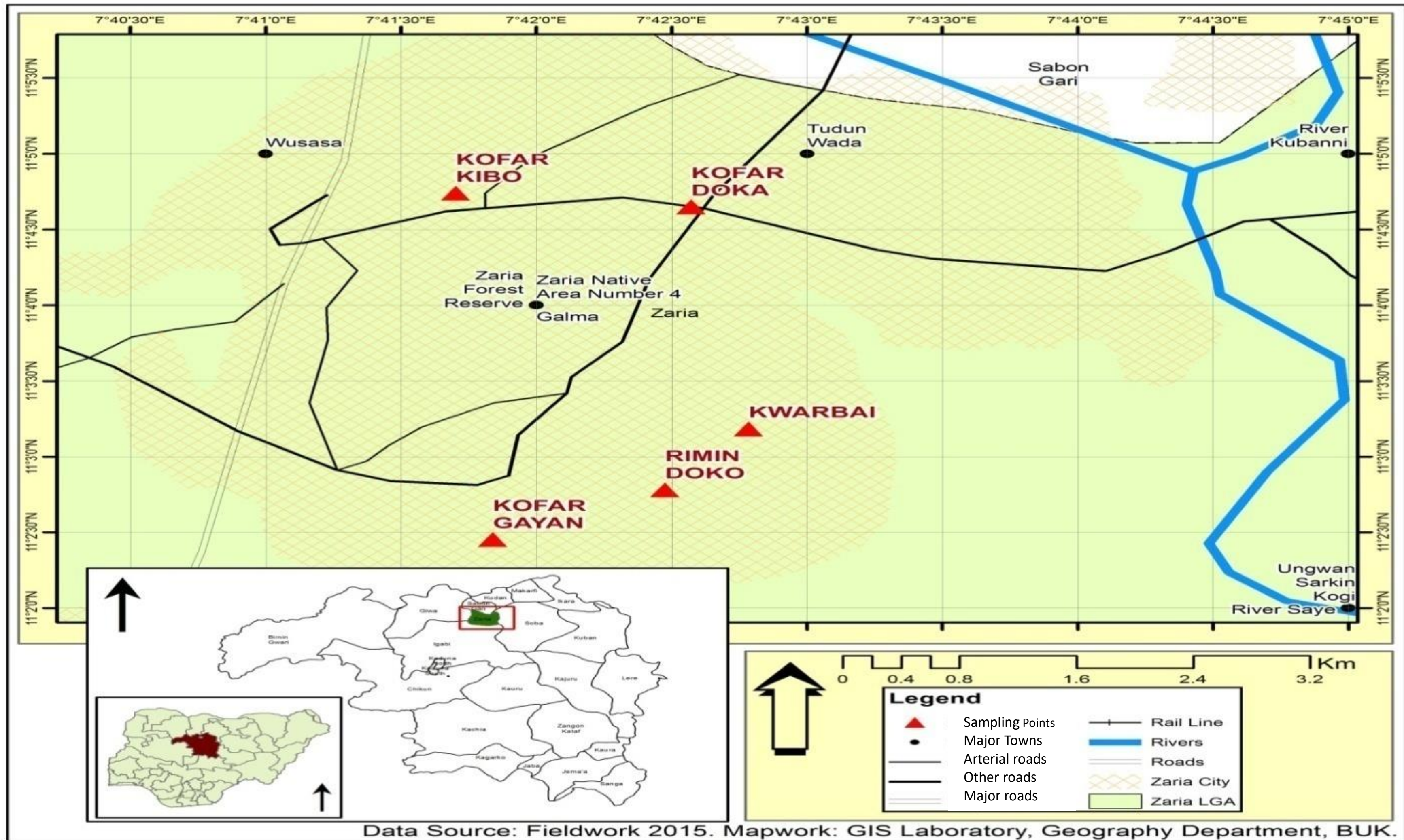

Figure 1: A Map of Zaria City Showing the Five Sampling Points

Collection, Identification and Preparation of Plant Materials for Phytochemical Analyses

The fresh leaves, young stems and roots of *C. pentandra* species were collected from the above mentioned sampling points. The plant samples were identified at the Herbarium of Biological Sciences Department, Ahmadu Bello University, Zaria, with a voucher specimen (No. 7059). Collected samples were transported to Biological Sciences Department, Bayero University, Kano for laboratory analysis. The plant samples were air-dried indoors, finely powdered using a sterilized grinder for the extraction procedure.

Preparation of Extracts

The extraction scheme was performed according to the standard method adopted by Okoye and Osadede (2009). One hundred grams (100g) of powdered plant materials were subjected to solvent extraction for a period of two days by cold maceration in 99% methanol with periodic shaking. The crude methanol extract was drained, filtered and concentrated almost to dryness under reduced pressure at 40°C using rotary evaporator (Rotavapo R210/215, Buchi).

Phytochemical Screening of Extracts

The methanol extracts obtained from the above method were subjected to preliminary phyto-chemical screening following standard protocols (Poongothai *et al.,* 2011).The extracts were screened for the presence of tannins, flavonoids, saponins, steroids, triterpenoids and reducing sugar. A portion of the extract (0.5mg) was treated with saturated solution of ferric chloride; a green-black or blue-black colour indicated the presence of tannin (Shamsuddeen *et al.,* 2009). Flavonoids detcetion was carried out in accordance with the method of Shamsuddeen *et al. (*2009), where piece of magnesium ribbon was added to 4mg/ml of each extract which was followed by the addition of concentrated hydrochloric acid (HCl), drop-wise. Appearance of Crimson to magenta colour indicated the presence of flavonoids. 0.5g of each extract was shaken in 10ml of distilled water. Production of persistent foam which lasted for 15 minutes indicated the presence of saponin (Shamsuddeen *et al.,* 2009).One milligram (1mg) of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by the side of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (Poongothai *et al.,* 2011).Ten milligram (10mg) of the extract was dissolved in 5ml of chloroform; 1ml of acetic anhydride was added following the addition of 2ml of conc. H_2SO_4.Formation of reddish violet colour indicates the presence of triterpenoids (Poongothai *et al.,* 2011).Test for detection of reducing sugars was carried out in accordance with the method of Poongothai *et al.* (2011) where 1ml of stock solution of each extract was diluted with 2ml of distilled water, followed by the addition of Fehling's solution (A+B) and then warmed. Brick red precipitates at the bottom of the tubes indicate the presence of reducing sugars.

Gas Chromatography-Mass Spectrometry Analysis of Extracts

GC-MS analysis of the extracts was performed using GCMS-QP2010 PLUS (Shimadzu Japan system). The sample (2 ml) was injected into a RTX-5 column (60m x 0.25mm internal diameter, film thickness 0.25 μm) of GC-MS. Helium was used as carrier gas at a constant column flow of 1.58 ml/min. at 108.0 kpa inlet pressure. Temperature programming was maintained from 80°C to 250°C with constant rise of 5°C/min. and then held isothermal at 250°C 10 min.; further the temperature was increased by 30°C/min. up to 310°C and again held isothermal at 320°C for 22 min. The injector and ion source temperature were 270°C and 230°C, respectively. Each of the extracts was dissolved in methanol, and in turn injected with a split ratio of 1:20. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and the total GC/MS running time 70 minutes. Interpretation and identification of the spectrum of the GC-MS was obtained using the database of library in GC-MS system of the National Research Institute for Chemical Technology (NARICT), Zaria.

RESULTS

Table 1: Preliminary Phytochemical Screening of *C. pentandra* Root Extracts from Five Sampling Points in Zaria City

Tests	K. Gayan	K. Kibo	R. Doko	Kwarbai	K. Doka
Flavonoids	+	+	+	+	+
Saponins	-	-	-	-	-
Steroids	-	-	-	-	+
Tannins	+	+	+	+	+
Triterpenoids	-	-	+	-	+
Reducing sugar	+	+	+	+	+

Key: + Detected
- Not detected

Table 2: Preliminary Phytochemical Screening of *C. pentandra* Stem Bark Extracts from Five Sampling Points of Zaria City

Tests	K. Gayan	K. Kibo	R. Doko	Kwarbai	K. Doka
Flavonoids	-	-	-	+	-
Saponins	-	-	+	-	-
Steroids	+	+	+	-	+
Tannins	+	+	+	-	+
Triterpenoids	+	+	+	-	+
Reducing sugar	-	+	-	-	+

Key: + Detected
- Not detected

Table 3: Preliminary Phytochemical Screening of *C. pentandra* Leaf Extracts from Five Sampling Points of Zaria City

Tests	K. Gayan	K. Kibo	R. Doko	Kwarbai	K. Doka
Flavonoids	-	+	+	+	-
Saponins	+	+	-	+	-
Steroids	+	+	-	-	-
Tannins	+	+	+	+	+
Triterpenoids	+	+	-	-	-
Reducing sugar	-	-	+	-	-

Key: + Detected
- Not detected

Table 4: Identified Metabolites Obtained at Different Retention Times from the Methanol Leaf Extracts

Retention time (Mins)	KofarGayan	RiminDoko	Kwarbai	KofarDoka	KofarKibo	Compound Empirical Formula	Names of Compounds
4.0-4.5	+	-	+	-	-	C_8H_{16}	1-octene
4.6-5.0	+	-	+	+	+	$C_4H_8O_2$	Formic acid
5.1-5.5	-	-	+	+	+	$C_6H_{14}O_3$	2-hydroxypropyl ether
5.6-6.0	-	-	-	-	-		
6.1-6.5	-	-	-	-	-		
6.6-7.0	-	-	-	-	-		
7.1-7.5	-	-	-	-	-		
7.6-8.0	+	-	-	-	+	$C_{17}H_{23}NO_2$	Anthranilic acid
8.1-8.5	-	-	-	-	-		
8.6-9.0	-	-	-	-	-		
9.1-9.5	-	-	-	-	-		
9.6-10.0	-	-	+	-	-	$C_{15}H_{24}$	1,3-dimethyl-8-(1-methylethyl)
10.1-10.5	-	-	-	-	+	$C_{15}H_{24}$	1,3-dimethyl-8-(1-methylethyl)
10.6-11.0	-	-	+	-	-	$C_{14}H_{22}$	5,9-tetradecadiyne
11.1-11.5	-	-	+	-	-	$C_{15}H_{24}$	1,3-dimethyl-8-(1-methylethyl)
11.6-12.0	-	-	-	-	-		
12.1-12.5	+	-	-	-	+	$C_{15}H_{30}O_2$	Tridecanoic acid ethyl ester
12.6-13.0	-	-	+	-	+	$C_{10}H_{16}O$	Bicyclo(3.1.1)heptan-3-one
13.1-13.5	-	-	-	-	+	$C_{10}H_{16}O_2$	7-oxabicyclo(4.1.0)heptane
13.6-14.0	+	-	+	-	-	$C_{11}H_{22}O_2$	Decanoic acid
14.1-14.5	-	-	-	-	-		
14.6-15.0	+	-	-	-	-	$C_{10}H_{21}F$	Decylfluoride
15.1-15.5	-	-	-	-	-		
15.6-16.0	-	-	-	-	-		
16.1-16.5	-	+	+	-	+	$C_{19}H_{36}O_2$	11-octadecenoic acid
16.6-17.0	+	+	+	+	+	$C_{11}H_{22}O_2$	Decanoic acid
17.1-17.5	-	-	-	-	-		
17.6-18.0	-	-	-	-	-		
18.1-18.5	+	+	+	+	+	$C_{15}H_{30}O_2$	Tridecanoic acid ethyl ester
18.6-19.0	-	-	-	-	-		
19.1-19.5	-	-	-	-	-		
19.6-20.0	+	+	+	+	+	$C_{17}H_{30}O_2$	9,12-hexadecadienoic acid
20.1-20.5	+	+	+	+	+	$C_{19}H_{36}O_2$	11-octadecenoic acid
20.6-21.0	+	-	+	+	+	$C_{18}H_{34}O_2$	Oleic acid
21.1-21.5	+	+	+	+	+	$C_{18}H_{36}O_2$	Methyl isoheptadecanoate
21.6-22.0	-	-	-	-	-		
22.1-22.5	+	-	+	+	+	$C_{14}H_{28}$	4-tetradecene
22.6-23.0	+	+	+	+	+	$C_{21}H_{42}O_2$	Nonadecyl acetate
23.1-23.5	-	-	-	-	-		
23.6-24.0	-	-	-	+	-	$C_{11}H_{20}O$	Dehydrogeraniol
24.1-24.5	+	+	+	+	+	$C_{18}H_{34}O$	9,12-octadecadien-1-ol
24.6-25.0	+	+	+	+	+	$C_{11}H_{24}$	5-methyldecane
25.1-25.5	-	-	-	-	-		
25.6-26.0	-	-	-	-	-		
26.1-26.5	-	-	-	-	-		
26.6-27.0	-	-	-	-	-		
27.1-27.5	-	-	-	+	-	$C_{18}H_{34}O_2$	Oleic acid
27.6-28.0	+	+	+	+	+	$C_{30}H_{50}$	Squalene

Table 5: Identified Metabolites Obtained at Different Retention Time Range from the Methanol Stem Bark Extracts

Retention time (Mins)	KofarGayan	RiminDoko	Kwarbai	KofarDoka	KofarKibo	Compound Empirical Formula	Names of Compounds
4.0-4.5	-	+	-	-	-	$C_5H_{12}O$	1,2-methyl-1-butanol
4.6-5.0	+	-	-	+	+	$C_6H_{14}O_3$	2-hydroxypropyl ether
5.1-5.5	-	-	-	-	-		
5.6-6.0	-	-	+	-	-	$C_5H_{10}O_3$	Butanoic acid
6.1-6.5	-	-	-	-	-		
6.6-7.0	-	-	-	-	-		
7.1-7.5	-	-	-	-	-		
7.6-8.0	+	-	+	-	-	$C_5H_{10}O_4$	Acetyl monoglyceride
8.1-8.5	-	-	-	-	-		
8.6-9.0	-	-	+	-	-	$C_6H_{10}O_4$	Ethylene acetate
9.1-9.5	-	-	-	-	-		
9.6-10.0	-	-	-	-	-		
10.1-10.5	-	-	-	-	-		
10.6-11.0	+	-	-	-	-	$C_{12}H_{26}O$	2-butyl-1-octanol
11.1-11.5	+	+	-	-	-	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester
11.6-12.0	-	-	-	-	-		
12.1-12.5	-	-	-	-	-		
12.6-13.0	+	-	-	-	-	$C_{14}H_{28}$	3-tetradecene
13.1-13.5	-	-	-	-	-		
13.6-14.0	+	+	-	-	-	$C_{14}H_{28}O_2$	Tridecanoic acid
14.1-14.5	-	-	-	-	-		
14.6-15.0	-	+	-	-	-	$C_{16}H_{32}O$	Hexadecylene oxide
15.1-15.5	-	-	-	-	-		
15.6-16.0	-	-	-	-	-		
16.1-16.5	+	+	-	-	-	$C_{23}H_{44}O_2$	13-docosenoic acid, methyl ester
16.6-17.0	+	-	+	+	+	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester
17.1-17.5	-	-	-	-	-		
17.6-18.0	-	-	-	-	-		
18.1-18.5	+	+	+	+	+	$C_{16}H_{32}O_2$	Hexadecanoic acid
18.6-19.0	-	-	-	-	-		
19.1-19.5	-	-	-	-	-		
19.6-20.0	+	+	+	+	+	$C_{19}H_{34}O_2$	Linolelaidic acid, methyl ester
20.1-20.5	+	+	+	+	+	$C_{19}H_{36}O_2$	6-octadecenoic acid, methyl ester
20.6-21.0	+	+	+	+	+	$C_{14}H_{26}O_2$	E-9-tetradecenoic acid
21.1-21.5	+	+	+	-	+	$C_{18}H_{32}$	1,E-11,Z-13-octadecatriene
21.6-22.0	-	-	+	-	-	$C_{14}H_{28}$	3-tetradecene
22.1-22.5	+	+	+	+	+	$C_{12}H_{26}O$	2-butyloctyl alcohol
22.6-23.0	+	+	+	+	+	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
23.1-23.5	-	+	+	-	-	$C_{18}H_{35}NO$	Oleic acid amide
23.6-24.0	-	+	+	-	-	$C_{20}H_{42}O$	1- Eicosanol
24.1-24.5	+	+	+	+	+	$C_{18}H_{34}O$	Octadecenyl aldehyde
24.6-25.0	-	+	+	+	+	$C_{10}H_{22}O$	n-propyl heptyl ether
25.1-25.5	-	-	-	-	-		
25.6-26.0	-	-	-	-	-		
26.1-26.5	-	-	-	-	-		
26.6-27.0	-	-	-	-	-		
27.1-27.5	-	-	-	-	-		
27.6-28.0	-	-	+	-	-	$C_{16}H_{32}O$	Palmitaldehyde

Table 6: Identified Metabolites Obtained at Different Retention Time Range from the Methanol Root Extracts

Retention time (Mins)	KofarGayan	RiminDoko	Kwarbai	KofarDoka	KofarKibo	Compound Empirical Formula	Names of Compounds
4.0-4.5	-	-	-	+	+	$C_{10}H_{20}$	1-decene
4.6-5.0	+	+	-	-	-	$C_6H_{14}O_3$	2-hydroxypropyl ether
5.1-5.5	+	+	+	+	+	$C_6H_{14}O_3$	2-hydroxypropyl ether
5.6-6.0	-	-	-	-	-		
6.1-6.5	-	-	-	-	-		
6.6-7.0	-	-	-	-	-		
7.1-7.5	-	-	-	-	-		
7.6-8.0	-	-	-	-	+	$C_{10}H_{16}O$	Alpha-citral
8.1-8.5	-	-	-	-	-		
8.6-9.0	-	-	-	-	-		
9.1-9.5	-	-	-	-	+	$C_{12}H_{20}O_2$	Linalool acetate
9.6-10.0	-	-	-	-	-		
10.1-10.5	-	-	-	-	-		
10.6-11.0	+	-	-	-	-	$C_{14}H_{26}O$	E-9-tetradecenal
11.1-11.5	-	+	-	-	-	$C_{14}H_{28}O_2$	Tridecanoic acid, methyl ester
11.6-12.0	-	-	-	-	-		
12.1-12.5	-	+	-	-	-	$C_{15}H_{30}O_2$	Pentadecanoic acid
12.6-13.0	-	-	-	-	+	$C_{10}H_{20}O$	Isocitronellol
13.1-13.5	-	-	-	-	-		
13.6-14.0	-	+	-	-	+	$C_{18}H_{36}O_2$	Stearic acid
14.1-14.5	-	-	-	-	-		
14.6-15.0	-	+	-	-	-	$C_{20}H_{40}O_2$	Acetic acid, octadecyl ester
15.1-15.5	-	+	-	-	-	$C_{14}H_{22}$	5,9-tetradecadiyne
15.6-16.0	-	-	+	-	-	$C_{21}H_{38}O_2$	11,14-eicosadienoic acid, methyl ester
16.1-16.5	-	-	+	-	+	$C_{12}H_{14}N_2O_6$	Acetic acid, (2,4-dinitro-6-s-butylphenyl
16.6-17.0	+	+	+	+	+	$C_{17}H_{34}O_2$	Heptadecanoic acid
17.1-17.5	-	-	-	-	-		
17.6-18.0	-	-	-	-	-		
18.1-18.5	+	+	+	+	+	$C_{16}H_{32}O_2$	Hexadecanoic acid
18.6-19.0	-	-	-	-	-		
19.1-19.5	-	-	-	-	-		
19.6-20.0	+	+	+	+	+	$C_{19}H_{34}O_2$	Linolelaidic acid, methyl ester
20.1-20.5	+	+	+	+	+	$C_{19}H_{36}O_2$	13-octadecenoic acid
20.6-21.0	+	+	+	+	+	$C_{18}H_{34}O_2$	Oleic acid
21.1-21.5	+	+	+	+	+	$C_{19}H_{34}O_2$	Linolelaidic acid, methyl ester
21.6-22.0	-	-	-	-	-		
22.1-22.5	+	+	+	+	+	$C_{10}H_{21}F$	Decyl fluoride
22.6-23.0	+	+	+	+	+	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
23.1-23.5	-	-	-	-	-		
23.6-24.0	-	-	+	-	-	$C_{13}H_{26}O$	11-tridecen-1-ol
24.1-24.5	+	+	+	+	+	$C_{11}H_{20}O$	Undecylenic aldehyde
24.6-25.0	+	+	+	+	+	$C_{10}H_{21}F$	Decyl fluoride
25.1-25.5	-	-	-	-	-		
25.6-26.0	-	-	-	-	-		
26.1-26.5	-	-	-	-	+	$C_{14}H_{26}O$	E-9-tetradecenal
26.6-27.0	-	-	-	-	-		
27.1-27.5	-	-	-	-	-		
27.6-28.0	-	-	-	+	-	$C_{16}H_{32}O$	E-2-hexadecacen-1-ol

DISCUSSION

Massive loss of valuable plant species in the past centuries and its adverse impact on environmental and socioeconomic values has triggered the conservation of plant resources. Appropriate identification and characterization of plant materials is essential for the successful conservation of plant resources and to ensure their sustainable use (Arif *et al.,* 2010).

The results of the phytochemical assessment from the five sampling points (Kofar Gayan, Rimin Doko, Kwarbai, Kofar Kibo and Kofar Doka) indicate relative variation among the extracts from the above mentioned sampling points which may have resulted from continuous cutting down of the trees for firewood and other domestic needs. It may also have been due to stress posed by environmental hazards such as pollution, drought, ultra violet

exposure, herbivory and pathogenic attack, (Ali and Alqurainі, 2006).

Phytochemicals are biologically synthesized chemical compounds in plants, which serve in protecting the plant cells against environmental hazards such as: pollution, stress, and drought, ultra violet exposure, herbivory and pathogenic attack (Ali and Alqurainі, 2006). Phytochemicals, also referred to as secondary metabolites, provide health benefits to humans; acting as synergistic agents, hence, allowing more efficient nutrient use by the body (Andre *et al.,* 2010). This study identified flavonoids, saponins, steroids, tannins, triterpenoids and reducing sugar (Tables 1, 2 and 3). Overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in the human body is responsible for the pathogenesis of some diseases. The scavenging of these oxidants is thought to be an effective measure to depress the level of oxidative stress of organisms. It has been reported that intake of plant parts is inversely associated with the risk of many chronic diseases, and antioxidant phytochemicals in the plants are considered to be responsible for these health benefits. They often possess strong antioxidant and free radical scavenging abilities, as well as anti-inflammatory action, which are also the basis of other bioactivities and health benefits, such as anticancer, anti-aging, and protective action for cardiovascular diseases, diabetes mellitus, obesity and neuro-degenerative diseases (Zhang *et al.,* 2015).

The presence of the range of phytochemicals in the different plant parts could be related to the use of the plant for medicinal purposes. Bairwa *et al.* (2010) reported protective activity of ethyl acetate fraction of methanol extract of stem bark of *C. pentandra* against induced liver damage in rats. *C. pentandra* root and bark extracts have been reported to have a hypoglycaemic effect (Djomeni-Dzeufiet *et al.,* 2006).

Tannins were found present in the leaves, bark, stem and the roots of *C. pentandra* extracts(Tables 1, 2 and 3). Tannins possess a characteristic feature of tan, i.e. they have the potential to convert things into leather (Sarker and Nahar, 2007), provide protection to the plant against microbial pathogens, harmful insects and other herbivores (Ali and Alqurainі. 2006).Tannins present in the extracts of *C. pentandra* have being also reported by Akinpelu and Onakoya (2006) as the main component for the treatment of intestinal disorders like diarrhea and dysentery. Triterpenoids found in these plant extracts serve as a major component of many essential oils, resins or oleoresins (Firn, 2010), and are widely used in herbal medicine (Edeoga *et al.,* 2005). Many sesquiterpene lactones with moderate antimicrobial activity have been isolated from the root bark of *C. pentandra* (Rao *et al.,* 1993). Flavanoids presented in the Tables 1, 2 and 3have a number of nutritional functions and have been described as biological response modifiers', mostly act as anti-oxidant and some have anti-inflammatory properties or as free radical scavengers (Kar, 2007). Flavonoids were also described as phyto-constituents serving as flavouring ingredients of spices and vegetables (Osuntokun *et al.,* 2017). Isoflavone glucosides isolated from the bark of *C. pentandra* showed inhibitory effects on cyclooxygenase-2-catalyzed prostaglandin biosynthesis indicating an analgesic potential (Noreen *et al.,* 1998). The steroids otherwise termed as steroid glycosides or cardiac glycosides are plant phyto-constituents having therapeutic applications as arrow poisons or cardiac drugs (Firn, 2012), they are also reported to have the ability to promote nitrogen retention in osteoporosis and in animals with wasting illness Madziga *et al.* (2010). Saponins as presented in Tables 2 and 3 possess hypolipidemic and anticancer activity, and therefore, necessary for cardiac glycosides activities, steroidal saponins are used in the commercial production of sex hormones for clinical use (Sarker and Nahar, 2007). The result of these phytoconstituents in *C. pentandra* further suggested the reason for the usage of the plant in curing many diseases such as colic in man, dressing of sores for tumors, whitlow, inflammatory diseases, cancer, fatigue, lumbago, gonorrhea, dysentery, anti-microbial and anti-fungal effects (Edeoga *et al.,* 2001). Since these phytochemicals are present in all the targeted organs in the plant, the use of root and stem bark of the plant should be discouraged to ensure sustainable usage of the plant, as the use of root in particular can result in destructive usage.

Variation in biosynthesis of these metabolites may be resulted from both genetic and environmental factors, which play important roles in the development of phenotypic variations in plants as reported by Salim *et al.* (2011).

Some of the compounds revealed in the GC-MS analysis includes: Squalene; a triterpene which is an intermediate for phytosteol biosynthesis in plants extracts (Table 4) and is highly appreciated in the cosmetic and pharmaceutical industry due to its remarkable properties; easy incorporation in cosmetic emulsions as effect of its light consistency and excellent spreadability, non- greasy texture, high stability, also due to the fact that the plant origin squalene is odourless and colorless with no harmful substances, as such serve as an potentiating for chimiotherapic drugs (Ioana *et al.,* 2014). Linalool or Linalyl acetate is a naturally occurring aromatic compound (Table 5), which is regarded as substantial criteria in the authenticity control of essential oils and also adopted for quality assurance by the flavor and fragrance industry (Kreis and Mosandi, 1992). Decanoic, Pentadecanoic, Hexadecanoic, Octadecanoic and Eicosanoic acids are saturated fatty acids (Tables 4-6) and serve as important component in the cosmetic industry (CHEMIK, 2014). They are primarily used as intermediates in the manufacture of corresponding alkali salts, which are in turn, used as emulsifiers, emollients, and lubricants in a variety of cosmetic creams, cakes, soaps and pastes (Mary, 1987), these compounds extracted from the leaves of *Sesuviumportulacastrum*revealed their ability to have the potential antimicrobial agents against tested human pathogenic microorganisms (Chandrasekaran, and Santhilkumar, 2011). Anthranilic acid and its derivatives as presented in Table 4 have applications in various sectors, including; food and perfumery industries, and are used in the synthesis of bioactive molecules (Wiklund and Bergman, 2006). It also exhibits anticancer, antimicrobial, insecticide, antiviral and anti-inflammatory activities (Pajor and Sun, 2013).

The above result was further collaborated and confirmed by metabolic fingerprinting of the Secondary metabolites (phytochemical markers) using GC-MS technique, thus, revealed the presence of pre- cursor molecules to the above mentioned compounds (phytochemicals). These compounds includes; 2-hydroxypropyl ether, E-9-tetradecenal, 11-tridece-1-ol, E-2-hexadecacen-1-ol, Palmitic acid methyl ester, Linolelaidic acid methyl ester, 13-octadecatriene, Acetic, (2,4-dinitro-6-S-butylphenyl), Butanoic acid, Hexadecanoic acid, Acetic acid octadecyl ester, Linolelaidic acid methyl ester, 6- octadecenoic acid, Isoeitronellol, Undecylenic aldehyde, Decylfluoride, Nanodecyl acetate, 9,12-otadecadien-1-ol, 5-methyl decane, Squalene among others. These compounds were found to be diverse among extracts based on their retention time range.

CONCLUSION

In conclusion, the result of this study revealed variation among *C. pentandra* species found around Zaria city, on the basis of the phytochemical markers analytes (secondary metabolites) produced by the extracts, there was relative diversity among the secondary metabolites contained in the extracts. Differences in metabolites at ranges of retention times were observed among the samples obtained from different sites. Analysis of the leaf extract at retention time of 4.0-4.5 minutes indicated the presence of 1-octene only in *C. pentandra* from K. Gayan and Kwarbai, Conversely, at a retention time ranging from 18.1-18.5 minutes, tridecanoic acid ethyl ester was identified in all the leaf samples. Furthermore, while most of the metabolites were observed to be present only in either the leaf, root or stem bark extracts, few others such as 2-hydroxypropyl ether were found to be common to all the extracts. Hence, the biotechnological tool (GC-MS) is one of the important techniques used for the improvement of plant species, which are important economically and medicinally, and may also have a positive impact in their conservation.

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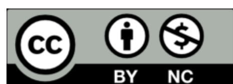

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