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Leaf microscopy, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analyses of *Croton zambesicus* Müll.-Arg. leaf (Family: Euphorbiaceae) in Nigeria

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This study was done to get information on the chemical composition and the internal structure of the plant which could aid in its further characterization. The study is aimed at determining the epidermal leaf anatomy, HPLC of the hot water extract, chemical constituents of the oil from the leaves by GC-MS and chemo-microscopic properties of *Croton zambesicus* for proper identification of the plant. The leaf epidermal microscopy of *C. zambesicus* showed abundant trichome on the abaxial surface arising from a multi-cellular base and radiating radially and paracytic stomata were observed on the lower surface of the leaf. Chemo-microscopic analysis of the leaves showed the presence of cellulose, lignin, starch, oxalate crystals, tannin, oils, proteins and absence of mucilage. The physicochemical parameters evaluated on the leaves had moisture content of 4.10% and total ash value of 11.91%. HPLC analysis of the hot water extract revealed 12 peaks with number 3 having the highest peak of 93 mAU at 3.5 min. The GC-MS analysis of the oil of the leaves had 57 components. Caryophyllene had the highest percentage composition (15.53%) followed by Copaene (11.38%), Phellandrene (8.65%), 1,6-Octadien-3-ol, 3,7-dimethyl (4.67%), Humulene (3.94%), Pinene (3.85%) and Ar-tumerone (3.41%).

Key words: *Croton zambesicus*, high performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), microscopy, Nigeria.

INTRODUCTION

Plants have been used by mankind for its medicinal value and the healing of different ailments. Quite a number of modern drugs are plant based and of natural sources. Many of these active agents isolated were based on their

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uses in traditional medicine (Boukhira et al., 2016). The material medica of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India (Manikandaselvi et al., 2016). The books on ayurvedic medicine such as *Charaka Samhita* and *Susruta Samhita* refer to the use of more than 700 herbs (Manikandaselvi et al., 2016).

Croton zambesicus Mull. Arg. has wide application in Africa for medicinal purposes (Kumar et al., 2011) and grown in many Nigerian communities for medicinal purposes (Ogundajo et al., 2014). The wood is pale yellow, fine-grained, hard, gives a good polish and the stems are used in parts of West Africa for hut-posts ((Irvine, 1961). The bark emits an aromatic smell; an infusion of bark is used in Nigeria in cases of malaria, and the leaves are considered strengthening (Bello et al., 2014). A soup made from the leaves is given to dysentery cases in Southern Nigeria, whereas in both Nigeria and Sierra Leone a leaf decoction is used as a wash and is taken internally for dysentery, fever, convulsions, headache, and as a vermifuge (Irvine, 1961). The fruits and bark are aromatic and are used in the Adamawa region of Nigeria to spice food and to prepare a sort of scent (Ogundajo et al., 2010). The seeds are said to have medicinal use in Togo. Examination of Nigerian material has shown the presence of a trace of alkaloid in the stem and leaf (Ogundajo et al., 2010).

About 80% of the people in developing countries rely mostly on traditional medicine for their health care needs, of which the use of plant extracts or their active principles are involved (Springfield et al., 2005). One of the criticisms of herbal medicine is lack of standardization and quality control profiles, as well as the correct identification of the species concerned, whether in the fresh, dried or powdered state (Springfield et al., 2005).

Phytochemistry deals with a variety of organic substances accumulated in plants (Ogundajo et al., 2014). Further, besides their chemical compounds like carbohydrates, protein, and lipids being used as food by man, other compounds like glycosides, alkaloids, and flavonoids are used as medicines by him in various ways. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is an important step in medicinal plant research (Kokate, 1994). Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso and Adeyemo, 2007).

C. zambesicus has wide application in Africa for medicinal purposes and other economic uses. However, there is insufficient information on the chemical constituents and internal structure of the plant which could aid in its further characterization. Based on the presented information, this study was done with a view to characterize the epidermal leaf anatomy, chemical constituents by HPLC and GC-MS analyses, and chemo-microscopic properties of *C. zambesicus* for proper identification and standardization of the species.

MATERIALS AND METHODS

Plant collection and identification

The plant material was collected from NIPRD, Abuja on 13th October, 2020. Identification was done at the Herbarium and Ethnobotany Unit, Medicinal Plant Research and Traditional Medicine (MPR and TM) Department, NIPRD-Abuja.

Leaf epidermal microscopy

Epidermal preparations (abaxial and adaxial surfaces) followed the methods of Ayodele and Olowokudejo (1997, cited by Ugbabe and Ayodele, 2008). Slides were labeled appropriately and examined under the light microscope (ACCU-SCOPE 3025 Microscope Series) while photographs of the micro morphological features were taken using camera (Industrial Design Camera E31SPM12000KPA) with magnifications x100 and x400. Terminologies are based on Metcalfe and Chalk (1979).

Physicochemical determination and chemo-microscopy

Two physicochemical parameters, moisture content and ash values were determined following WHO guidelines (African Pharmacopoeia, 1986).

Chemo-microscopic studies of the comminuted dried leaf sample was carried out using the methods of Adamu et al. (2018) to test for the presence of different metabolites (African Pharmacopoeia, 1986; Evans, 2002).

High performance liquid chromatography (HPLC)

The method described by Adamu et al. (2018) was used with some modifications. The dried samples (0.2 g) were weighed into clean and well labeled sample bottles, 10 ml of 70% ethanol was added to each sample bottle, allowed to stand for 24 h and the mixture was filtered into clean bottle. An aliquot of each sample was taken with the aid of a 2-ml syringe, filtered through a 0.45-µm Millipore membrane filter and then transferred into HPLC vial before injecting into the HPLC machine. The HPLC operating conditions were programmed to give the following: solvent B: 20% at a flow rate of 0.6 ml/min; and column oven was set to 40°C temperature. The total run time was 20 min.

Gas chromatography–mass spectrometry (GC-MS) analysis

Extraction of oils by hydro distillation

The methods of Okhale et al. (2018) were used where fresh *C. zambesicus* leaf sample was chopped into pieces and subjected to hydro-distillation for 4 h using Clevenger-type apparatus. The essential oil obtained was dried over a hydrous sodium sulphate and used immediately for GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy =70Ev), scan range =45400 amu, and scan rate = 3.99 scan/s], and Shimadzu GCMS solution data system). The percentage of each component was reported as raw percentages based on the total ion current (Okhale et al., 2018).

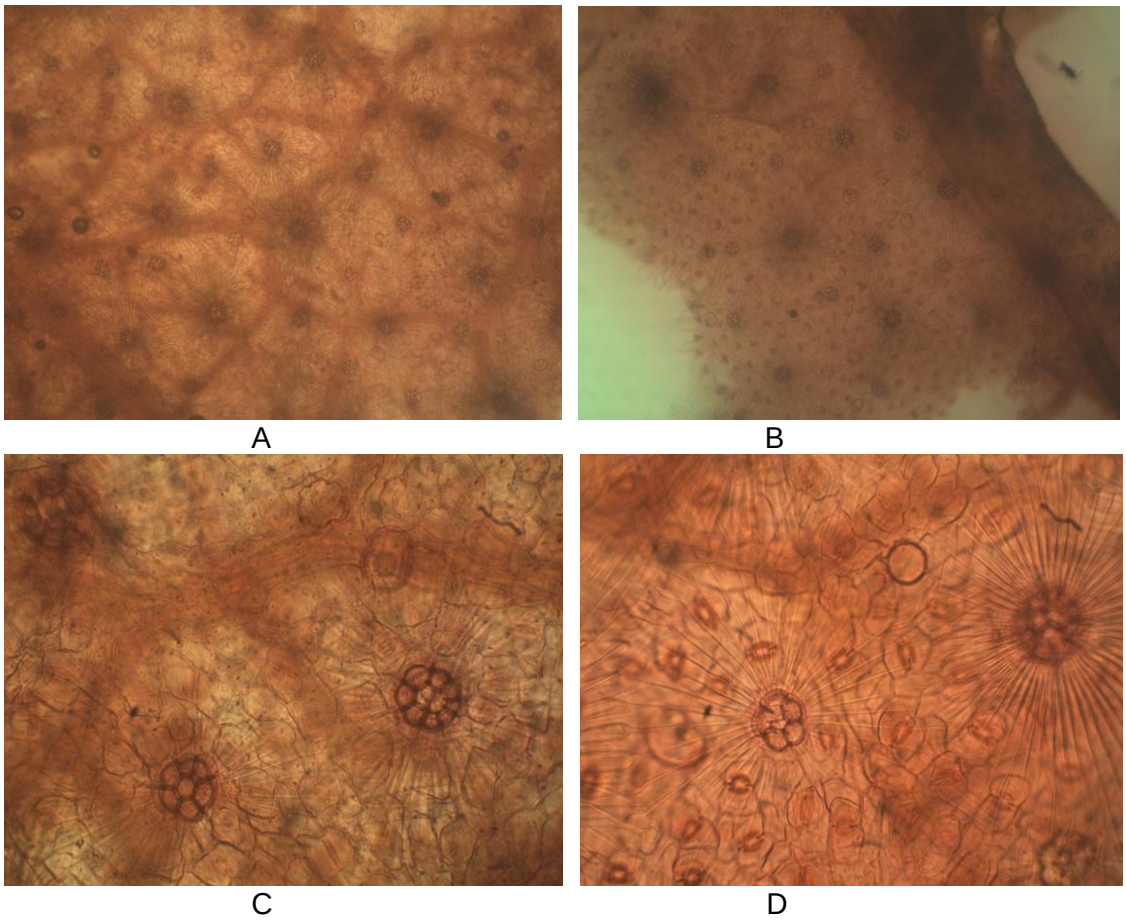


Figure 1. Leaf epidermal microscopy of *Croton zambesicus (*A) Abaxial surface X 100 showing the outline of the epidermal cells and reticulate venation on the leaf surface (B) Abaxial surface X 100 showing the outline of the epidermal cells (C) Abaxial surface X 400 showing trichomes radiating from multi-cellular base and glandular trichome with a rounded head, and D) Abaxial surface X 400 showing paracytic stomata.

RESULTS

Leaf epidermal microscopy

Figures 1 and 2 showed the *C. zambesicus* leaf epidermis qualitative characters. It revealed a paracytic stomata on the abaxial surface while the adaxial surface has no stomata. Trichomes are numerous radiating from a multicellular base radially. The cells are polygonal with nearly straight cell walls on the adaxial surface (Figure 1).

The powdered leaf microscopy revealed oil globules, calcium oxalate crystals and trichomes arising from a base and branching radially (Figure 3).

Chemo-microscopic evaluation of *C. zambesicus* leaf

The chemo-microscopic evaluation of the leaf of *C. zambesicus* revealed the presence of lignin, cellulose, tannins, starch, calcium, oxalate crystals, oils, protein and the absence of mucilage. The leaf has a moisture content of 4.10 and an ash value of 11.91 (Table 1).

HPLC analysis of *C. zambesicus* leaf extract

The HPLC analysis revealed 12 peaks; number 3 has the highest peak (Figure 4) and retention time of 3.214 min. (Table 2 and Figure 4).

GC-MS analysis of *C. zambesicus* leaf oil

The chromatogram of the GC-MS analysis of the leaf of *C. zambesicus* had 57 compounds (Figure 5 and Table 3). The major compounds in the analysis were Caryophyllene (15.53%); beta-copaene (11.38%); alpha-myrcene (8.65%); 1,6-octachen3-ol-1,7-dymethyl- (4.77%); alpha-pinene (3.85%) and Ar-turmerone (3.41%).

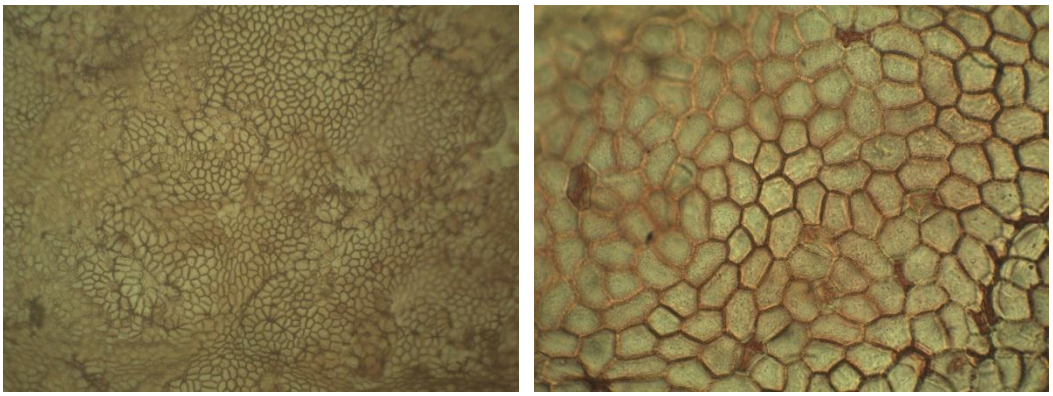


Figure 2. Leaf epidermal microscopy of *Croton zambesicus* A) Adaxial surface ×100 showing the outline of the epidermal cells B) Adaxial surface ×400 showing nearly straight cell walls and have no trichomes and no stomata.

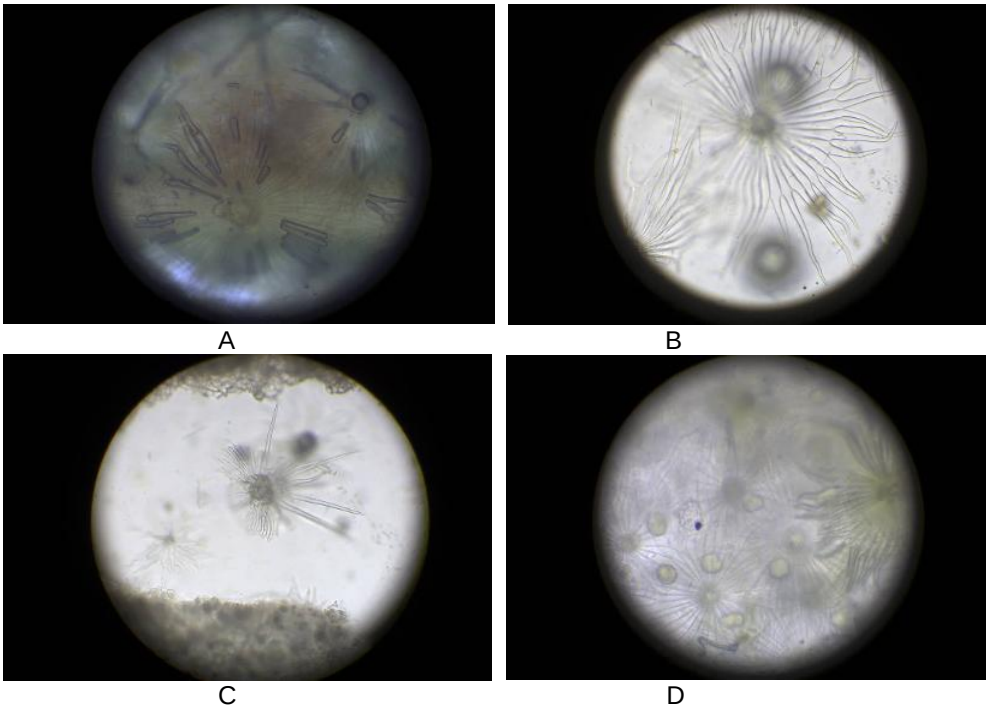


Figure 3. Powdered leaf microscopy of *Croton zambesicus.* A) Calcium oxalate crystals, (B) Trichomes radiating from multi-cellular base and branching radially, (C) Trichome and D) Showing oil globules and trichomes.

Chemical structures of some compounds in *C. zambesicus* leaf oil

Some compounds in *C. zambesicus* leaf oil and their chemical structures are as presented in Scheme 1. These include Caryophyllene, Ar-turmerone, alpha-Phellandrene, alpha-Myrcene, 1,6-octachen3-ol-1,7-dymethyl and beta-copaene.

DISCUSSION

The leaf epidermal microscopy of *C. zambesicus* showed abundant stellate trichomes arising from a multi-cellular base and branching radially on the abaxial surface with paracytic stomata also observed on the lower epidermis of the leaf. The comminuted dried leaf of *C. zambesicus* showed stellate trichome with paracytic stomata. Chemo-

Table 1. Chemo-microscopy and physico-chemical evaluation of *Croton zambesicus* leaf.

Parameter	Inference
Lignin	+
Mucilage	_
Cellulose	+
Tannins	+
Starch	+
Calcium Oxalate crystal	+
Oil	+
Proteins	+
Moisture content	4.10%
Total ash value	11.91%

+= present, - = absent.

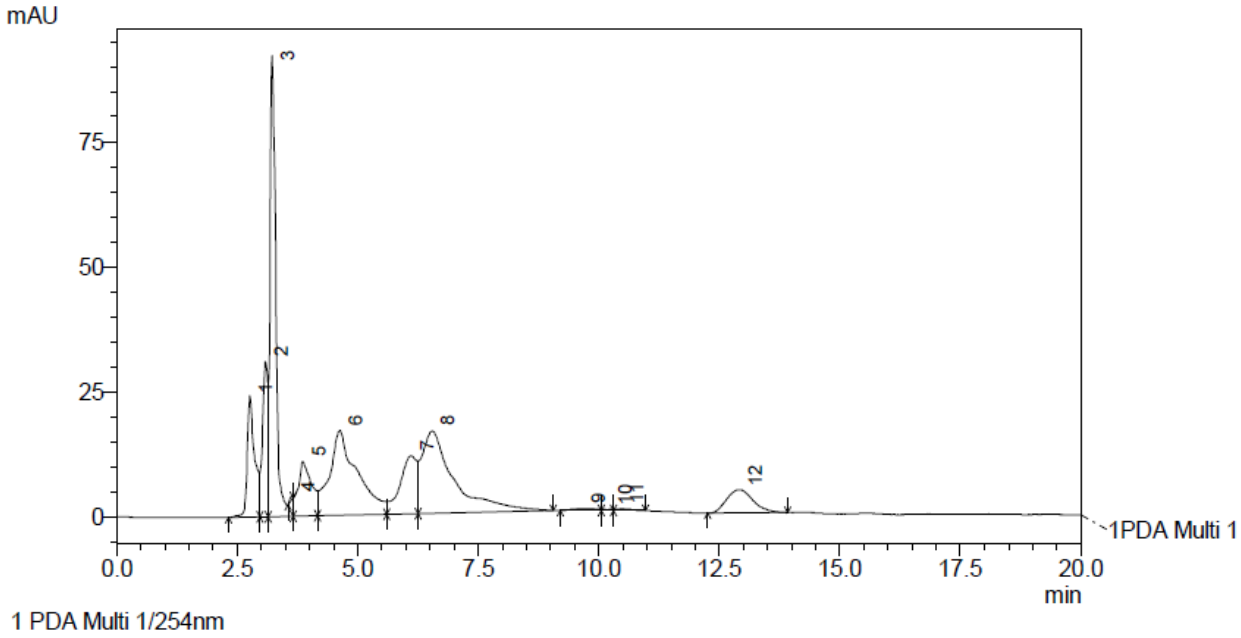


Figure 4. HPLC chromatogram of *Croton zambesicus* leaf extract.

microscopic analysis of leaves revealed the presence of cellulose, lignin, starch, oxalate crystals, tannin, oils, proteins and absence of mucilage in the leaves. The physicochemical parameters evaluated were: moisture content (4.10%) and total ash value (11.91%). The HPLC analysis had 12 peaks with number 3 having the highest peak. The chromatogram of the GC-MS analysis of the leaf of *C. zambesicus* had 57 compounds (Figure 5 and Table 3). The major compounds in the analysis were Caryophyllene (15.53%), beta-copaene (11.38%), alpha-myrcene (8.65%), 1,6-octachen3-ol-1,7-dymethyl- (4.77%), alpha-pinene (3.85%) and Ar-turmerone (3.41%).

Stomata are the microscopic pores on the leaf that facilitate gas and water exchange with the atmosphere and have a characteristics associated with photosynthesis and transpiration (Mansfield et al*.,* 1990). Stomata vary in size and density in different species and are greatly influenced by the environment. Both glandular and non-glandular trichomes were observed in *C. zambesicus*. Trichomes are tiny outgrowths from the plant epidermis that have ability to secret water, nectar, resins, mucilage and terpenes (Mansfield et al*.,* 1990). They serve as physical and chemical protection for the leaf against microbial organisms, aphids and insects ((Mansfield et al*.,* 1990). The cell wall provides mechanical strength and

Table 2. HPLC chromatogram of *Croton zambesicus* leaf extracts.

Retention time	Area
2.757	265244
3.082	245547
3.214	755307
3.616	3538
3.854	213504
4.623	680552
6.102	283292
6.536	766816
9.658	14719
10.219	3622
10.452	8637
12.912	179337

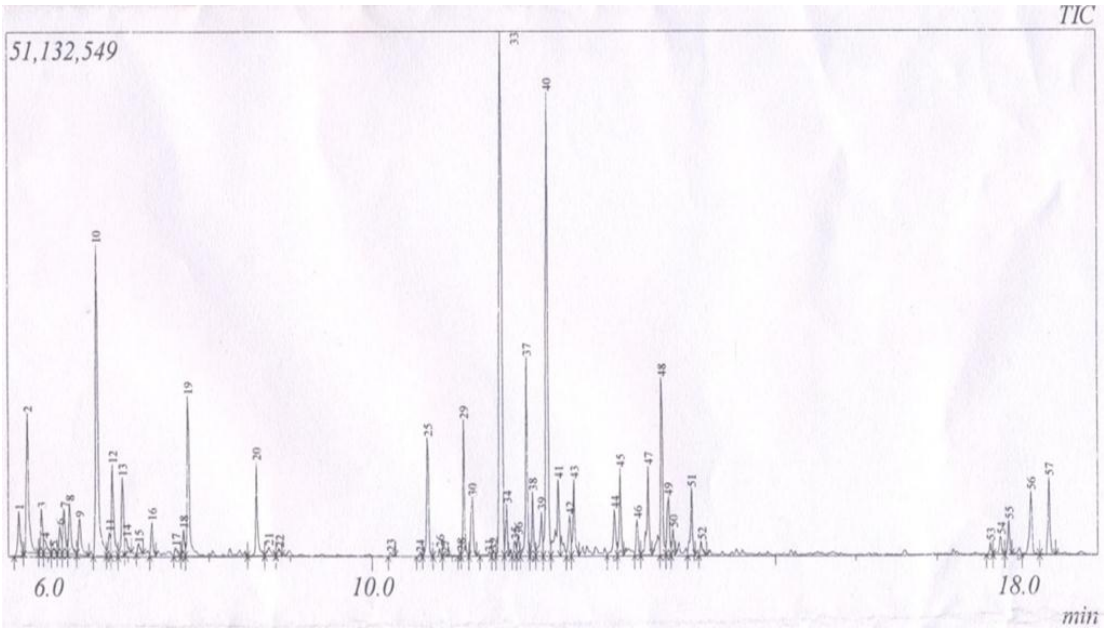


Figure 5. Chromatograph of GC-MS analysis of *Croton zambesicus* leaf oil.

support to the plant. Moisture content determination will lead to activation of enzymes and give the proliferation of living organism. Moisture content of crude plant is a function of its shelf life; the lower the moisture contents the longer the shelf life. Ash value is useful in determining authenticity and purity of samples and also these values are important qualitative standards. The ash value and moisture content for *C. zambesicus* is found to be 4.10 and 11.91% respectively. The ash value of *C. zambesicus* is slightly above the standard and the moisture content is within the acceptable value.

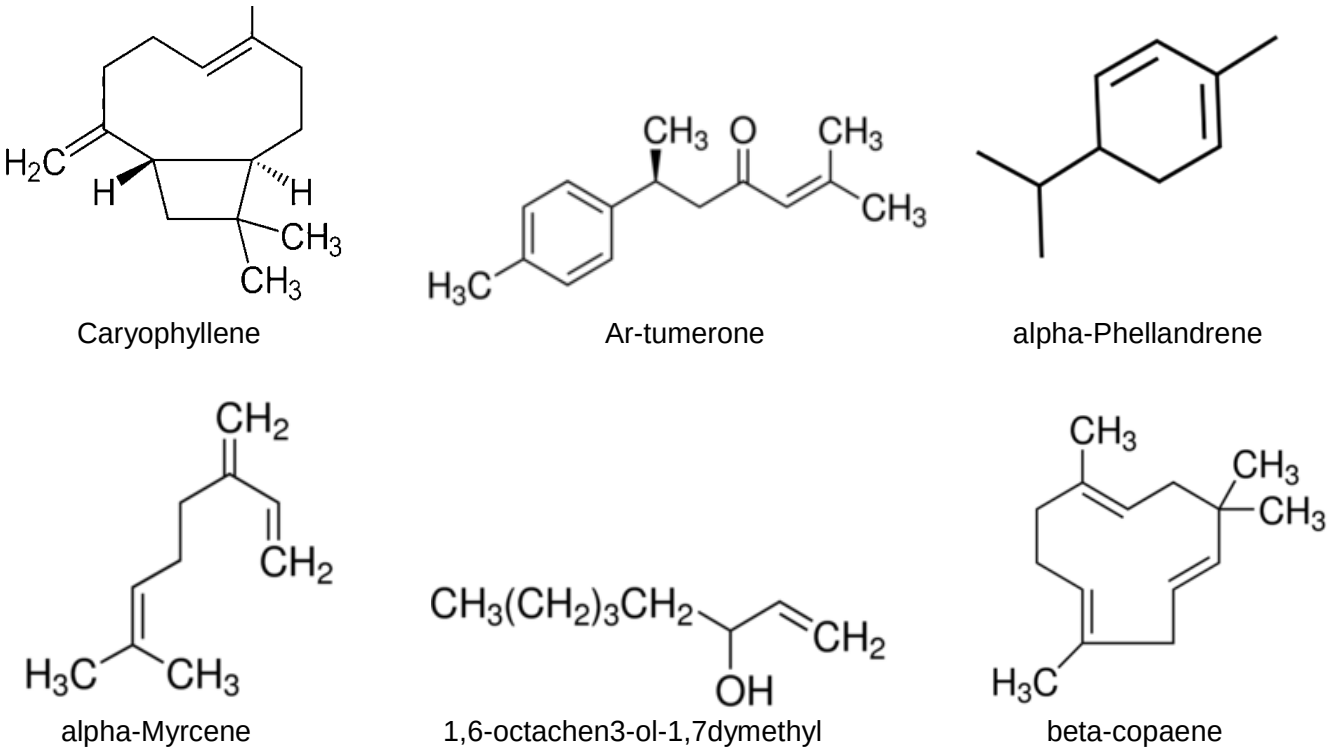
HPLC is a tool used in the standardization of complex drug which has the ability to estimate the presence of active marker quantitatively and qualitatively. GC-MS is a method that combines gas-chromatography and mass spectrometry to identify different substances within a test sample in drug detection and identification of unknown sample (Sparkman et al*.,* 2011). Caryophyllene is a terpene used for its pain-relieving and antimicrobial properties; beta-caryophyllene improves mood, and may also help prevent osteoporosis (Jürg et al., 2008). Terpenes are common compounds in plants responsible for distinct aromas and flavors in essential oils (Jürg et al., 2008). Due to the smell, taste, and ability to act as a dietary cannabinoid, beta-caryophyllene can be used as a flavoring agent or food additive (Jürg et al.,2008). It can also be used in cosmetics, creams, toothpaste, and other commercial products to enhance their therapeutic effects.

Table 3. Chromatographic profile of the oil of *Croton zambesicus* leaf.

Name of compound	Retention Time	Molecular formula	% Composition
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	5.633	$C_{10}H_{16}$	1.28
alpha.-Pinene	5.741	$C_{10}H_{16}$	3.85
Camphene	5.918	$C_{10}H_{17}$	1.03
Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl	5.956	$C_{11}H_{18}O$	0.44
Benzene, 1,2,4-trimethyl	6.067	C_9H_{12}	0.11
- 1-Octen-3-ol	6.150		0.66
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl	6.201	$C_{10}H_{16}$	1.16
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	6.273	$C_{10}H_{16}$	1.35
beta.-Myrcene	6.398	$C_{10}H_{16}$	1.08
alpha.-Phellandrene	6.615	$C_{10}H_{16}$	8.65
Cyclohexene, 4-methyl-3-(1-methylethylidene	6.770	$C_{10}H_{16}$	0.61
o-Cymene	6.809	$C_{10}H_{14}$	2.24
Cyclohexene, 1-methyl-5-(1-methylethenyl)-,	6.933	$C_{10}H_{16}$	1.96
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	6.975	$C_{10}H_{16}$	0.35
beta.-Ocimene	7.129	$C_{10}H_{16}$	0.28
gamma.-Terpinene	7.294	$C_{10}H_{16}$	0.77
Benzene, 1-ethyl-3,5-dimethyl-	7.585	$C_{10}H_{14}$	0.26
Cyclohexene, 1-methyl-4-(1-methylethylide	7.683	$C_{10}H_{16}$	0.56
1,6-Octadien-3-ol, 3,7-dimethyl-	7.742	$C_{10}H_{18}O$	4.67
endo-Borneol	8.586	$C_{10}H_{18}O$	2.15
Terpinen-4-ol	8.734	$C_{10}H_{18}O$	0.36
alpha.-Terpin	8.861	$C_{15}H_{24}$	0.22
4,4-Dimethyl-cyclohex-2-en-1-ol	10.227	$C_{10}H_{16}O$	0.09
gamma.-Elemene	10.593	$C_{15}H_{24}$	0.07
Cyclohexene, 4-ethenyl-4 -methyl-3 -(1-methylethenyl	10.694	$C_{15}H_{24}$	2.37
alpha.-Cubebene	10.838	$C_{15}H_{24}$	0.17
alpha.-Guaiene	10.899	$C_{15}H_{24}$	0.06
alpha.-ylangene	11.099	$C_{15}H_{24}$	0.08
alfa.-Copaene	11.142	$C_{15}H_{24}$	2.51
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl	11.248	$C_{15}H_{24}$	1.67
Caryophyllene	11.453	$C_{15}H_{24}$	0.18
1H-Cycloprop [e]azulene, 1a,2,3,4,4a,5,6,7b- octahydro:	11.508	$C_{15}H_{24}$	0.14
Caryophyllene	11.616	$C_{15}H_{24}$	15.53
1H-Cyclopenta [1,3] cyclopropa[1,2] benzene,	11.673	$C_{15}H_{24}$	1.07
Isoledene	11.774	$C_{15}H_{24}$	0.33
gamma.-Muurolene	11.814	$C_{15}H_{24}$	0.42
Humulene	11.923	$C_{15}H_{24}$	0.42
Alloaromadendrene	11.993	$C_{15}H_{24}$	1.27
.gamma.-Muurolene	12.101	$C_{15}H_{24}$	1.32
beta.-copaene	12.182	$C_{15}H_{24}$	11.38
gamma.-Elemene	12.312	$C_{15}H_{24}$	2.46
Naphthalene, 1,2,3,4,4a,5,6,8a- octahydro-7-methyl-4-methylene	12.452	$C_{15}H_{24}$	1.11
Naphthalene,1,2,3,5,6,8a-hexahydro- 4,7-dimethyl	12.506	$C_{15}H_{24}$	1.68
(-)-Spathulenol	13.011	$C_{15}H_{24}O$	1.34
Caryophyllene oxide	13.083	$C_{15}H_{24}O$	1.59
Globulol	13.292	$C_{15}H_{26}O$	0.84
1H- Cycloprop[e]azulen-7-ol, decahydro-1,1	13.432	$C_{15}H_{24}O$	2.16
Ar-turmerone	13.601	$C_{15}H_{200}$	3.41
Turmerone	13.682		1.48
1- Naphthalenol, decahydro-1,4a- dimethyl-7-(1-methylethylidene	13.741	$C_{15}H_{26}O$	0.52
Curlone	13.970	$C_{15}H_{22}O$	1.55

Table 3. Contd.

Tetradecanal	14.088	$C_{14}H_{28}O$	0.35
Acetic acid, 10- hydroxy-12a-methyl- 7-oxo-1,2,3,3a,3b,4	17.657	$C_{20}H_{28}O_4$	0.23
Trachylobane	17.785	$C_{20}H_{32}$	0.46
Podocarp-7-en-3-one, 13.beta.-methyl-13-vinyl	17.888	$C_{20}H_{30}O$	0.68
Podocarp-7-en-3-one, 13 a.-methyl-13-vinyl	18.167	$C_{20}H_{30}O$	1.77
Podocarp-7-en-3.beta.-ol, 13.beta.-methyl-13	18.391	$C_{19}H_{28}O_2$	1.72



Scheme 1. Chemical structures of some compounds in *Croton zambesicus* leaf oil.

Beta-copaene is a sesquiterpenoids and was first reported by Türkez et al. (2013).

Conclusion

The analyses carried out in this study can be used for identification and standardization of the plant. *C. zambesicus* has wide application in Africa for medicinal purposes and other economic uses. However, there is insufficient information on the chemical constituents, and internal structure of the plant which could aid in its further characterization. Hence, this study is aimed at bridging this research gap by determining the epidermal leaf anatomy, chemical constituent of the oil from the leaves by GC-MS analysis, HPLC analysis from the leaf extract, chemo-microscopic properties of *C. zambesicus* for proper identification, standardization of the plant as well as drug manufacturing from isolation of the various beneficial compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adamu A, Esievo KB, Ugbabe G, Okhale SE, Egharevba HO (2018). High performance liquid chromatography-diode array detection (HPLC-DAD) profiling, antioxidant and anti-proliferative activities of ethanol leaf extract of Berlinia grandiflora (Vahl) Hutch. Dalziel. Journal of Pharmacognosy and Phytotherapy 10(11):187-194.

African Pharmacopoeia (1986). General Methods for Analysis. OAU/SRTC Scientific Publications Lagos pp. 137-149.

Ayodele AE, Olowokudejo JD (1997). Systematic Importance of leaf and epidermal characters in West African species of family Myrtaceae. Boletim da Sociedade Broteriana 68:35-72.
Banso A, Adeyemo SO (2007). Phytochemical and antimicrobial evaluation of ethanolic extract of *Dracaena manni* Bark. Nigerian Journal of Biotechnology 18(1-2):27-32.
Bello MO, Hammed MA, Adekunle AS, Fasogbon OT (2014). Nutrient Contents and Fatty Acids Profiles of Leaves and Seeds of *Croton zambesicus*. Advance Journal of Food Science and Technology 6(3):398-402.
Boukhira S, Balouiri M, Bousta F, Moularat S, Taleb MS, Bousta D (2016). Antimicrobial Activities of Essential Oil of Five Plant Species from Morocco against Some Microbial Strains. International Journal of Pharmacognosy and Phytochemical Research 8(11):1901-1906.
Charak Samhita, Charaka Chandrika (2007). Hindi commentary, by Dr. Brahmanand Tripathi and Dr. Ganga Sahay Pandey, Siddhi Sthana Chapter 12, Verse No. 39. Chaukhamba Surbharti Prakashan P 1342.
Evans MC (2002). Trease and Evans pharmacognosy. 15th Edition. WB Sanders. London, pp. 183-393.
Irvine FR (1961). Woody plant of Ghana. Oxford University Press. London.
Jürg G, Marco L, Stefan R, Ildiko R, Jian-Zhong C, Xiang-Qun X, Karl-Heinz A, Meliha K, Andreas Z (2008). Beta-caryophyllene is a dietary cannabinoid. Proceedings of the National Academy of Sciences of the United States of America 105(26):9099-9104.
Kokate CK (1994). Practical Pharmacognosy, 4th Edition. Vallabh Prakashan, New Delhi pp. 112-120.
Kumar RS, Rajkapoor B, Perumal P (2011). Antioxidat Potetial *of Digofera liaeialia* vitro study. Pharmacology online 1: 710-720.
Manikandaselvi S, Vadivel V, Brindha P (2016). Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant. Research Journal of Medicinal Plants 10:127-139.
Mansfield TA, Hetherington AM, Atkinson CJ (1990). Some current aspects of stomatal physiology. Annual Review: Plant Physiololgy. Annual Review of Plant Biology 41(1):55-75.
Metcalfe CR, Chalk L (1979). Anatomy of Dicotyledons. Vol. I. Second Edition. Oxford University Press, New York.
Ogundajo IA, Akintayo L, Ogunwande HG. Gbadamosi RG, Guido F (2014). Chemical Composition of the Leaf Essential Oils of Croton zambesicus Müll.-Arg. Grown in Lagos, South-West Nigeria. European Journal of Medicinal Plants 4(12):1524-1533.
Ogundajo LA, Adeniyi OO, Denloye, OAL (2010). Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry Lagoons, Lagos, Nigeria.
Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L (Rubiaceae) collected from North Central Nigeria. International Journal of Bioassays 7:5630-5637.
Sparkman DO, Penton Z, Kitson FG (2011). GC-MS a practical guide. Academic press. ISBN 98-0-08-092015-3.
Springfield EP, Eagles PK, Scott G (2005). Quality assessment of South African herbal medicines by means of HPLC fingerprinting. Journal of Ethnopharmacology 101(1-3):75-83.
Türkez H, Celik K, Togar B (2013). Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology 66(4):597-603. DOI: 10.1007/s10616-013-9611-1.
Ugbabe GE, Ayodele AE (2008). Foliar epidermal studies in the family Bignoniaceae Juss. in Nigeria. African Journal of Agricultural Research 3(2):154-166.