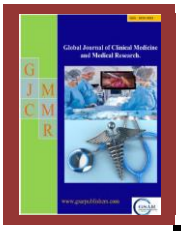


Global Journal of Clinical Medicine and Medical Research [GJCMMR]
ISSN: 2583-987X (Online)
Abbreviated key title: Glob.J.Clinic.Medici.Medica.Res.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjcmmr-home/

Gas Chromatographic Analysis of Bioactive Compounds in *Rothmannia whitfieldii and Penthacletra macrophylla, Lonchocarpus cyanescens Curcuma longa* and *Duranta repens*

By

Adjero, L.A.*[1], Ezejiofor, T.I.N.,[2] Udebuani, A.C.[2] Duru, C.M.,[1] Nwachukwu, M.O.[1] & Ibeh, O.O.[1]

[1]Department of Biology, Federal University of Technology, Owerri, Imo State Nigeria
[2]Department of Biotechnology, Federal University of Technology, Owerri, Imo State Nigeria

Article History

Received: 05/04/2025
Accepted: 15/04/2025
Published: 17/04/2025

Vol – 3 Issue – 4

PP: -07-13

Abstract

This study characterized the bioactive components in Rothmannia whitfieldiiand Penthacletra macrophylla, Lonchocarpus cyanescens Curcuma longa and Duranta repens to establish its usefulness in replacing costly synthetic dyes. Identification and quantification of the bioactive attributes were performed using gas-liquid chromatography with flame ionization detector (GC-FID) after extraction with n-Hexane. The results further revealed the identity of the following bioactive compounds present in the GC-MS analysis carried on the plant extracts: 2-Methoxy-4-vinylphenol in LCE & CLE; LCE, Dodecanoic acid, methyl ester in DRE, & RWSHE; Methyl tetradecanoate in LCE, DRE, & RWSHE; Di-sec-butyl phthalate in LCE, DRE, & RWSHE; Hexadecanoic acid, methyl ester in LCE, CLE RWSHE, RWSE, & PME; n-Hexadecanoic acid in LCE, DRE, & RWSE; Oleic Acid in LCE, DRE, RWSE, & PME; 6-Octadecenoic acid, (Z)- in LCE, DRE, & PME; 2,3-Dihydroxypropyl elaidate in LCE & PME; Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- and aR-Turmeronein CLE & PME; cis-13-Octadecenoic acid, methyl ester in CLE & RWSE; Dodecanoic acid in DRE, RWSE, RWSHE, & PME; Methyl 13-methyltetradecanoate in DRE & RWSHE; 9-Octadecenoic acid in DRE, RWSE, RWSHE, & PME; Hexadecanoic. The plant used in this study (Curcuma longa, Pentclethra macrophylla, Duranta repens, Lonchocarpus cyanescens, and Rowthmania whitfieldii) may serve as an eco-friendly alternative to their synthetic counterparts and cost effective biological stains.

Keywords: *Gas Chromatographic, Bioactive Compounds, Rothmannia whitfieldiiand, Penthacletra macrophylla, Lonchocarpus cyanescens Curcuma longa, Duranta repens*

Introduction

Pentaclethra macrophylla equally known as African oil bean; and in Nigeria as Ugba/Ukpaka in Igbo, Apara in Yoruba and Ukana in Efik [1] is an evergreen tree growing to about 21m in height and 6m in girth, in well drained acidic medium loamy and heavy clay soils (Keay, 1989). The tree has a peculiar branching habit which allows enough quality of light under its canopy [2]. The back has a reddish-brown to grey colour with irregular patches. The leaves are glabrous measuring about 20-45 centimetres long and covered with rusty hairs of 10-20 pairs of stout pinnal [3]. The tree trunk is buttress and crooked, oozing out reddish orange coloured fluid when cut [4]

It occurs from Senegal to Angola, Islands of Pimcipe and Sao Tome and endemic to the humid and sub humid zones of West Africa with the annual rainfall of 1000 – 2000mm and mean temperature of 18 – 25^{0}C [5].

Pentaclethra macrophylla is planted on the fringes of compound farms, sides of road as shade trees and around communities as cash crops [6]. It is a major component of Agro-forestry system [8]. Leaves shade during dry season is believed by farmers to contribute to soil fertility [7]. Its empty fruit pods and wood is highly suitable for fuel and charcoal making [2]. Flowers from the tree produced twice a year in March and April and between June and November which are colourfully yellow and pinkish-white, sweet smelling attracts a lot of insects including the honey bee [3].

Curcuma longa (L.) (Turmeric) also known as “Golden spice” or “Indian saffron” is an erect perennial underground stem rhizomatous herb of the ginger family *Zingiberaceae*growing up to the height of one meter (1m) [9]/ Turmeric is native to

*Corresponding Author: **Adjero, L.A***

the South and Southeast Asian region, but with India being the largest producer, consumer, and exporter in the world (Lal, 2012), having a strong connotation with their socio-cultural life. It has also gained widespread uses in the tropics of Austria and Africa, Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, and the Philippines. They thrive marvelously on different soil types, ranging from light black, sandy loam, and red soils to clay loams. They require a temperature between 20^0C and 30^0C, with considerable moderate amount of annual rainfall [5].

Duranta repens, also known as *Duranta erecta* is a flowering shrub or small tree in the verbenaceae family that can grow up to 6m tall and can spread to an equal width [10]. It is a native plant of Asia, Africa, and South and Central America [11]. *D. repens* is a weed of disturbed sites, waste areas, roadsides, wetter pastures, open woodlands and densely forested areas, and particularly along waterways in sub-tropical and tropical regions, which is widely cultivated as an ornamental plant throughout the world, and has become naturalized in many places [1]. Common names include Brazilian sky flower, Brazilian skyflower, duranta, forget me not bush, forget me not tree, golden dew drop, golden dewdrop, golden dewdrop duranta, golden dewdrops, golden tears, pigeon berry, pigeon-berry, pigeon berry, sky flower, sky-flower, skyflower [12].

Rothmannia whitfieldii is an evergreen tree belonging to the family of Rubiaceae. They are found widely distributed within tropical Africa, spreading from East of Senegal, Sudan, South Angola, Nigeria, Zinbabwe, Gabon [13]. Its common names are; Diola in Senegal, Kissi in Sierra Leone. In Nigeria, the local names are 'Okunkin', 'Obong', 'Nsun, 'Uri' [14]. *Rothmannia* comprises about 30 species distributed in Africa, Madagascar, and Asia. About 18 species are present in tropical Africa.This plant is usually found in forest undergrowth, usually in secondary forest, but also in savannah woodland at elevation up to 1,700 meters.

The genus *Rothmannia* is one of the few that has three leaves appearing from the same node. The leaves are opposite, simple, and entire. It has pinnate venation with 8-15 pairs of lateral veins. The enormous slightly scented pendant flowers with huge enlarged stigma hanging on a thread-like style make it easily recognized. In the absence of flowers and fruits, they are readily identified by the leaves that are up to 30cm long with dense velvety hairs on their under surface. The stipules are triangular and about 6mm long. Propagation is by seed. The fruit is globose, being 3-7cm in diameter, smooth and ribbed [15] They appear velvet brown pubescent when young but glabrescent. The pod contains many seeds crowned by a persistent calyx. The seeds are lens-shaped with a dimension of 7-11mm × 3-4mm [13] Rothmannia has variations in its flowers. They can be long tubed or short tubed.

Lonchocarpus cyanescens is a woody climbing shrub that grows to about 2.5 meters tall, with pinnate compound leaves and large pinnacles of reddish flowers turning blue [1]. It is well distributed and cultivated in West Africa but recently introduced to peninsular Malaysia.

In Nigeria, *L. cyanescens* is found growing wild with common names as Anunu by the Igbos, Elo in Yoruba, Talaki in Hausa, Suru in Tiv and Ebelu in Edo ([16]. The aerial parts of the plant yields indigo; a useful colorant known as 'gara' for dying [17].

The plant is utilized in traditional medicine were the leaves and roots are applied as a pouttice to treat skin diseases and ulcers in Sierra Leone, leprosy in Ghana, laxative in as well as for intestinal disorders and dysentery in Benin [18]. Equally, its leaf and root decoction is used in Nigeria to treat arthritis, venereal diseases and diarrhoea [3].

Lonchocarpus cyanescens contains alkaloids, flavonoids, tannins and saponins [19]. Its leaves have in doxyl, oleanane derivatives and glycyrrhetinic (Moronkola & Oladosu, 2013). Indoxyl yields indigotin contained in dye stuffs, *L. cyanescens* anti-inflammatory, anti-arthritic and its relief on ulcer due to the bioactive substances; oleanane against inflammations and ulcer and triterpenes against arthritis has been demonstrated by [4].

Materials and methods

Collection of Plant Samples

Plant samples were collected from different parts of Imo State; Akabor (Mbaise) (Ahiazu Mbaise Local Government area), Ihiagwa, and Obinze (Owerri West Local Government area) all in Imo state. *Rothmannia whitfieldii*and *Penthacletra macrophylla* were collected from Akabor in Mbaise, *Lonchocarpus cyanescens* and *Duranta repens* from FUTO campus, while the rhizome of *Curcuma longa* wasprocured from Ihiagwa market. Field location and character was captured using the Global Positioning System (GPS). The habit and morphological features of the specimens were captured using FUJI film digital camera, Fine pix S4250. The collected plant samples were taken to a taxonomist in the Department of Biology, Federal University of Technology owerri for proper identification and classification. Then, the samples were labeled appropriately and analyzed at the laboratories of the Departments of Biological science, Science Lab technology (SLT), and Crop science, Federal University of Technology, Owerri. The Location Name and Geographic Coordinates of plant sampling areas is shown in Table 1.

Table 1: Location Name and Geographic Coordinates of plant sampling areas

S/N	Name Of Location	Longitude	Latitude
1	Ahiazu Mbaise	5.7070° N	6.7909° E
2	Ihiagwa	6.4002^0N	4.5370^0E
3	Obinze	4.8396° N	6.9112° E

GC/MS Experimental Procedures

The procedures used to carry out the analysis in the laboratory are as follows:

*Corresponding Author: **Adjero, L.A**

1. The standard used were straight alkanes, aromatic halide alkyl halide, aromatic ether, and alkanes.
2. The console was entered and GC/MSD icon was clicked to open the GC/ MSD.
3. The analysis was then set-up by clicking on the green arrow.
4. The "Ok" and "Run method" button was clicked to begin the GC/MS automation
5. As run progresses, and the approximate 3 minute solvent delay has passed, peaks started appearing in the smaller sub-windows.
6. Useful data were obtained from the sub- windows. The peaks retention time was displayed at the top of the mass spectrum sub window.
7. In the mass spectrum sub- window, a library comparison search was performed by double right clicking the lower – sub- window.
8. The entire spectrum were analyzed to ensure that the snap shot included every peak.
9. As soon as the run was completed, another sample was introduced. The instrument required a few minutes to return the GC oven temperature back to the 40 OC starting point.

Chromatographic conditions

HP Gas chromatograph 5890 series II, software - HP ChemStation Rev.A 09.01[1206], Column type – HP -5MS and HP INNOWax, Agilent Technologies India Pvt. Ltd, Hyderabad, India. Injection temperature - split injection; split ratio - 20:1; carrier gas – nitrogen, inlet temperature – 250^{o}C; column dimension (30 m × 0.25 mm × 0.25 µm); detector and detector temperature – fid (320^{o}C); flow rate – 1.0 ml/min; hydrogen gas pressure – 30 psi; compressed air – 35 psi; oven programs (Carotenoids and sterols - initial temperature @ 60^{o}C. first ramping @ 10 ^{o}C/min for 20 min, maintained for 2 min second ramping @ 15^{o}C/min for 4 min.).

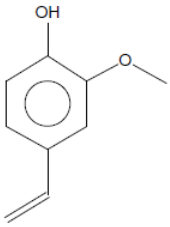
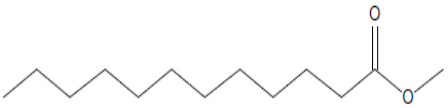
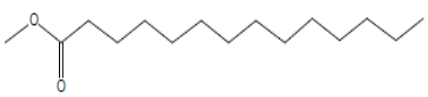
Results and discussion

Gas chromatography-mass spectroscopy profiling of the plant extracts

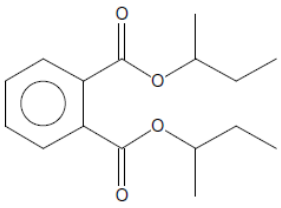
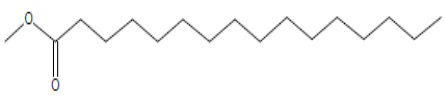
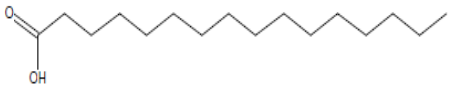
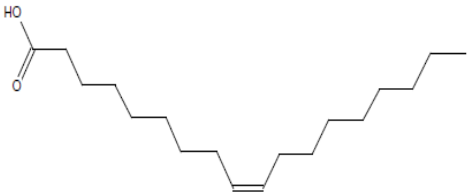
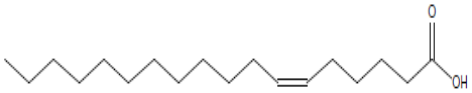
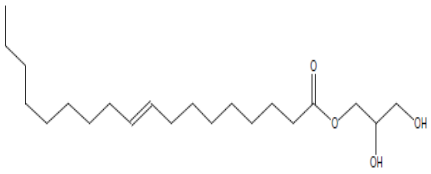
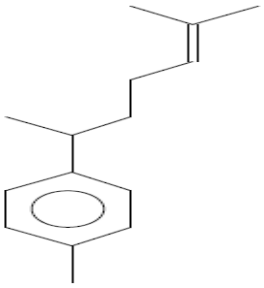
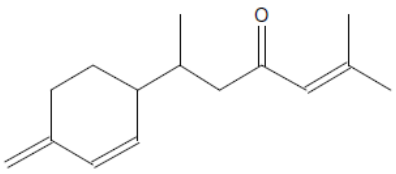
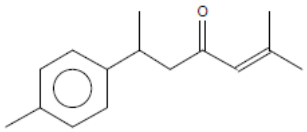
Results of the GC-MS analysis of the plant extracts indicating the identity, chemical structure and molecular formula of different compounds present in the plant extracts at various retention times used in the study is shown in Table 2. The results showed that a total of 20 compounds were identified from the GC-MS analysis of methanol fraction of *Curcuma longa, Pentclethra macrophylla, Duranta repens, Lonchocarpus cyanescens, and Rowthmania whitfieldii)* leaves exhibiting various phytochemical activities.

The results further revealed the identity of the following bioactive compounds present in the GC-MS analysis carried on the plant extracts: 2-Methoxy-4-vinylphenol in LCE & CLE; LCE, Dodecanoic acid, methyl ester in DRE, & RWSHE; Methyl tetradecanoate in LCE, DRE, & RWSHE; Di-sec-butyl phthalate in LCE, DRE, & RWSHE; Hexadecanoic acid, methyl ester in LCE, CLE RWSHE, RWSE, & PME; n-Hexadecanoic acid in LCE, DRE, & RWSE; Oleic Acid in LCE, DRE, RWSE, & PME; 6-Octadecenoic acid, (Z)- in LCE, DRE, & PME; 2,3-Dihydroxypropyl elaidate in LCE & PME; Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- and aR-Turmeronein CLE & PME; cis-13-Octadecenoic acid, methyl ester in CLE & RWSE; Dodecanoic acid in DRE, RWSE, RWSHE, & PME; Methyl 13-methyltetradecanoate in DRE & RWSHE; 9-Octadecenoic acid in DRE, RWSE, RWSHE, & PME; Hexadecanoic acid, ethyl ester in DRE & RWSHE; cis-Vaccenic acid, Methyl stearate, Octadecanoic acid, ethyl ester, Tetradecanoic acid, Undecanoic acid, 10-methyl-, methyl ester, 9-Octadecenoic acid, methyl ester, (E)-, 11-Octadecenoic acid, Phthalic acid, ethyl pentyl ester in DRE, RWSE, RWSHE, & PME DRE, RWSE, RWSHE, & PME DRE & RWSHE DRE, RWSE, RWSHE, & PME RWSE & PME DRE & RWSE RWSE & RWSHE respectively.

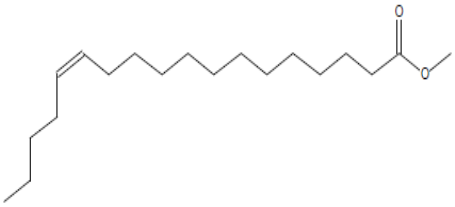
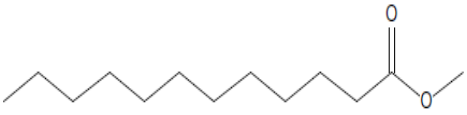
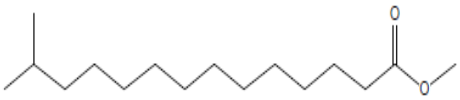
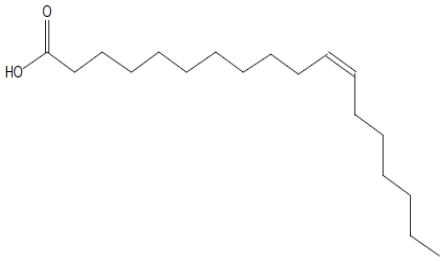
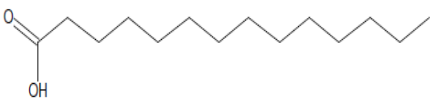
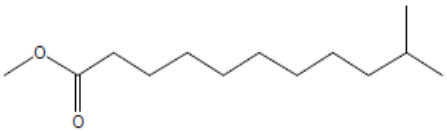
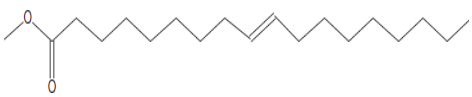
Table 2: Analyzed GC-MS Results of the plant extracts used

R.et. time (Min)	Identity of compound	Chemical structures	Mol. weight	Molecular formular	Present in
4.062	2-Methoxy-4-vinylphenol		150	$C_9H_{10}O_2$	LCE & CLE
5.205	Dodecanoic acid, methyl ester		214	$C_{13}H_{26}O_2$	LCE, DRE, & RWSHE
6.382	Methyl tetradecanoate		242	$C_{15}H_{30}O_2$	LCE, DRE, & RWSHE

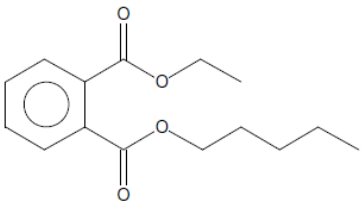
*Corresponding Author: **Adjero, L.A**

7.200	Di-sec-butyl phthalate		278	$C_{16}H_{22}O_4$	LCE & PME
7.457	Hexadecanoic acid, methyl ester		270	$C_{17}H_{34}O_2$	LCE, CLE RWSHE, RWSE, & PME
7.954	n-Hexadecanoic acid		256	$C_{16}H_{32}O_2$	LCE, DRE, & RWSE
8.600	Oleic Acid		282	$C_{18}H_{34}O_2$	LCE, DRE, RWSE, & PME
8.794	6-Octadecenoic acid, (Z)-		282	$C_{18}H_{34}O_2$	LCE, DRE, & PME,
10.783	2,3-Dihydroxypropyl elaidate		356	$C_{21}H_{40}O_4$	LCE & PME
5.016	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-		202	$C_{15}H_{22}$	CLE & PME
6.382	2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one		218	$C_{15}H_{22}O$	CLE & PME
6.177	aR-Turmerone		216	$C_{15}H_{20}O$	CLE & DRE

*Corresponding Author: **Adjero, L.A**

8.629	cis-13-Octadecenoic acid, methyl ester		296	$C_{19}H_{36}O_2$	CLE & RWSE
5.205	Dodecanoic acid		200	$C_{13}H_{26}O_2$	DRE, RWSE, RWSHE, & PME
6.937	Methyl 13-methyltetradecanoate		256	$C_{16}H_{32}O_2$	DRE & RWSE
5.800	9-Octadecenoic acid		282	$C_{18}H_{34}O_2$	DRE, RWSE, RWSHE, & PME
7.788	Hexadecanoic acid, ethyl ester		284	$C_{18}H_{34}O_2$	DRE & RWSHE
8.200	cis-Vaccenic acid		282	$C_{18}H_{34}O_2$	DRE, RWSE, RWSHE, & PME
8.434	Methyl stearate		298	$C_{19}H_{38}O_2$	DRE, RWSE, RWSHE, & PME
8.737	Octadecanoic acid, ethyl ester		310	$C_{20}H_{38}O_2$	DRE & RWSHE
6.714	Tetradecanoic acid		310	$C_{14}H_{28}O_2$	DRE, RWSE, RWSHE, & PME
5.205	Undecanoic acid, 10-methyl-, methyl ester		214	$C_{13}H_{26}O_2$	RWSE & PME
8.326	9-Octadecenoic acid, methyl ester, (E)-11-Octadecenoic acid		296	$C_{19}H_{36}O_2$	DRE & RWSE

*Corresponding Author: **Adjero, L.A**

7.200	Phthalic acid, ethyl pentyl ester		264	$C_{15}H_{20}O_4$	RWSE & RWSHE

Discussion

The GCMS composition of *Curcuma longa, Pentclethra macrophylla, Duranta repens, Lonchocarpus cyanescens, and Rowthmania whitfieldii* was in concordance with the findings of [19] who identified some volatile compounds such as acids, esters, alcohols, aldehydes, phenol, etc which were also identified in this study. Among the identified bioactive components, 2,3-Dihydroxypropyl elaidate has highest percent peak area. This is comparable to the finding of [20] in their study on GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. This compound has been reported to poses antioxidant and antibacterial properties. 2-Methoxy-4-vinylphenol has antimicrobial, antiseptic, hair-conditioning agent, and skin-conditioning agent-emollient properties. n-Hexadecanoic acid has antioxidant, 5- alpha-reductase inhibitor, anti-fibrinolytic, hemolytic, antimicrobial activity, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, and hemolytic properties [21]. 9-Octadecenoic acid has anti-inflammatory, cancer preventive, hepatoprotective, antioxidant, and hypocholesterolemic properties. These compounds were reported by other scholars to have antimicrobial, antioxidant, hepatoprotective, and hypocholesterolemia as well as cancer preventive activities.

Similarly, hexadecenoic acid is found mostly in plants, animals, or micro-organism as a form of saturated fatty acid. It is used as release agents, soap production, and cosmetics. The following therapeutic benefits of hexadecanoic acid have been documented: hypocholesterolemia, antioxidant, antifungal, and antibacterial activity [22]. Diets high in unsaturated fatty acids have generally been shown to lower total cholesterol, which lowers the chance of developing chronic heart disease by a considerable amount. These bioactive substances have the potential to greatly increase the nutritional and therapeutic benefits of DRE & RWSHE used in this study.

References

1. Ali, H. M., Shehata, S. F. & Ramadan, K. M. A. (2016). Microbial decolorization and degradation of crystal violet dye by *Aspergillus nigerInternational Journal of Environmental Science and Technology*, 13(12): 2917-2926.
2. Alim-un-Nisa, Naseem, Z. & Yasha, N. B. (2016). Sudan Dyes and Their Potential Health Effects. *Pakistan Journal Biochem. Mol. Biol*ogy, 49(1): 29-35
3. Almeida, L. P. (2011). Caracterização de pigmentos da Curcuma longa L., avaliação da atividade antimicrobiana, morfogênese in vitro na produção de curcuminóides e óleos essenciais. Belo Horizonte, 2006. 120 p. [Thesis of PhD degree. Faculty of Pharmaceutical Sciences, Federal University of Minas Gerais].
4. Alturkistani, H., Tashkandi, F., & Mohammedsaleh, Z. (2015). Histological Stains: A Literature Review and Case Study. *Global Journal of Health Science*, 8(3): p.72.
5. Alupului, A., (2012). Microwave extraction of active principles from medicinal plants. U.P.B. Science Bulletin, Series B 74(2).
6. Amini, M. & Younesi, H. (2009). Biosorption of Cd(II), Ni(II) and Pb(II) from aqueous solution by dried biomass of *Aspergillus niger*. Application of response surface methodology to the optimization of process parameters. *Clean*, 37: 776–786
7. Anderson, J. (2011). An introduction to Routine and special staining. Retrieved from http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-routine-and-special-staining February 18, 2020.
8. Antai, A. B., Anaele, B. A., & Etta, K. M. (2005). Hypoglycemic action of a medicinal herbs. *Rothmannia hispida* in diabetic rats. *Mary Slessor Journal of Medicine*, 5(2): 21-24.
9. Bar-Sela, G., Epelbaum, R., & Schaffer, M, (2010). Curcumin as an anti-cancer agent: review of the gap between basic and clinical applications. *Current Medicinal Chemistry*, 17: 190-197.
10. Bassey, R., Oremosu, A., & Osinubi, A. (2012). *Curcuma longa*: staining effect on histomorphology of the testis. *Macedonia Journal of Medical Sciences*, 5(1): 26-29.
11. Bather, M. (2009). “Clothes Make the (Hu) Man”. *Science*, 325(5946): 1329
12. Baxter, R. (2020). Interpretation of histological sections: Stains used in histology. Retrieved from https://www.kenhub.com/en/library/anatomy/interpretatio n-of-histologic-sections-stains-used-in-histology November 25, 2020
13. Beeker, K., Harmsen, D., Mellmann, A., Meier, C., Schumann, P., Peters, G., & Von Eilf, C. (2004). Development and evaluation of a quality controlled ribosomal sequence database for 165 ribosomal DNA based identification of *Staphylococcus species. Journal of Clinical Microbiology*, 142(11): 4988-4995.
14. Beveridge, T. J. (2001). "Use of the Gram stain in microbiology". *Biotechnic and Histochemistry*, **76** (3): 111–118.

*Corresponding Author: **Adjero, L.A**

15. Bhatia, S. C. (2017). Pollution control in textile industry WPI Publishing. Pp. 34-45.
16. Bhattacharyya, N. (2010). Natural dyes and their eco-friendly application. IAFL, New Delhi. Pp 123.
17. Bhuyan, K., Singh, S. B., & Bhuyan, P. K. (2013). Application of generalized singular value decomposition to ionospheric tomography, *Ann. Geophys*., 22: 3437–3444
18. Antai, A. B., Ofem, O. E., Nwosu, O. J., Ukafia, S. O., Iyadi, K. C., & Nia, R. (2010). Comparative effect of *Rothmannia hispida* leaves extract and protamine – zinc insulin on alloxan-induced diabetic rats. *Journal of Diabetes and Endocrinology*, 101(4): 52-58.
19. Aquino, J. M., Rocha-Filho, R. C., Ruotolo, L. A., Bocchi, N. & Biaggio, S. R. (2014). Electrochemical degradation of a real textile wastewater using β-PbO2 and DSA anodes. *Chemical Engineering Journal*, 251: 138-145.
20. Arora, J., Agarwal, P., & Gupta, G. (2017). Rainbow of Natural Dyes on Textiles Using Plants Extracts: Sustainable and Eco-Friendly Processes. *Green and Sustainable Chemistry*, 7, 35-47
21. Araujo, C. C. & Leon, L. L. (2001). Biological activities of Curcuma longa L. *Mem Inst Oswaldo Cruz*, 96: 723-28.
22. To Biological Activity. *Current Nutrition & Food Science*, 5: 225-237.

*Corresponding Author: **Adjero, L.A**