Standard Scientific Research and Essays Vol 11(2): 035-044, February, 2023 (ISSN: 2310-7502)
http://www.standresjournal.org/journals/SSRE

Research Article

Comparative Study on the Anti-Anaemic Potential of *Jatropha tanjorensis* (Hospital too far) Leaf Extract with a Standard Blood Syrup and the Identification of the Bioactive Compounds in the Leaf Extract

***Nnawuihe GC and Achilike JJ**

Department of Chemistry/Biochemistry, Federal Polytechnic Nekede, Owerri, Imo State, Nigeria

*Corresponding Author Email: gidychris@gmail.com

Accepted 10, February, 2023

Abstract

***Jatropha tanjorensis* (Hospital too far) contain numerous bioactive compounds which are greatly responsible for their pharmacological properties. In this study, the Anti-Anaemic Potential of *Jatropha tanjorensis* (Hospital too far) Leaf Extract was compared with a Standard Blood Syrup and the Identification of the Bioactive Compounds in the Leaf Extract was also carried out. The leaves were air-dried and ground into powder using a pulverizing machine, the powdered samples were extracted using ethanol. The result of the anti-anaemic properties of the extract showed that the haemoglobin (HB) level ranged from 13.20±0.10 g/dl to 15.35±0.15g/dl, the packed cell volume (PCV) ranged from 38.00±1.00% to 46.50±0.50%, the White blood cell ranged from 3.85×10^{9}/L ±0.05 to 9.25×10^{9}/L ±0.05, the Red blood cell ranged from 5.35×10^{12}/L ±0.15 to 5.60×10^{12}/L ±0.10, the Platelet count ranged from 107.00×10^{9}/L ±1.00 to 365.00×10^{9}/L ±15.00. The result of the GC-MS and FTIR characterization showed that the following bioactive compounds were found in the leaf; 1-propanol, Erythritol, 2-dimethyl(prop-2-enyl) silyloxypropane, threitol, 2-o-methyl-D-mann opyranose, 2-pentadecanoic-6,10,14-trimethyl, pentadecanoic acid, n-hexadecanoic acid, 9-octadecenoic acid, phytol, 11-octadecenoic acid, oleic acid, methyl stearate and methyl 20-methyl-heneicosanoate which appears at different peaks from their FTIR spectrum. However, the bioactive compounds present in the plant has been found from literatures to be responsible for the following pharmacological properties; antibacterial, antifungal, antidiuretic, antimalaria, antiasthma, anticancer, cholinomimetic, antiarrhythmic, analgesic, antihperglycemic etc**

Keywords: Bioactive compounds, anti-anaemic, pharmacological properties, characterization

INTRODUCTION

Jatropha tanjorensis (Hospital too far) is a perennial herd that belongs to the family Euphorbiaceac which common name includes: lapalapa, Catholic vegetable, and in Yoruba language is called “Iyana Ipaja”, in Igbo language, it is called “Ugu Oyibo” (Oyewole and Akingbala, 2011).
It is a traditionally used medicinal plant in south-eastern Nigeria with many claims from consumers that it possesses

blood replenishing properties.

Many researchers have reported that *Jatropha tanjorensis* leaves contain beta blockers, anti-cancers agents, anti-microbial activities, anti-plasmodia and anti-oxidant effects against oxidative stress induced by malaria parasite (Duff, 2018).

Also, it has been researched that *Jatropha tanjorensis* leaf is a natural remedy against diseases and it is highly nutritious and edible, and possesses antiseptic and anti-hypertensive properties (Oboh and Masodje, 2019).

As earlier mentioned, *Jatropha tanjorensis* leaf is traditionally used as a medicinal plant in boosting or replenishing blood in the body. This indicates that *Jatropha tanjorensis* leaf has bioactive properties that enhance blood building in the body.

After a comparative study on the anti-anaemic potential of *Jatropha tanjorensis* leaf with well known standard blood syrup, it is therefore imperative to characterize the extract of *Jatropha tanjorensis* leaf in order to establish the bioactive compounds for the potency of *Jatropha tanjorensis* leaf (Iwualewa *et al*., 2015).

Aneamia is a medical condition in which the capacity of the blood to transport oxygen to the tissue is reduced either because of too few red blood cells or because of too little haemoglobin resulting in pallor and fatigue.

Anti-anaemic medications are medications administered in order to inhibit (to stop) anaemic activities in the body. *Jatropha tanjorensis* leaf as traditionally used is believed to be an anti-anaemic medication, but not scientifically proved, especially in comparism with a known standard blood medication (Prabakaran and Sujatha, 2021).

The identification of the bioactive compounds in the leaf extract of *Jatropha tanjorensis* is also important, owing to the fact that the result of the identification of the bioactive compounds of the leaf extract will help us to know the chemical compounds, possible functional groups responsible for the various health/medicinal properties of the plant. However, the present study is aimed at comparing the anti-anaemic potential of *Jatropha tanjorensis* leaf with standard blood syrup in order to ascertain the level of anti-anaemic potential of *Jatropha tanjorensis* leaf.

MATERIALS AND METHODS

Materials and Reagents

The materials used in this study were Hewlett-Packard (HP) 6890 gas chromatograph, Shimadzu FTIR–8400s, Rotary Evaporator, Conical flask, Whatman No. 44 filter paper, Sahli'shaemoglobin meter, Neubaurs counting chamber, Capillary tube, Haematocrit centrifuge, Pippete, Microscope. The reagents used were distilled water, Ethanol (C_2H_5OH), Phenyl hydrozane, N-hexane (C_6H_{14}) Silica gel, Potassium bromide (KBr). All reagents used were of Analytical grade (AG) so further purification were not required.

Sample collection and Preservation

Fresh mature leaves of *Jatropha tanjorensis* were collected from Akwete, Ezigaragu, Enyiogugu in Aboh Mbaise local government area of Imo state and was authenticated by Mr. Duru, N.C. The fresh leave of were washed with distilled water to remove dirts from the leave; the leaves were cut into different sizes and air-dried for one week. The dried leave was grinded into fine particles using a pulverizing machine and stored in an air-tight container.

Sample Extraction

The powdered sample was soaked with methanol in a clean container with a tight cover for 72 hours (three days). The soaked sample was filtered using a Whatman No. 44 filter paper. The filtrate was concentrated with rotary evaporator to get the crude ethanolic extract.

Anti-anaemic study

A total of 20 albino rats of different sexes were used for this experiment. All the animals were randomly divided into four groups (5 Albino rats per group) as shown below;

Number of group	Number of albino rats	
Group 1	5	Normal saline (Control)
Group 2	5	40mg/kg of Phenyl hydrazine + 500mg of standard blood syrup (Standard control)
Group 3	5	40mg/kg of Phenyl hydrazine + 200mg of crude extract
Group 4	5	40mg/kg of Phenyl hydrazine + 400mg of crude extract

Induction of Anaemia

From the above, 40mg/kg of phenyl hydrazine of hydrochloric acid of anaemia were induced. On the fourth day after treatment, blood samples were withdrawn from the orbital plexus of Albino rats in Ethylene diaminetetraacetic acid (EDTA) vials and were evaluated for blood parameters using Sahli's hemoglobin meter for hemoglobin determination and Neubaurs counting chamber for Red blood cell count.

Treatment with *Jatropha tanjorensis* extracts will continue for all groups except Group 1(vehicle control) for a further period of 10days. Blood samples will also be collected again from all the albino rats and were evaluated for hematological parameters such as Red Blood Cell (RBC) count, hemoglobin determination and Packed Cell Volume (PCV).

Determination of Haemoglobin (HB)

0.02ml (20ul) of blood was added to 5ml of Drabkins solution in a test tube. The solutions were properly mixed well and allowed to stand for 10mins. The absorbance calorimetrically were read at 540 (green filter) with Drabkinngs solution as blank. The absorbance of the standard, were read the same way. The result was recorded.

Determination of Parked Cell Volume (PCV)

The capillary tubes were filled with blood**,** one end of the capillary tubes were sealed with plastacine The sealed end were placed to the center of the haematocrit centrifuge and will spin at 12000g for 5mins**.** The spun tubes were placed on a designed scale and the parked cell volume were read out as percentage.

Determination of White Blood Cell (WBC)

1:200 of blood were prepared in a diluting fluid in thoma pipette. 0.02ml of blood was added to 0.38ml of diluting fluid. Neubauer counting chamber were charged with a well mixed diluted blood. The cells were allowed to settle in a moist chamber for 3-5 minutes. The cells were evenly distributed and well checked. The total numbers of white blood cells in the four large corners square were counted.

Determination of Red Blood Cell (RBC)

1:200 of blood were prepared in a thoma pipette by adding 0.02ml of blood to 0.98ml of diluting fluid. Neubauer counting chamber were charged with the well mixed diluted blood. The cells were allowed to settle in a moist chamber for 3-5mins. The rule areas of the counting chamber with the 10x objective of the microscope were located. 40 x objective were used to count the total number of red blood cells.

Determination of Platelet Count (PC)

1:200 dilution of the blood was used with the diluting fluid in a Thoma pipette. 0.02ml of blood were added to 3.98ml of diluting fluid. Neubauer counting chamber were charged with the well mixed diluted blood. The platelets were allowed to settle in a moist chamber for 3-5 minutes. The ruled areas of the counting chamber under 10x objective was located. Illuminations were reduced by closing the iris diaphragm.

CHEMICAL CHARACTERIZATION

GC-MS Analysis

The methanolic extract was then analyzed on a Hewlett-Packard (HP) 6890 gas chromatograph with a wet needle of the sample material being directly inserted into the inlet (spotless mode), and equipped with a Hp1206 software (chemstation Rev. A09.01). The column consisted of a HP-5MS fused silica capillary of 30 m × 0.25 mm × 0.25 mm film thickness. The temperature of the oven was programmed from 40 to 200°C at 5°C per min, and held isothermally at 200°C for 2 min. The injector temperature was 150°C, and the carrier gas was hydrogen with a flow rate of 1.0 ml/min. Percentage compositions of individual components was obtained from electronic integration using flame ionization detector (FID, 300°C). The volume injected was 0.2 ml with a 20:1 split ratio.

FTIR Analysis

Fourier transform infrared spectroscopic (FTIR) analysis of the extracts was carried out using Shimadzu FTIR–8400s Fourier transform infrared spectrophotometer, Japan. The samples were oven dried to get powders of the different solvent extracts used for FTIR analysis. The dried extracts powder (10 mg) was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc and analysis was carried out by scanning the samples through a wave number range of 400 to 4000 cm^{-1} with a resolution of $2cm^{-1}$. FTIR analyses were performed and the different peaks present and possible chemical interactions were examined.

RESULTS AND DISCUSSION

Table 1. Anti-anaemic Properties

GROUPS	HB (g/dl)	PCV (%)	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	PLATELET ($\times10^9$/L)
Control	14.10±0.10 ^{b}	42.50±0.50 ^{b}	4.05±0.05 ^{b}	5.40±0.20 ^{a}	213.50±0.50 ^{d}
Group 1	13.20±0.10 ^{b}	38.00±1.00 ^{a}	3.85±0.05 ^{b}	5.60±0.10 ^{c}	107.00±1.00 ^{c}
Group 2	14.35±0.05 ^{c}	41.50±0.50 ^{b}	7.45±0.15 ^{a}	5.30±0.10 ^{c}	365.00±15.00 ^{a}
Group 3	15.10±0.10 ^{b}	45.50±0.50 ^{b}	9.05±0.05 ^{b}	5.35±0.15 ^{b}	313.50±1.50 ^{b}
Group 4	15.35±0.15^a	46.50±0.50 ^{b}	9.25±0.05 ^{b}	5.50±0.20 ^{a}	315.00±1.00 ^{c}

Values are expressed as Mean ± SEM for two determinants per group (n=2). Values with the same superscript letter are not significantly differently at 95% confidence level (P<0.05)

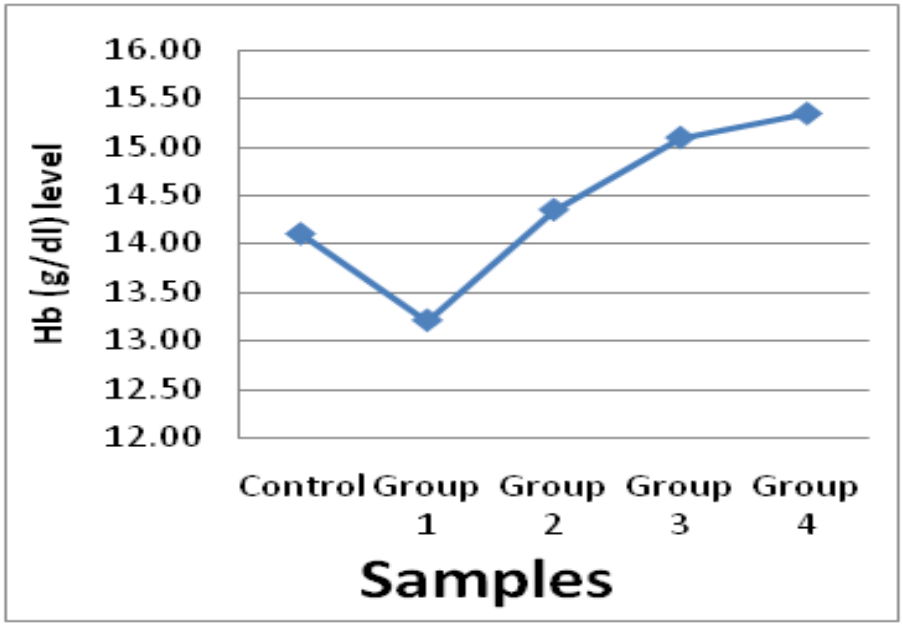


Figure 1. Plot of Heamoglobin level (g/dl)

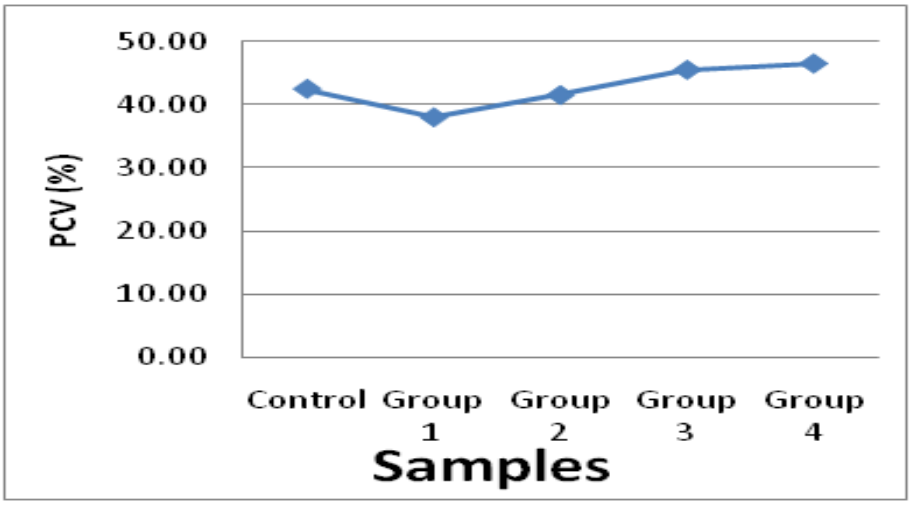


Figure 2. Plot of PCV (%)

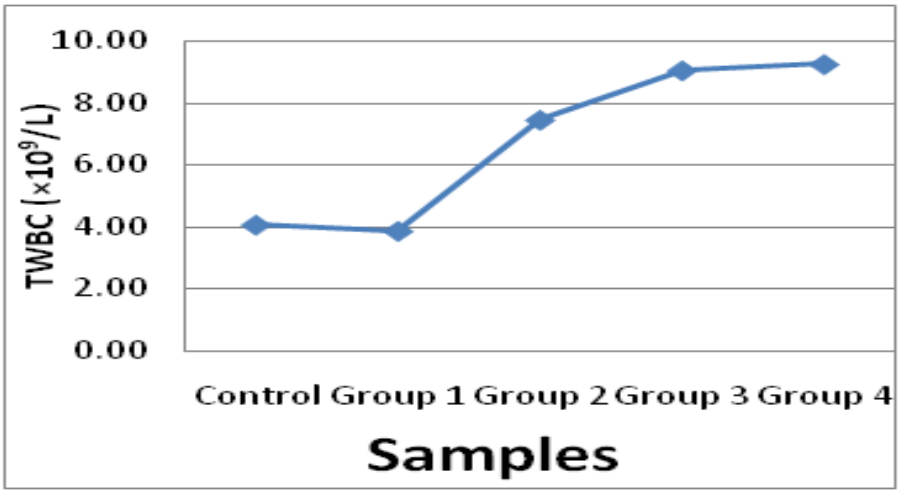


Figure 3. Plot of WBC ($\times 10^9$/L)

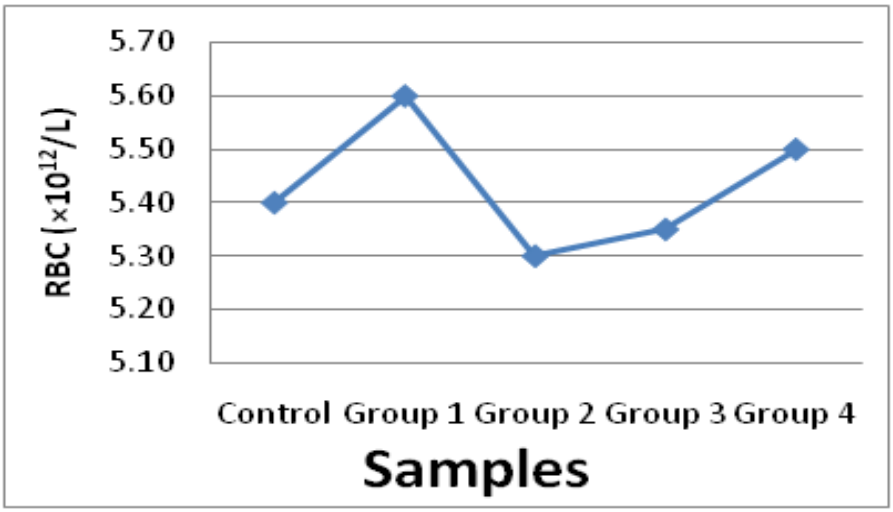


Figure 4. Plot of RBC ($\times 10^{12}$/L)

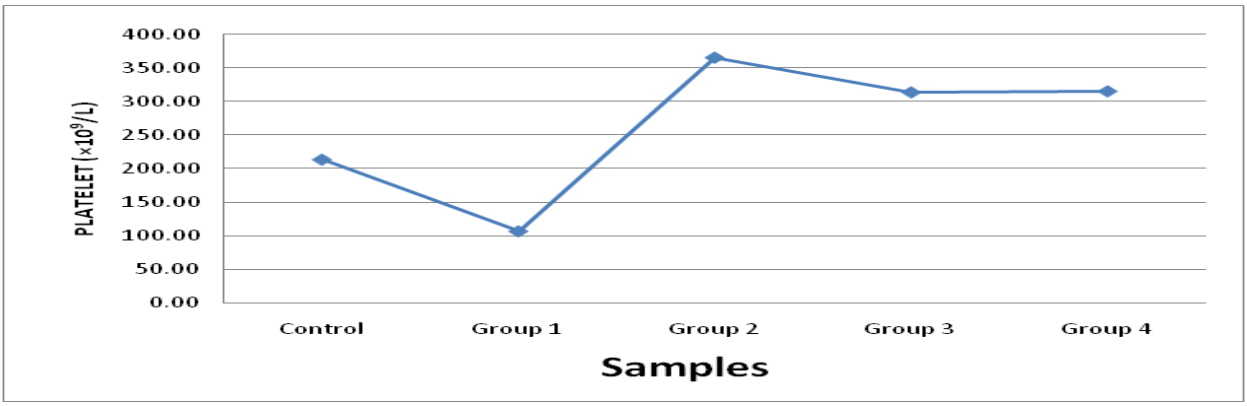


Figure 5. Plot of RBC (×10^{12}/L)

Table 2. GC-MS Analysis Result

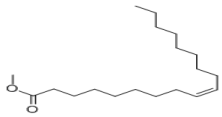
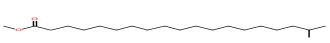
S/N	Name of structure	Structure	Molecular formular	Molecular weight
1.	Actinobolin		$C_{12}H_{18}N_2O_6$	286.12
2	Acetic acid (TBDMS derivatives)		$C_8H_{18}O_2Si$	174.31
3.	Butanal		$C_9H_{13}NO_2$	167.21
4.	Pentadecanoic acid, 1,14-methyl methyl este		$C_{15}H_{30}O_2$	242.22
5.	Dibutyl phthalate		$C_{18}H_{34}O_2$	282.46
6.	9-Octadecenoic acid methyl ester		$C_{19}H_{36}O_2$	296.49
7.	11-Octadecenoic acid methyl ester		$C_{18}H_{34}O_2$	282.46
8.	Phytol		$C_{20}H_{40}O$	296.31
9.	Methyl Stearate		$C_{19}H_{38}O_2$	298.50
10.	3-Azabicylo[3.2.2]nonane		$C_7H_{13}N$	111.18
11.	Methyl 18-methyl nonadecanoate		$C_{20}H_{39}O_2$	311.30
12	Docosanoic acid, methyl ester		$C_{19}H_{38}O_2$	298.50
13	N-desmethyltapentadol		$C_{12}H_{19}NO$	193.29

Table 3. FTIR Analysis Result

S/N	Frequency (cm^{-1})	Group	Compound Class
1	1243	S=O	Sulfon/Chloride
2	1420	OH	Alcohol
3	1630	C=C	Alkene
4	1631	C=C	Alkene
5	1880	C-H	Aromatic compounds
6	2059	N=C=S	Isothiocyanate
7	2493	S-H	Thiol
8	2766	C-H	Alkane

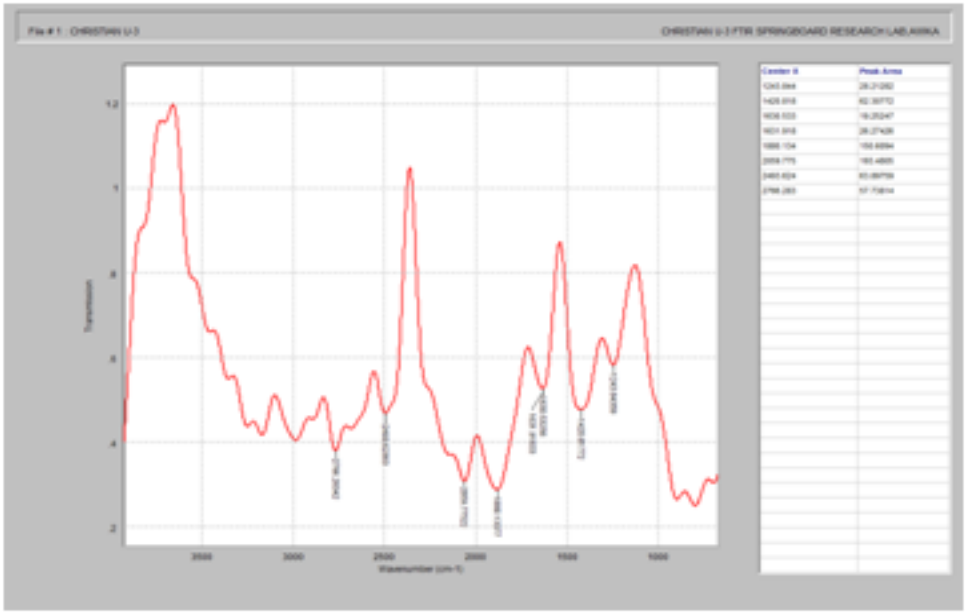


Figure 6. FT-IR spectrum of methanolic extract of *Jatropha tanjorensis* (Hospital too far)

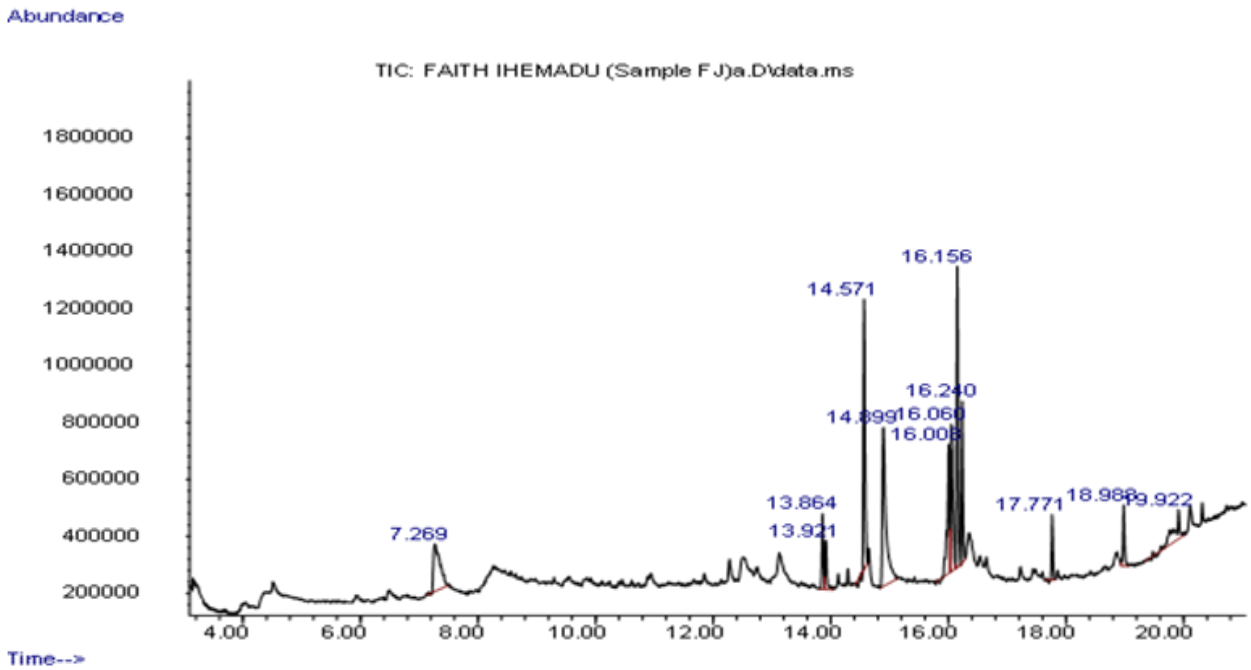


Figure 7. GC-MS spectrum of methanolic extract of *Jatropha tanjorensis* (Hospital too far)

DISCUSSION

The result of the Anti-anaemic Properties of the ethanolic crude extract of *Jatropha tanjorensis* (Hospital too far) as shown in table 1 revealed that the test samples increased the haemoglobin level of the albino rats with an increase in dosage, it also showed that the standard sample induces the haemoglobin level of the albino rats from 14.10±0.10 to 13.20±0.10%. The result was found to agree with the report of Stabler(2013).

The result of the Packed Cell Volume (PCV) showed that the PCV (%) level of the albino rats increased with an increase in dosage of the ethanolic leaf extract of *Jatropha tanjorensis* while the standard blood syrup induces the PCV level from 42.50±0.50 to 38.00±1.00 which was similar to the report of Ilondu and Enwa(2013).

The result of the White Blood Cell (WBC) revealed that the leaf extract of *Jatropha tanjorensis* increases the white blood cell of the albino rats as the dosage increases from 2ml to 6ml while the standard blood syrup had a slight inducement in the albino rats from the control sample from 4.05±0.05 to 3.85±0.05.

The result of the Red Blood Cell (RBC) revealed that the standard blood syrup increased the RBC level of the albino rats from 5.40±0.20 to 5.60±0.10. However, there was an inducement in Group 2 and Group 3 while there was an increase in red blood cell of the albino rats in Group 4. This showed that the phenyl hydrozane is incapable of inducing Red blood cell along. Hence, the leaf extract were unable to boost the red blood cell except in higher amount.

The Platelet result showed that there was a great reduction in the platelet level of the albino rats in Group 1 to group 2, there was an increase in the platelet level of the albino rats in group 2 while group 3 animals experienced a reduction from that of group 2 and slightly in group 4.

However, the reduction of the standard blood syrup as shown in figure 1, 2, 3 and 5 is due the inhibitory effect of phenyl hydrozane to the blood of the albino rats and the inability of the standard blood syrup to increase the blood level. Hence, the ethanolic leaf extract has higher ability of increasing the blood levels of the albino in each of the parameters tested. The increase is a function of the amount of the extract used. The result also showed that the higher the amount of the extract, the higher the increase in the blood level.

The result of the GC-MS analysis of the methanol crude leaf extract of *Jatropha tanjorensis* as shown in table 2 revealed that the leaf is enriched with Actinobolin, Acetic acid, butanal, pentadecanoic acid, dibutyl phthalate, 9-octadecenoic acid, 11-Octadecenoic acid, phytol, methyl stearate, 3-azabicyclo[3.2.2]nonane, methyl 18-methylnonadecanoate, docosanoic acid and N-desmethyltapentadol. The bioactive compounds were found to posses' good medicinal properties and may be broadly classified as alkaloids, steroids, flavonoids, saponins, tannins etc.

Actibolin has been reported to possess antibiotic properties and it has been used in the bio-synthesis of vitamins which is a blood boosting parameter (Oudhia, 2021).

Pentadecanoic acid has been reported by previous studies carried out by Omobuwajo et al. (2011) to possess antioxidant, anti-inflammatory, hypocholesterolemic, antiandrogenic, 5-α reductase inhibitor and hemolytic activities. Omigie and Agorevo (2014) also observed its anticancer and antimicrobial activities respectively. The anti-inflammatory activity of Pentadecanoic acid was revealed from structure and kinetic study carried out by Malomo (2020) to be due to its ability to inhibit PLA2 competitively. Other compounds identified in the extracts with anti-inflammatory, antioxidant, antimicrobial and anticancer activities were octadecenoic acid, 9-octadecanoic acid etc. phytol has been used as fragrance in industries.

The FTIR analysis result as shown in table 3 showed that eight (8) peaks were identified from the FTIR spectrum. The results revealed that the frequency 1243 cm^{-1} correspond to the (S=O), Sulfon/Chloride group, 1420 cm^{-1} correspond to (OH) Alcohol group, 1630 cm^{-1} and 1631 cm^{-1} correspond to (C=C) Alkene, 1880 cm^{-1} corresponds to (C-H) Aromatic compounds, 2059 cm^{-1} corresponds to (N=C=S) Thiol group while 2766 cm^{-1} corresponds to (C-H) Alkane group.

CONCLUSION

Based on the findings therein, the albino rats induced with phenylhydrozane and the ethanolic leaf extract of *Jatropha tanjorensis* gave higher amount of Haemoglobin (Hb), Packed Cell Volume (PCV), White Blood cell (WBC), Red blood cell (RBC) and platelet compared to the ones induced with phenylhydrozane and standard blood syrup. However, the standard blood syrup were unable to increase the blood level of the albino rats due to the inducement done by the phenylhydrozane except for the Red blood cell. However, the ethanolic leaf extract of *Jatropha tanjorensis* is capable of boosting blood levels compared to other blood syrup sold in the market and this is as a result of the various bioactive compounds identified in the compound which possesses known medicinal application. Hence, the wide use of the plant in traditional medicine in treating various diseases. Further research is ongoing to examine pharmacological activities of

the plant especially in treatment of oxidative stress and inflammation related disorders and other pharmacological potentials such as antimalaria, antibacterial anti-diabetic etc.

Acknowledgement

The author is grateful to the Management of Tertiary Education Trust Fund (TETFUND) for supporting this research and sponsoring it 100% through Federal Polytechnic Nekede for 2020 (Merged) TETFUND Intervention in Research Project.

References

Duff S(2018). Types of Anaemia,effects and cytopreactive potentials of nine edible vegetables in southwest. Nigeria J. Medicinal Food. 8: 5939-5944.

Ilondu EM, Enwa FO(2013). Commonly used medicinal plants in the management of sickle cell anaemia and diabetes mellitus by the local people of Edo state, Nigeria. Int. J. Pharmaceutical, Biological and Chemical Sci. 2(2): 14-19.

Iwualewa EO, Adewumi CO, Omisore NO, Adebanji OA, Azike CK(2015). Proantioxidant effects and crytoprotective potentials. J. Med. Food. 8(4):539-544

Malomo SO(2020). Medicinal plants and traditional medicine in Africa. Nigeria J. Biochem. Molecular Biology. 1: 33 – 35.

Oboh FO, Masodje H(2019). Nutritional and antimicrobial properties of *Jatropha tanjorensis* leaves. American Gurasian J. Sci. Res. 4 (1): 7 – 10.

Ogbe RJ, Adoga G, Abu AH(2010). Antianaemic potentials of some plant extracts on phenyl hydrazine induced anaemia in albino wister rats. J. Med. Plant Res. 4(8): 680-684

Oluwole IO, Akingbala FP(2011). Phytochemical analysis and hypolipidemic properties of *Jatropha tanjorensis* leaf extract. Eur. J. Med. Plants. 1(4): 180-185.

Omanklelin J, Owelabi E, Omogbai KI, Osahon O(2017). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigelia Africana (Bignoniaceac) Stem bark. Afr. J. Biotechnol. 6(14):1671-1680.

Omigie MI, Agoreyo FO(2014). the effect of *Jatropha tanjorensis* on blood electrolyte concentration of Albino wistar rates. Nigerian J. Pharmaceutical and Appl. Sci. Res. 3(1): 50-53.

Omobuwajo OR, Alade GO, Akanmu MA, Obuofor EM, Osasan SA(2011). Microscopic and Toxicity studies on the leaves of *Jatropha tanjorensis.* Afr. J. Pharmacology. 5(1): 12-17.

Oyewole IO, Akingbala FP(2011). psychochemical analysis and Hypolipidemic properties of *Japtropha tanjorensis* leaf extract. Eur. Med. Plants. 1(4): 180-185.

Prabakaran AJ, Sujatha M(2021). *Jatropha tanjorensis* and Saroja, a natural interspecfic hybrid occuring in tamil Nadu. India. Gen. Res crop. Evol. 46:213-218

Sofowora A(2013). Medicinal and traditional medicine in Africa spectrum books limited second edition. Pp. 130.

Stabler PS(2013). Clinical practice vitamin B2 deficiency. The New England J. Med. 368(3): 149-160.

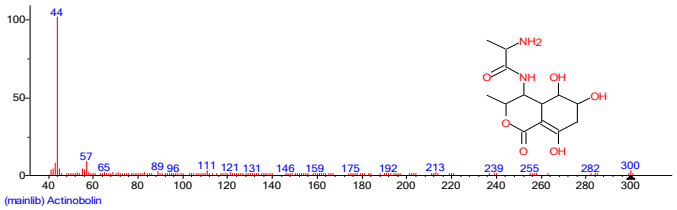
Subramani S. Casmir CA(2012). Flavonoids and antioxidant activity of Georgia grown Tannin acid. J. Agric. Food Chem. 50(19): 5338-5342.

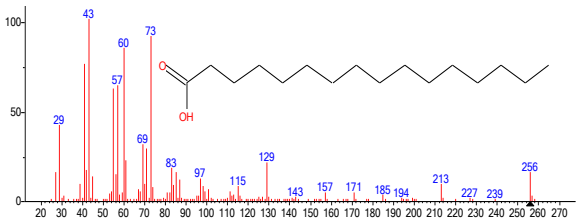
Ucar, K. (2012). clinical presentation and management of haemdyticanaemia oncology, 7: (16): 163-170

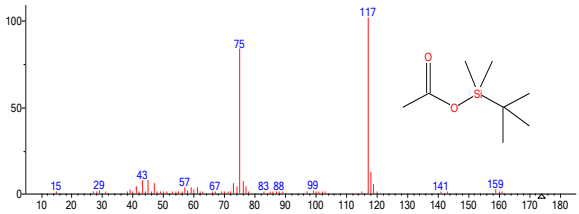
Unesco (2020). FT/504-RAF-48 temina report promotion of ethnobotany and sustainable use of plant resources in Africa paris. Pp. 60.

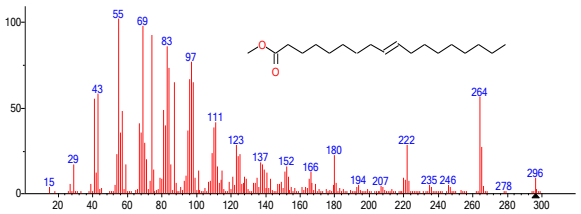
World Health Organization(WHO) (2006). Technical consultation on prevention and control of Iron deficiency in infants and young children in malaria-edemic area –food and nutrition bulletin. 28:489-631.

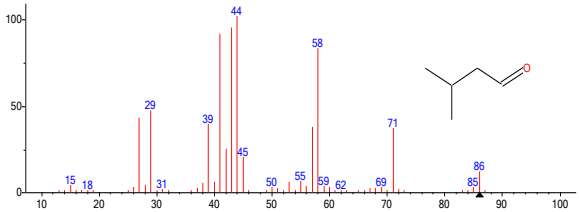
Appendix

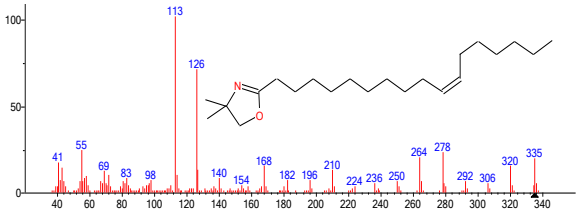

(mainlib) Actinobolin

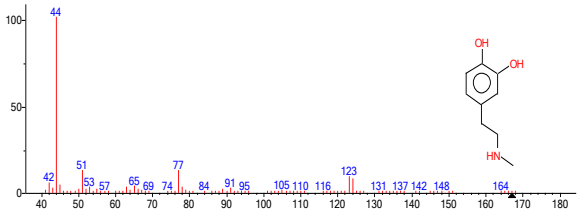

(mainlib) n-Hexadecanoic acid

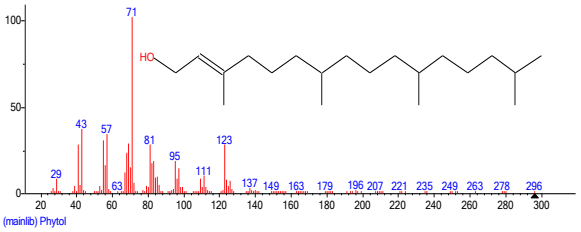

(mainlib) Acetic acid, TBDMS derivative


(mainlib) 9-Octadecenoic acid, methyl ester, (E)-

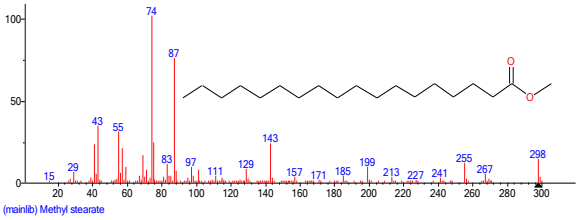

(mainlib) Butanal, 3-methyl-

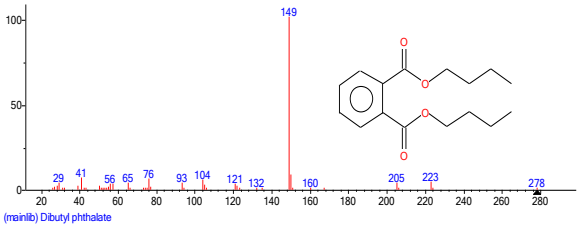

(mainlib) 11-Octadecenoic acid, DMOX derivative

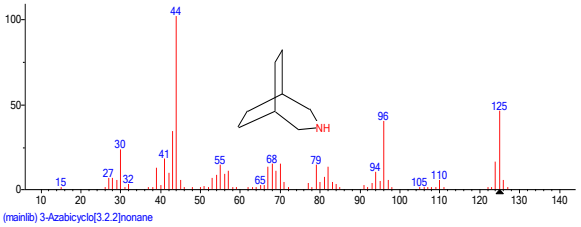

(mainlib) 1,2-Benzenediol, 4-[2-(methylamino)ethyl]-


(mainlib) Phytol


(mainlib) Pentadecanoic acid, 14-methyl-, methyl ester


(mainlib) Methyl stearate


(mainlib) Dibutyl phthalate


(mainlib) 3-Azabicyclo[3.2.2]nonane