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LC-MS, GC-MS and Hematological Profile of *Mucuna pruriens* Extracts in Alloxan Induced Diabetic Albino Rat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mucuna pruriens also known as velvet beans is an annual climbing shrub that has been claimed traditionally to possess anti anemic potentials. The present study evaluated bioactive constituents and hematological profile of *Mucuna pruriens* seed and leaf extracts on alloxan induced diabetic male albino rat using standard analytical method. Result of LC-MS investigation on the seed shows the presence of Hydroxystreptozolin (Oxazolidene) and Dihydrocapsaicin (Meltoxyphe.nol) while the leaf reveals the presence of four(4) bioactive compound: Furocoumurine acid, Epioxylubianin, Acetyllaustoinid and Quassin. The GC-MS studies on *Mucuna pruriens* seed indicates the presence of twelve bioactive compound. 2-methyl-z,z-3,13-octadecadienol cis-13-octadecadienoic acid, methylester hexadecanoic acid, methylester pentadecanoic acid, 9,12,octadecadienoic acid (z, z)-methylester, 11-octadenoic acid, methylester q-octadecenoic acid, Octadecanamide-N-(2-hydroxyethyl) dodecanamide,N-(2-hydroxyethyl), urea,triethyl-Ethanamine, N, N-dimethyl (phenylmethoxy), 13-Hexyloxacyclotridec carbonitrile,3,5bis[C2dimethylamino]ethylthio, 12-octadecadienoic acid(z, z)-cis-13-octadecenoic acid, 9,12-octadecadienoicacid(z,z)-2-hydroxy-1(Hydroxymethyl) ethylester, cyclopentadecanane,2-hydroxy-9,12-octadeadienol,LE, etc while GC-

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MS analysis of the leaf reveals the presence of twenty bioactive compounds such as Nonyl alcohol, 3-Undecene, 1-Dodecene etc. Findings from this study indicates significant improvement on the hematological indices in treated groups relative to untreated. The restorative hematological efficacy of *Mucuna pruriens* extract is indicative of its traditional relevance in medicinal and therapeutic applications and hence may justify its local claims as having anti-anemic functionality.

Keywords: Mucuna pruriens; hematological; profile; alloxan.

1. INTRODUCTION

Treatment of diseases with plant-based therapy has been an ancient folk healing method throughout the world. Chemical substances of plant origin are the backbone of traditional medicine predominant in less developed countries. *Mucuna pruriens* is a leguminous herb of *Fabaceae* family. The plant is well known for its itching attribute. The seeds are covered with clustered pods [1]. Natarajan et al., [2] posited that *Mucuna pruriens* leaf is used in traditional treatment of diseases such as infertility, diabetes mellitus and cancer whereas the seeds have several medicinal functions such as management of free radical-mediated diseases. The leaf of this plant is boiled and taken by most people in southern part of Nigeria as a blood boaster especially under anemic conditions. The need to ascertain the bioactive constituents and evaluate the local claims as possessing anti anemic properties motivated this research work.

2. METHODOLOGY

2.1 Plant Collection

Fresh seeds of *Mucuna pruriens* were collected from a botanical garden in Mararaba Akunza Area of Nasarawa state, Nigeria and was authenticated by a botanist in the Department of Plant Science and Biotechnology, Federal University of Lafia.

2.2 Induction of Diabetes

Single intraperitoneal injection of 160 mg per body weight of alloxan was adopted. Alloxan was dissolved in 0.1% normal distill water as vehicle. Animals with blood glucose greater than 200mg/dl were considered diabetic after 2 days of induction and were used for the experiment.

2.3 Experimental Animal

Forty-eight (48) healthy male albino rats weighing between 109- 120 g obtained from the Animal house Federal College of Animal Health and Production Technology, Vom in Jos Plateau State were used for each of the experiment. They were acclimatized for one week prior to induction and treatment. The animals were grouped into twelve (12) groups of four (4) rat. Group 1 served as control. Group 2 diabetic control group were fed rat feeds and water. Group 3 diabetic group treated with 200 mg/kg body weight of aqueous extract of *Mucuna pruriens* seed Group 4 diabetic group treated with 500 mg/kg body weight of aqueous extract of *Mucuna pruriens* seed. Group 5 diabetic group treated with 200 mg/kg body weight of ethanol extract of *Mucuna pruriens* seed Group 6 diabetic group treated with 500 mg/kg body weight of ethanol extract of *Mucuna pruriens* Seed. The same grouping was repeated for the groups treated with *Mucuna pruriens* leaf extracts.

2.4 Determination of Bioactive Compounds Using LC-MS

LC-MS Analysis (Generic Method) using LC Waters e2695 separation module with W2998 PDA and couple to ACQ-QDA MS.

The samples were analyzed using liquid chromatography (LC) tandem mass spectrophotometer (MS) as described by [3] with some modifications. The extracted samples were reconstituted in Methanol and filtered through polytetrafluoroethylene (PTFE) membrane filter with 0.45 µm size. After filtration, the filtrate (10.0 µl) was injected into the LC system and allowed to separate on Sunfire C18 5.0µm 4.6mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, Sample and Column temperature at 25^{o}C. The compounds were identified on the basis of the following information, elution order, and retention time (tR), fragmentation pattern, and Base m/z.

2.5 Determination of Bioactive Compounds Using GC-MS

The samples for Gas Chromatography/Mass Spectrometry were prepared by dissolving 3 g of

extracted powder in methanol solvent. For the analysis, GC-MS-QP 2010 SHIMADZU instrument was used. To analyze the sample the column oven temperature and Injector temperature was set at 800°C and 200°C respectively. The flow control mode was maintained in linear velocity with a split injection mode split ratio of 20. The column flow was 1.46 ml/min with a helium carrier gas of 99.9995% purity. Then the phytochemical was identified based on the hits returned after comparing the unknown peak value and the chromatogram from GC-MS against the known chromatogram, peak value from the NIST library data base. Subsequently, the detail about their molecular formula, molecular weight, structures was obtained.

2.6 Determination of Hematological Parameters

Packed Cell Volume (PCV) (%), Red Blood Cells (x109/L) and white blood count were determined using standard hematological procedure as described by Ochei and Kolhatkar [4]. Platelets (x109/l) was determined using the method described by Cheesbrough [5] while Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) where determined by calculation according to Ochei and Kolhatkar [4].

2.7 Statistical Analysis

The data generated were expressed as Mean ± Standard Deviation (M ± SD). One-way analysis of variance followed by Duncan Multiple Range Test (DMRT) was used for the analysis using SPPS V25.0. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

Table 1 shows LC-MS profile of *Mucuna P* seed. Two compounds were identified. 9-hydroxystreptozolin with mass of 224 and retention time of 2.19 while Dihydrocapsaicin has retention time of 12.66 and mass of 308.

Table 2 shows LC-MS profile of methanol extract of *Mucuna P* leaf. Four compounds were identified. The compounds identified are Furanocounarins with retention time of 6.02 and mass of 367, Epioxylubirim with retention time of 10.52 and mass of 270, Acetyllaustonulin with retention time of 6.77 and mass of 365 and Quanssin with retention time of 8.41 and mass of 389.

Table 1. LC-MS profile of *Mucuna P* seed

Peak no	RT (Min)	Candidate $_{mass}$	Name of compound	Molecular formular
1	2.19	224	9-hydroxystreptozolin	$C_{11}H_{13}NO_4$
2	12.66	308	Dihydrocapsaicin	$C_{18}H_{29}NO_3$

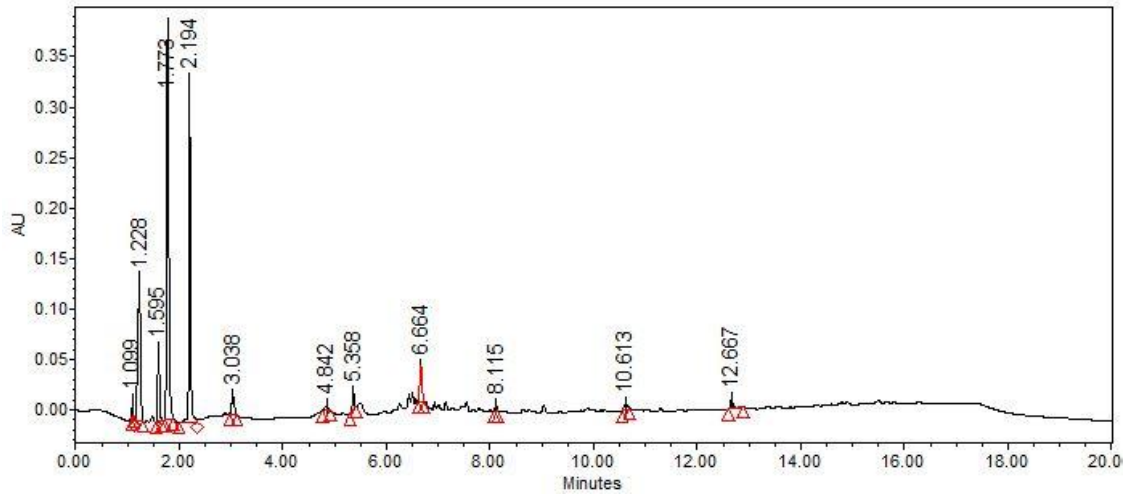

Fig. 1. LC-MS chromatogram of *Mucuna pruriens* seed

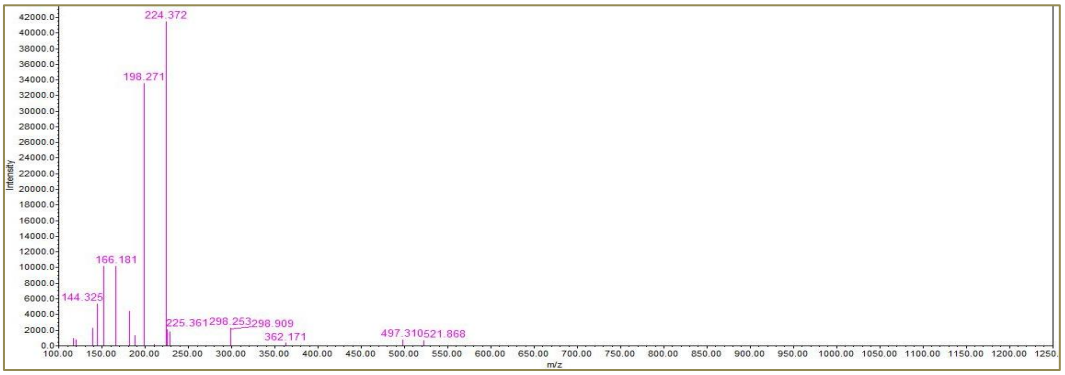

Fig. 2. Hydroxystreptozolin (Oxazolidene) identified in *M.p* seed (RT 2.19)

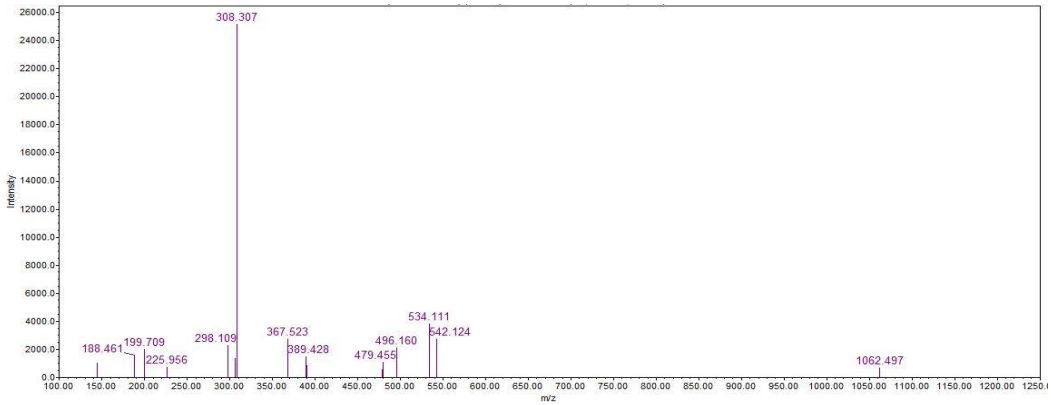

Fig. 3. Dihydrocapsaicin (Meltoxyphenol) identified in *M.p* seed (RT 12.66)

Table 2. LC-MS profile of methanol extract of *Mucuna P* leaf

Peak no	RT (Min)	Candidate mass	Name of compound	Molecular formular
1	6.02	367	Furanocounarins	$C_{12}H_80_4$
2	10.52	270	Epioxylubirim	$C_{21}H_{25}CLO_5$
3	6.77	365	Acetyllaustonulin	C_2H_3O
4	8.41	389	Quanssin	

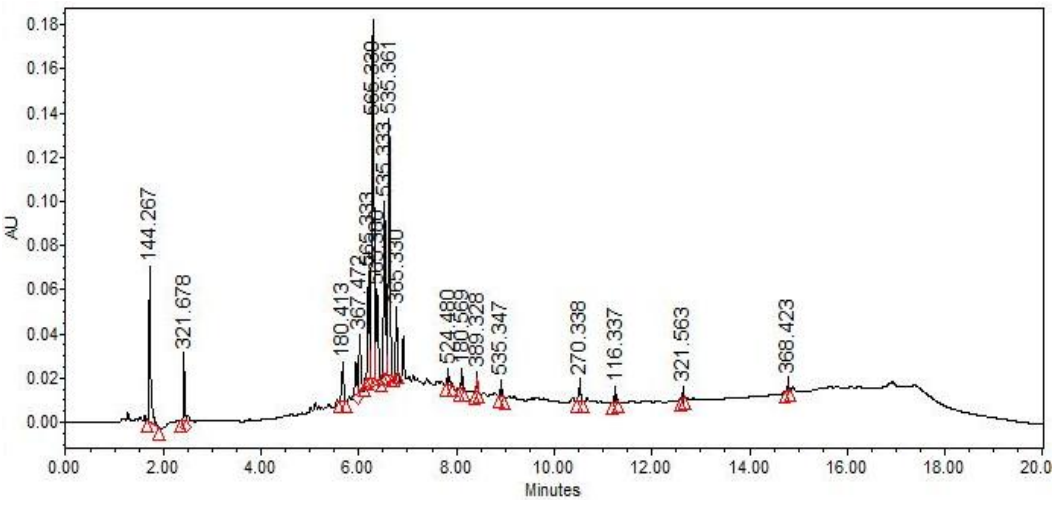

Fig. 4. LC-MS *Mucuna P.* leaf chromatogram

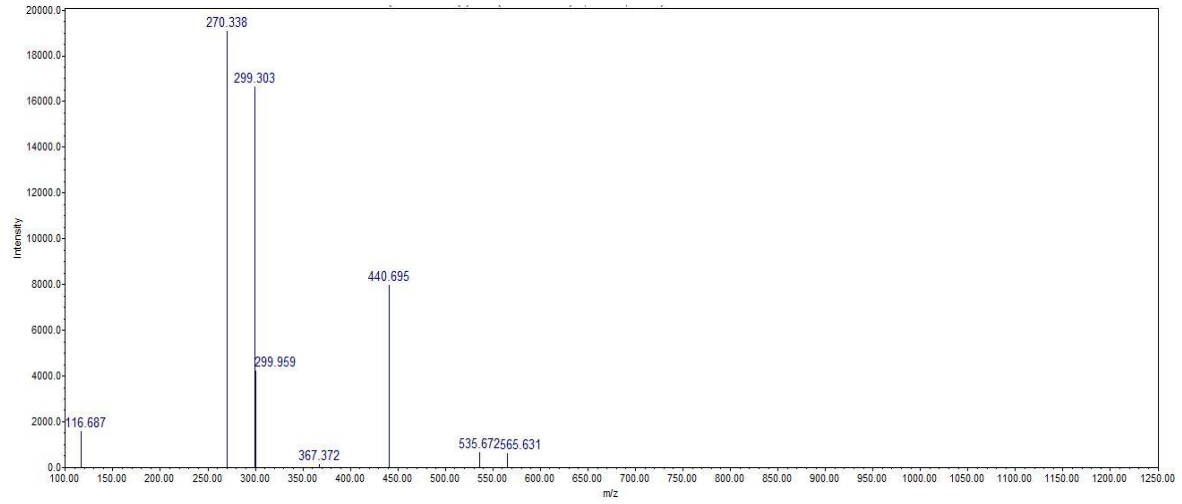

Fig. 5. Mass spectrum for epioxylubianin (Sesquineperoxide)) identified in *M. p* leaf (RT 10.52)

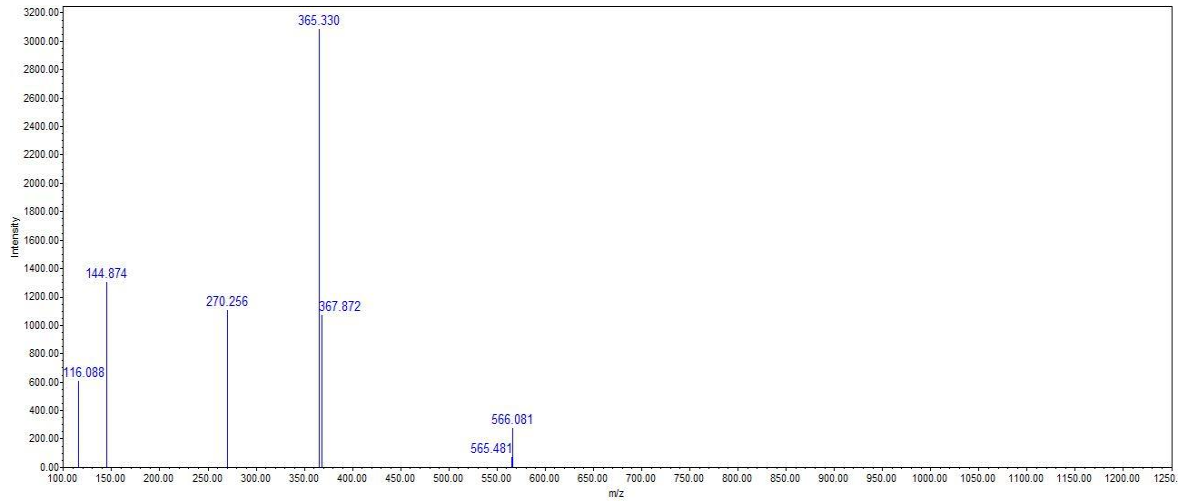

Fig. 6. Mass spectrum for acetyllaustoinide (Diterpernoid) identified in *M.p* leaf (RT 6.77)

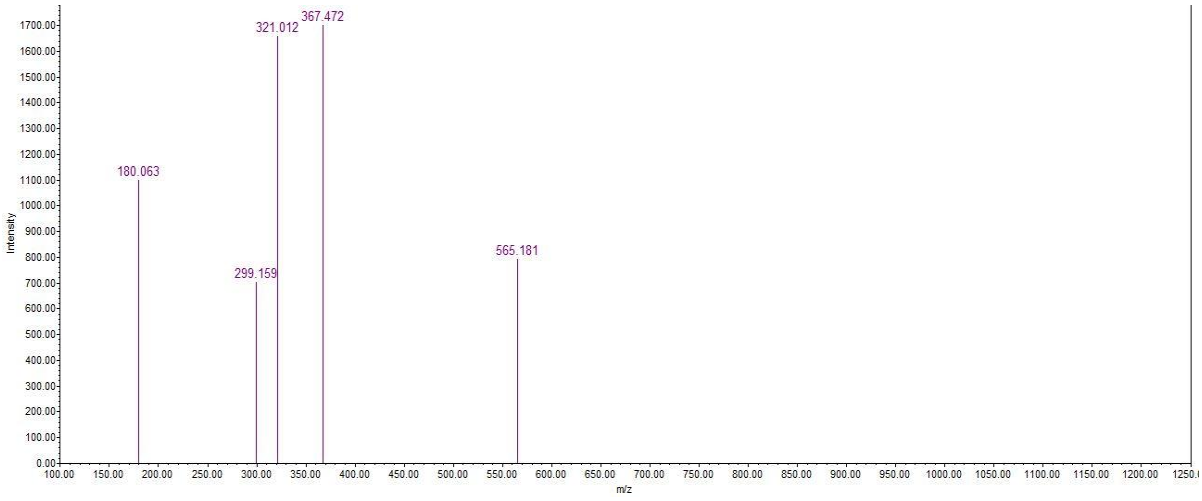

Fig. 7. Furocoumurine acid glycoside (phenolic glycoside) identified in *M. p* leaf (RT 6.02)

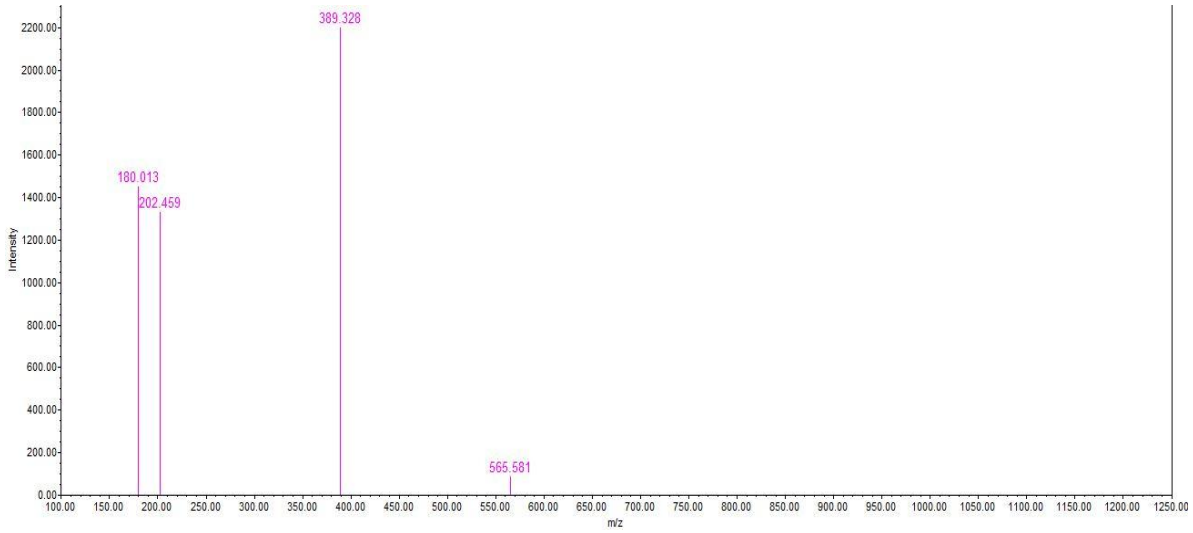

Fig. 8. Mass spectrum for Quassin (Quaesinoids) identified in *M. p* leaf (RT 8.41)

Table 3. GC-MS profile of *M. prureins* seeds

S/N	Retention Time(min)	Components	Molecular Fomular	Molecular Weight	% Peak
1	5.237	2-methyl-z,z-3,13-octadecadienol cis-13-octadecadienoic acid	$C_{19}H_{34}O_2$	294.5	34.39
2	12.053	Methylester Hexadecanoic acid methylester pentadecanoic acid	$C_{18}H_{36}O_2$ $C_7H_{14}O_2$	284.5 130.1849	2.20
3	14.191	9,12,octadecadienoic acid (z,z)-methylester	$C_{19}H_{34}O_2$	294.4721	6.68
4	14.260	11-octadenoic acid methylester q-octadecenoic acid	$C_{18}H_{34}O_2$ $C_{19}H_{36}O_2$	282.5 296.5	7.84
5	14.822	Octadecanamide,N-(2-hydroxyethyl)dodecanamide,N-(2-hydroxyethyl)	$C_2H_{14}NO_2$	327.5	3.90
6	16.795	urea,triethyl-Ethanamine,N,N-dimethyl-2-(phenylmethoxy)	$C_{23}H_{29}NO_8$	447.75	2.60
7	17.263	13-Hexyloxacyclotridec-10-en-2-one 9 12-octadecadienoyi chloride (z,z)-phenol	$C_{18}H_{32}O_2$ $C_9H_{12}O$	280.4 136.19	9.98
8	19.294	Hexadecanal,2-methyl-isothiazole-4-carbonitrile 3,5-bis[C2-dimethylamino]ethylthio	$C_{16}H_{32}O$ $C_{22}H_{24}N_4O$	240.42 360.5	7.25
9	20.406	12-octadecadienoic acid(z,z)-cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.468	6.48
10	23.037	9,12-octadecadienoic acid (z,z)-2-hydroxy-1(Hydroxymethyl)ethylester	$C_{21}H_{38}O_4$	354.5240	17.88
11	23.241	cyclopentadecanane,2-hydroxy-9,12-octadeadienol	$C_{10}H_{12}O_2$	164.20	0.43
12	27.023	LE,2-1,3,12-Nonadecatriene(IS,4as,4bs,7s,8as,10as)-7-isoproyl 4a-dimethyltetradecahydrophenanthrene	C_3H_8O $C_{11}H_{13}NO$	60.10 175.23	6.27

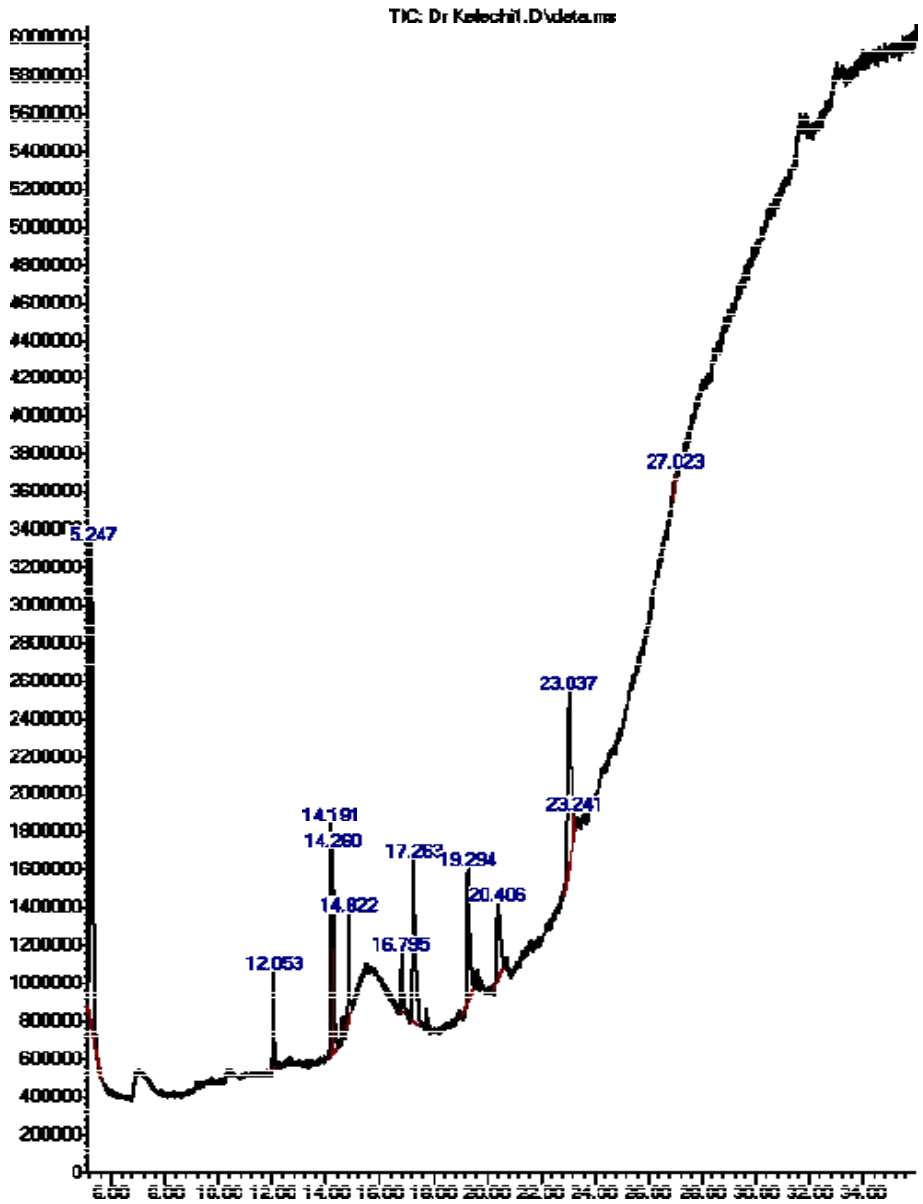

Fig. 9. GC-MS Chromatogram of *Mucuna pruriens* seed extract

Table 4. GC-MS result of *M. pruriens* leaf

S/N	Retention Time	Name of Compound	Molecular formula	Area %
1	3.835	Nonyl alcohol, 3-Undecene, 1-Dodecene	C_6H_{14}	0.55
2	7.713	2H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl, l-Mevalonic acid lactone	$C_{15}H_{30}O_2$	0.45
3	10.656	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	1.04
4	11.524	n-Hexadecanoic acid, Pentadecanoic acid	$C_{15}H_{30}O_2$	5.00
5	12.957	Octanoic acid, methyl ester, Tridecanoic acid, Decanoic acid	$C_9H_{18}O_2$	1.10
6	13.760	4-Methyloctanoic acid, Eicosanoic acid, n-Hexadecanoic acid, Octadecanoic acid	$C_{16}H_{32}O_2$	4.85
7	15.677	Palmitic acid, methyl ester, Methyl heptacosanoate	$C_{21}H_{42}O_2$	4.93

S/N	Retention Time	Name of Compound	Molecular formula	Area %
8	17.111	n-Hexadecanoic acid, Nonadecanoic acid, Pentadecanoic acid,	$C_{22}H_{44}O_4$	21.07
9	18.893	9,12-Octadecadienoic acid, methyl ester,	$C_{21}H_{38}O_2$	5.17
10	18.962	11-Octadecenoic acid	$C_{19}H_{36}O_2$	8.04
11	19.314	Decanoic acid, ridecanoic acid	$C_{17}H_{34}O_2$	2.04
12	20.147	Oleic Acid, E-2-Octadecadecen-1-ol, Z-8-	$C_{18}H_{34}O_2$	26.04
13	20.365	Methyl-.beta.-D-arabinopyranoside,	$C_6H_{12}O_5$	12.82
14	21.650	n-Propyl heptyl ether, Acetic acid,	$C_{15}H_{28}O_4$	0.72
15	21.902	Pentanoic acid, Heptacosanoic acid, Tetradecanoic acid	$C_7H_{14}O_2$	0.28
16	23.521	3-n-Hexylthiolane, S,S-dioxide	$C_{11}H_{20}Cl_2O_2$	1.96
17	23.900	Docosanoic acid, Heneicosanoic acid	$C_{23}H_{46}O_2$	0.95
18	25.001	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate,	$C_{26}H_{54}O_2$	1.21
19	25.611	Heneicosanoic acid, Hexacosanoic acid	$C_{18}H_{36}O_2$	0.57
20	26.384	2H-Pyran, 2-(7heptadecynyloxy)tetrahydro	$C_{22}H_{36}O_2$	1.19

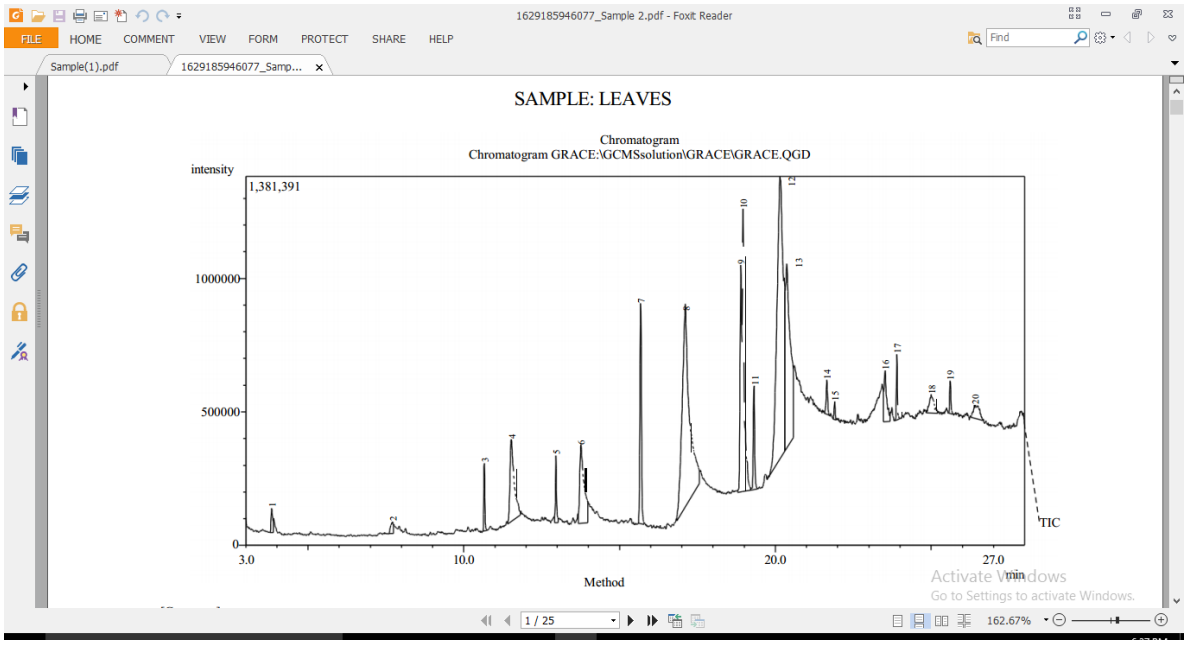

Fig 10. GC-MS chromatogram of *M. p* leaf

Each value represents mean ± SD, n=3, mean values with different superscripts are significantly different (P<0.05). Group 1= Normal Control; Group 2= (Diabetes Control, Alloxan-induced untreated rats); Group 3= (Treated with 200 mg/kg body weight of aqueous extract); Group 4= (Treated with 500mg/kg body weight of aqueous extract); Group 5= (Treated with 200 mg/kg body weight of ethanol extract); Group 6= (Treated with 500 mg/kg body weight of ethanol extract (Tbale 5).

Values represent mean of triplicate determination Mean± SD. Values in the same column having different alphabets are statistically significant (P ≤ 0.05). Group 1= Normal Control; Group 2= (Diabetes Control, Alloxan-induced untreated rats); Group 3= (Treated with 200 mg/ kg body weight of aqueous extract); Group 4= (Treated with 500mg/kg body weight of aqueous extract); Group 5= (Treated with 200 mg/kg body weight of ethanol extract); Group 6= (Treated with 500 mg/kg body weight of ethanol extract (Table 6).

Table 5. Effect of *M. prureins* seeds extract on hematological parameters

	Parameters							
Gp	**RBC(10^{12}/l)**	**PVC(%)**	**Hb(g/dl)**	**WBC(10^{9}/l)**	**Platelets(%)**	**MCV(%)**	**MCH(pg)**	**MCHC(%)**
1	6.66 ± ± 03^{C}	41.72±0.03^{e}	13.63±0.03^{e}	7.01±0.02^{f}	513.12±0.03^{f}	62.11±0.02^{f}	20.31±0.01^{e}	32.76±0.03^{a}
2	5.35±0.03^{e}	35.42±0.02^{f}	11.61±0.01^{f}	12.43±0.03^{a}	567.01±0.01^{e}	67.73±0.02^{a}	22.31±0.01^{d}	32.43±0.03^{b}
3	7.25 ±.25±.03^{a}	46.42±0.01^{a}	14.62±0.02^{d}	7.82±0.03^{d}	583±0.04^{c}	64.73±0.03^{e}	20.32±0.02^{c}	31.35±0.02^{f}
4	0.41 ± 0.01.41±0.01	46.41±0.03^{b}	14.63±0.03^{c}	7.83±0.01^{c}	583.84±0.02^{b}	64.73±0.03^{d}	20.32±0.02^{b}	31.35±0.03^{e}
5	645 ±45±3^{d}	43.11±0.01^{d}	14.75±0.03^{b}	8.03±0.02^{e}	570.97±14.2^{d}	65.21±0.02^{c}	20.31±0.01^{f}	31.41±0.01^{d}
6	6.8± 0.03 ^{b}	44.31±0.01^{c}	14.91±0.01^{a}	8.13±0.03^{b}	593±0.7^{a}	66.04±0.04^{b}	20.34±0.01^{a}	31.45±0.02^{c}

Table 6. Effect of *Mucuna pruriens* leaf extracts on hematological indices of alloxan induced diabetic male albino rat

Groups	**Parameters**							
	RBC (10^{12}/l)	**PCV (%)**	**Hb (g/dl)**	**WBC (10^{9}/l)**	**Platelets (%)**	**MCV (%)**	**MCH (pg)**	**MCHC (%)**
Group 1	6.69±0.26^{b}	41.71±1.50^{b}	13.64±0.93^{b}	7.02±1.69^{a}	513.14±15.32^{a}	62.12±0.64^{a}	20.31±0.63^{c}	32.78±0.96^{f}
Group 2	5.38±0.39^{a}	35.43±2.88^{a}	11.61±0.97^{a}	12.45±3.93^{f}	567.00±13.81^{f}	67.76±2.08^{e}	22.31±1.24^{f}	32.46±1.35^{c}
Group 3	6.91±0.52^{d}	43.57±3.69^{c}	13.94±0.33^{c}	8.34±1.44^{e}	523.71±15.41^{c}	62.69±1.11^{b}	20.30±1.25^{b}	32.62±2.06^{d}
Group 4	7.47±0.17^{f}	49.71±2.29^{f}	14.93±0.74^{f}	8.21±1.60^{d}	542.86±28.81^{d}	66.15±2.5^{d}	19.68±1.18^{a}	30.30±2.16^{a}
Group 5	6.78±0.50^{C}	45.71±2.21^{e}	14.70±0.42^{e}	8.02±1.96^{c}	520.86±12.58^{b}	70.51±1.76^{f}	21.76±0.45^{e}	31.11±0.45^{b}
Group 6	7.16±0.23^{e}	45.43±1.51^{d}	14.90±0.27^{d}	7.88±1.28^{b}	56.86±36.37^{e}	63.76±1.05^{c}	20.57±1.03d	32.71±1.13^{e}

4. DISCUSSION AND CONCLUSION

Bioactive substances exert remarkable effect in humans due to its health-promoting potentials and they constitute the backbone for medicinal functionality of plants. The LC-MS and GC-MS analysis indicate the presence of bioactive compounds that may be of medicinal relevance. Furanocoumarins detected in the plant has been posited to exhibit strong phototoxic properties hence, their presence in plant is a protective mechanism against phytopathogenic microorganisms and herbivores. They have also been implicated in antiproliferative activities against cancer cell through modulation of molecular pathways [6]. Some of the compounds such as 13-octadecadienol, triethyl-Ethanamine,N,N-dimethyl-2-(phenylmethoxy)acids are reported to quicken healing of wounds [6]. Also report have shown that Methylester Hexadecanoic, uric acid, hydroxyethyl) dodecanamide, and N-(2-hydroxyethyl) possess antimicrobial and antifungal activity [7]. Antidiabetic properties of cyclopentadecanane,2-hydroxy-9,12-octadeadienol,methylester and q-octadecenoic acid have been reported [8]. Pathania et al., (2018) also reported that Nonadecatriene (IS,4as,4bs,7s,8as,10as)-7-isoproyl are potent bioactive compounds against infertility. The determination of hematological indices provides physiological information on the general blood picture and the immune system. Administration of *Mucuna p* extracts at different concentrations for both aqueous and ethanol ameliorated the damage by significantly increasing ($p<0.05$) the level of TWBC and TRBC in this groups (group 3-6) relative to group 2 which serves as the negative control. The level of amelioration is with increase in concentration of the extract. Also, neutrophils and lymphocytes levels were significantly ($p<0.05$) raised by the administration of the extract in group 3-6 compared to group 2 that did not receive the extract. This may have resulted from acceptability of the extract by the experimental animals as well as plants bioactive constituents. Similar finding has been reported by Sonpetkars et al., [9] who opined that ameliorative effect of *M. p* extracts is dose and concentration dependent. Some substances which found their way into the blood stream triggered the generation of WBCs, thus playing a defensive role against any of the substances which might likely be toxic to the immune system. The increased concentration of WBCs in rats administered with extract is an indication of the functionality of the immune system in defense against toxins. This finding is also indicative of the abilities of *Mucuna p* extracts to curtail and restore hematological abuses in the defense system of the diabetic rats. Ndukwe et al. [10], also reported a significant ($p<0.05$) increase in white blood cell (WBC) in rats feed with *Mucuna p* seed extract. Findings from this study indicates significant improvement on hematological indices in *Mucuna pruriens* treated groups compared to untreated. This may justify the local usage as a blood boaster and suggest that *Mucuna pruriens* seed and leaf extracts may be of medicinal relevance in management of anemia. Researchers [11] have reported some toxic compounds in *M.p* hence proper detoxification procedures should be employed to encourage holistic utilization of the plant for medicinal and therapeutic application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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