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Separation and Identification of the Components of Acacia Sieberiana Stem Bark Plant Extract and Study its Safety as a Treatment Drug for Diarrhea

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Abstract

The Safety of the stem bark of Acacia Siberian, as an antidiarrheal, in Albino rats (four groups) has not yet been evaluated. This study to identify the effect of the acetone extract of the stem bark of Acacia Siberian (AESBAS) on Albino rats, Separation and identification of the acetone extract of the stem bark of Acacia Siberian by GC-MS-TMS analysis. Albino rats (four groups) were subjected to the sub-chronic oral administration of the AESBAS for Twenty-eight days. Phytochemical screening test of the AESBAS confirmed by GC-MS-TMS analysis. Non-significant effect ($p>0.05$) in mean weight gain during the end of the experiment between groups. Also, some hematological analysis in the twenty-ninth day was estimated using standard steps. The AESBAS had non-significant effect ($p>0.05$) on some hematological analysis on study groups compared with the control group, that were given varying doses of the AESBAS. The components of AESBAS confirmed by GC-MS-TMS analysis. The results of this study suggest the AESBAS does not possess haemotoxic activities that could limit therapeutic use as an antidiarrheal. Phytochemical screening of the AESBAS by GC-MS-TMS analysis, confirms the existence of various compounds with different chemical structures, analysis of the AESBAS confirms the results data tests for phytochemical screening.

Keywords: Acacia Sieberiana; Antidiarrheal; Toxicity; Sub-chronic oral administration; Haematological parameters; GC-MS-Derivatization analysis; Phytochemical screening

Abbreviations: AESBAS: Acetone extract of stem bark of Acacia Sieberiana; **ANOVA**: Analysis of variance; **BSTFA**: Bis (trimethylsilyl) trifluoroacetamide; **CLA**: conjugated linoleic acids; **EI:** Electron ionization; GC: Gas Chromatography; **GC-MS:** Gas Chromatography-Mass Spectrometry; **Hb:** Hemoglobin; **IE:** Ionization energy; **MCH:** Mean corpuscular haemoglobin; **MCHC:** Mean corpuscular haemoglobin concentration; **MCV:** Mean corpuscular volume; **NIST:** National Institute Standard and Technology library data; **NRC:** National Research Council; **NVRI:** National Veterinary Research Institute; **PDW:** Platelet distribution width; **PLT:** Platelet; **RBC:** Red blood cells; **RDW:** Red cell distribution; **TMS:** tri-methyl silyl group; **WBC:** White blood cell; **WHO:** World Health Organization

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Introduction

Herbal medicines are widely used for the treatment and prevention of various diseases in Africa and other developing countries of the world. These herbs are generally accessible, affordable and acceptable by most of the consumers [1]. According to the WHO about Eighty-five percent of traditional medicine involves the use of medicinal plant extracts [2].

In recent years, there has been interest in the use of herbal medicine in the treatment of a number of illness amongst which is diarrhea. In the developing countries, Diarrhea is the main cause of death in children, deaths estimated at about 4.600.000 to 5.000.000 every year in children under the age of 5 years **[**3,4].

Acacia Sieberiana (English name: White thorn) (**Figure 1a**). A member of the Family Fabaceae – Mimosoideae, is a tree that grows up to 15 m high with light-colored bark and often with a flat crown. The leaves ten to fifteen centimeter long have straight white thorns at their base, the branches and often the leaves are covered with yellow hairs. The seeds are contained in straight pods, Eight to twelve centimeters long and Two to three centimeterbroad. The flower heads are spherical shape and cream colored. Acacia Sieberiana grows in various regions in the world and Africa [5].

Medicinal plants are a promising source of antidiarrheal drugs, due to the fact that they are widely used by traditional healers and livestock owners in the management and control of diarrhea [6]. The incidence of diarrhea still remains high despite the intervention of government health agencies to halt the trend [7]. The fundamental reason in the medicinal properties of plants, the presence of varieties of different compounds such as alkaloids, flavonoids, tannins and terpenes [8].

Many medicinal plant species and extracts used to treat patients with diarrhea in Africa and other developing country has been reported. Moreover, several bio-remedies have been identified and confirmed to have antidiarrheal properties [9] including Acacia Sieberiana [10].

Some plant extracts show adverse effects on animal models, hence, it is necessary to evaluate the toxicological profiles of such plants before its eventual recommendations for public use or development into medical application. Test examinations are needed to determine the safety of each plant before they can be recommended for medical use [11].

In the previous ten years, there were a number of dramatic advances in analytical techniques including GC-MS that were strong tools for isolation, identification and structure determination of phytochemicals [12]. Phytochemicals are responsible for the medicinal activity of plants [13].

AESBAS (Acetone extract of stem bark of Acacia Sieberiana) slowed down the propulsion of charcoal meal through the gastrointestinal tract, though not in a dose- dependent manner and with the highest inhibition at 600 mg/kg. The AESBAS (300, 600 or 1200 mg/kg) also exhibited a significant inhibition of castor oil-induced diarrhea in a dose-dependent manner with the highest inhibition ($p<0.001$) at 84% with the 1200 mg/ kg. The results of this investigation show that AESBAS contains phytochemical substances with antidiarrheal properties. This provides the rationale for the use of AESBAS as an anti-diarrheal remedy by traditional healers [14].

Materials and Methods

Collection of plant material and extraction

The fresh samples of AESBAS, were collected on the premises of the National Veterinary Research Institute (NVRI) Vom, Jos Nigeria. The collected samples were cut into small pieces and dried (**Figure 1b**). The bark portion containing the dead cells was scrapped off while the fresh part was cut into smaller pieces before they were air dried in a well-ventilated room. The dried samples were pounded and pulverized into fine powder (**Figure 1c**) using pestle and mortar.

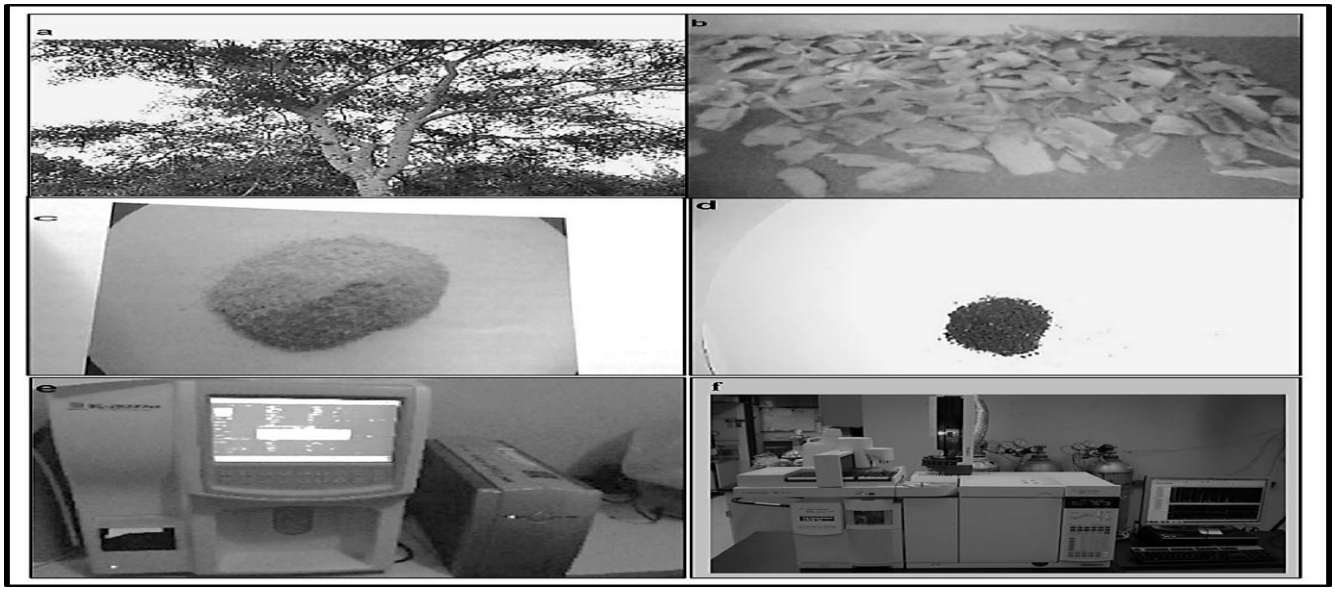


Figure 1:(a) Acacia Sieberiana tree. (b) The dried Fresh samples of stem bark of Acacia Sieberiana. (c) The acacia sieberiana powder after the dried bark was pulverized. (d) The dried AESBAS. (e) BC-2800 Auto Hematology Analyzer. (f) Gas Chromatography-Mass Spectrometry (GC-MS) Instrument.

The resultant powder obtained was then weighed and put into an adequately labeled receptacle in preparation for extraction. Acetone as an organic solvent for the extraction preparation. 1000 g of the powdered sample was mixed with 2 L of acetone and left on the bench for 48 hours at room temperature.

The mixture was then filtered twice using a laboratory test sieve of 150µ pore size. The filtrate was then allowed to settle and then decanted and filtered using Whatman filter paper No. 1. The resulting filtrate was dried at room temperature, using a steady stream of air current generated by a laboratory fan. When dried, the extract **AESBAS** (**Figure 1d**) was collected into a container, sealed and stored at room temperature for phytochemical screening and administration to the test animals (albino rats).

Experimental Animal and Treatment protocol

Experimental site

The method of this study was the sub-chronic oral toxicity test method on some Hematological parameter on albino rats Using BC-2800 Auto Hematology Analyzer (**Figure 1e**), to study the safety of AESBAS as a treatment drug for diarrhea, separation and identification bioactive components of AESBAS using GC- MS Derivatization analysis (**Figure 1f**).

Acquisition of experimental animals

Forty albino rats (equal numbers of male and female), weighing between 100-200 g used in this study were acquired from the Small Animal Experimental Station, NVRI, Nigeria. The experiments on albino rats in accordance with the principles and guide for the care and use of laboratory animals (NRC, 1996), according to the animal welfare and the ethics board of the NVRI, Nigeria.

Rats in group 1 served as control and were treated with 1 ml/kg of distilled water, while groups 2, 3 and 4 were treated with graded doses of AESBAS (300 mg/kg, 600 mg/kg and 1200 mg/kg body weight of the extract respectively) by oral gavage using feeding tubes. These regimens were administered every day for 28 days of the experiment and the animals were weighed on day 0, 7, 14, 21 and 28. The animals were also monitored for clinical signs of toxicity and death during this period. On the 29th day, the animals were sacrificed by jugular venesection.

Statistical analysis

The data were presented as mean ± SEM (standard error of mean) and analysed by one way analysis of variance ANOVA using Graph Pad Prism version 4.03 *P* values less than 0.05 is significant.

Qualitative Phytochemical Analysis

Phytochemical screening

AESBAS were subjected to qualitative phytochemical screening using the method of Trease and Evans [15] for a screening test for the presence of alkaloids, Tannins, Flavonoids, Saponins, Cardiac Glycosides, Steroids and Terpenes.

Gas Chromatography-mass spectrometry derivatization analysis (Improve chromatographic behavior or detectability)

The method of chemical compound improvement to produce a new compound which has properties that is stable and more suitable for analysis using a GC. Many chemical structure does not produce a sharp chromatograph that difficult to detect (i.e., multiple peaks, or one big blob), or the sample of interest goes undetected. Derivatization can also be used to decrease volatility to allow analysis of very low molecular weight compounds, to minimize losses in falsity data, separate sample peaks from solvent peak and serves to indicate the differences in the sample compounds to facilitate the chromatographic separation. Silylation is a method of derivatizations that produces silyl derivatives (TMS), are more stable compounds [16].

Derivatization of extract

2mg dried extract of AESBAS derivatized (by silylation methods) with 2 µl of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine (2 µl), vial heated in a water bath for 30 min to 80ºC, 2 µl of these solutions was employed for GC/MS analysis.

GC analysis

The GC-MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS (5% Diphenyl-95% dimethylpolysiloxane) fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). For GC-MS detection, an EI system with IE of 70 eV, Helium gas as the carrier gas, flow rate of 1mL/min. The injector and MS transfer

line temperature were set at 280ºC. The oven temperature was programmed at an initial temperature 85 ºC (hold 10 min) to 310ºC as a final temperature at an increasing rate of 3ºC /min (hold 5 min).

Identification of components

The qualitative of all the identified components was scanned using a percent relative peak area. The Separation and identification of the compounds according to the comparison of relative RT and MS with those of National Institute Standard and Technology (NIST) at least 220,000 mass spectra. When the NIST library is available, the computer can check for spectra that might match from library data of the GC-MS system.

Results

Effect of sub chronic administration of Acacia Sieberiana stem bark extract on Albino Rats

At the end of the experiment, all groups gained weight, and none lost weight comparison with the initial Weight (**Figure 2a**).

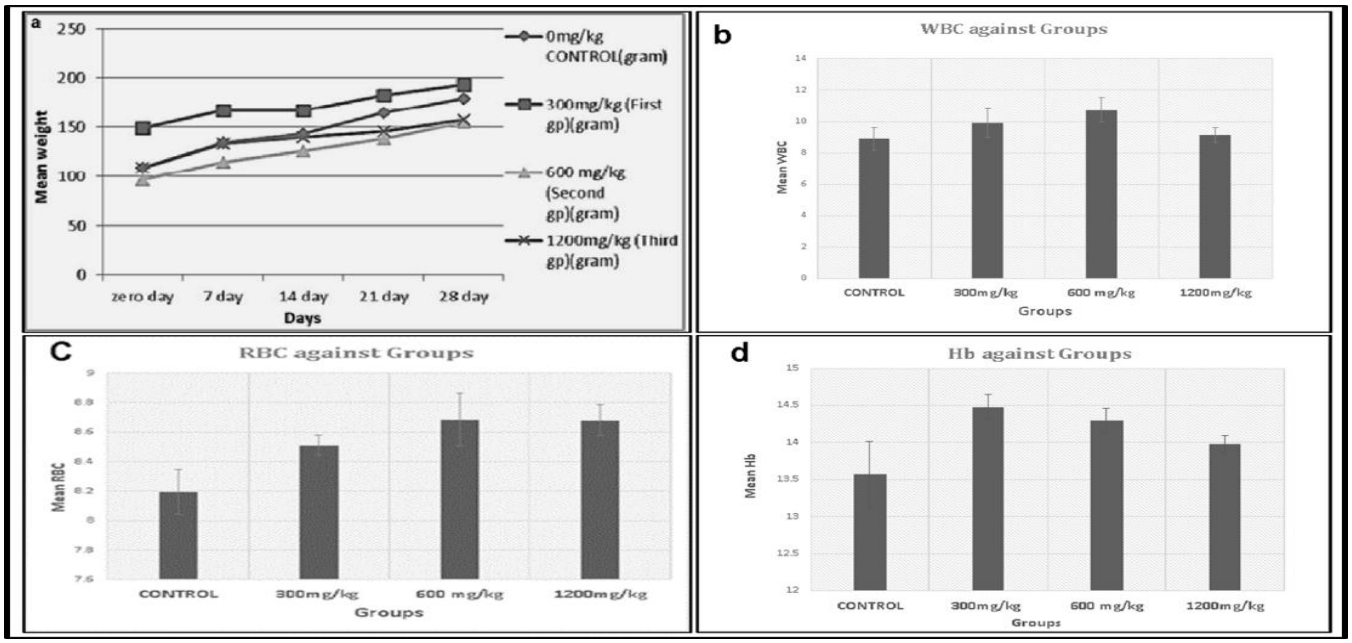


Figure 2: (a) Effect of AESBAS on Mean weights of the experimental groups. (b) Effect of AESBAS on the WBC level of albino rats. (c) Effect of AESBAS on the RBC level of albino rats. (d) Effect of AESBAS on the Hb level of albino rats.

Study of some Haematological parameters

From (**Table 1**) There was no significant difference in the Haematological parameters which include WBC (**Fig. 2b**), RBC (**Figure 2c**), Hb (**Figure 2d**), MCV (**Figure 3a**), MCH (**Figure 3b**), MCHC (**Figure 3c**), RDW (**Figure 3d**), PLT (**Figure 3e**) and PDW (**Figure 3f**) of control rats relative to the separate rats groups that were given varying doses.

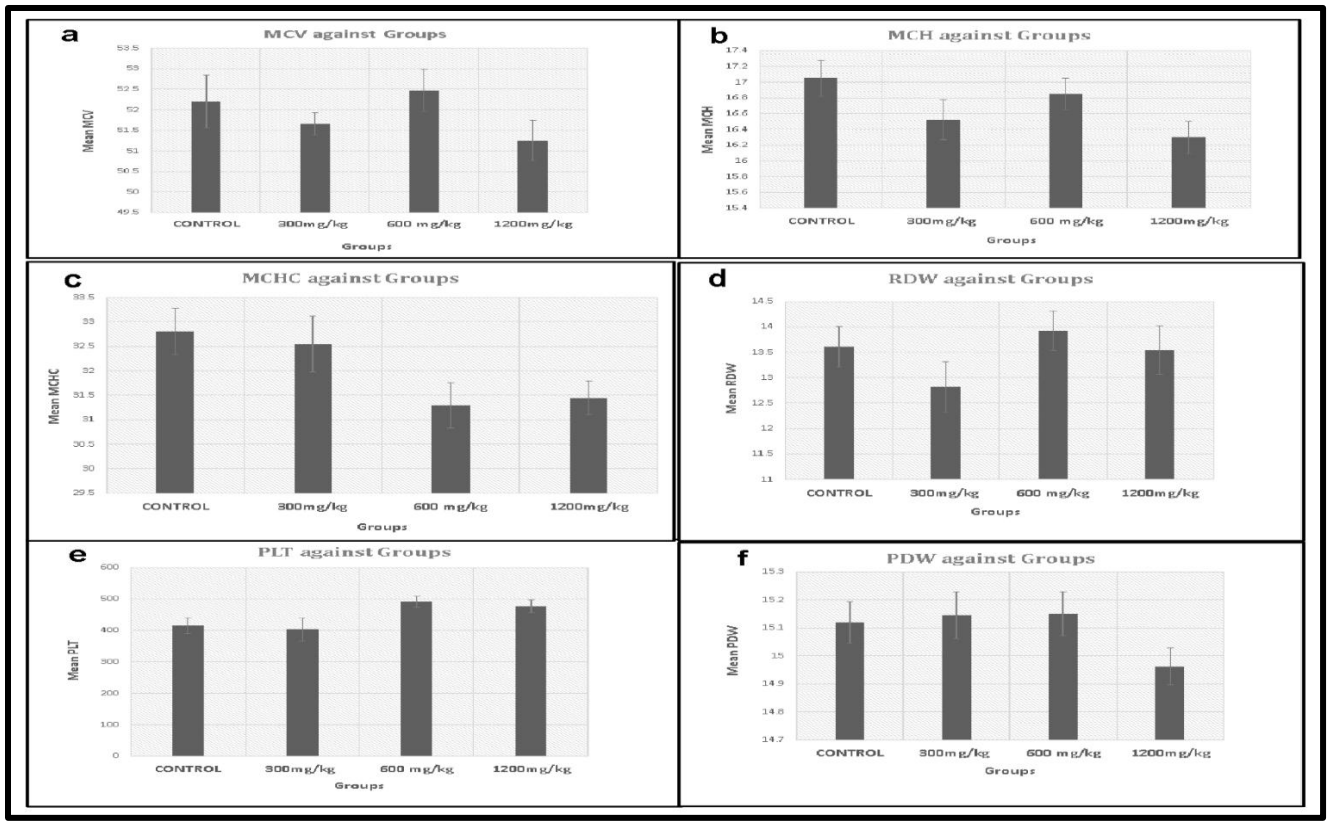


Figure 3: (a) Effect of AESBAS on MCV level of albino rats. (b) Effect of AESBAS on MCH level of albino rats. (c) Effect of AESBAS on MCHC level of albino rats. (d) Effect of AESBAS on the RDW level of albino rats. (e) Effect of AESBAS on the PLT level of albino rats. (f) Effect of AESBAS on the PDW level of albino rats.

Phytochemical Screening and GC-MS-TMS analysis

Phytochemical analysis of AESBAS analysis confirms the presence of many secondary metabolites like tannins, saponin, flavonoids, terpenoids and glycosides (**Table 2**).

The results pertaining to GC-MS-TMS analysis led to the separation and identification of a number of compounds from the GC fractions of AESBAS. From The present results in (**Table 3**), Separation and identification of the compounds present in AESBAS were identified by GC-MS –TMS Chromatogram analysis (**Figure 4**). The major components present in AESBAS were 2-Ethyl-3-methylnaphtho [2,3-b] Thiophene-4,9-Dione, Peak area%(10.61%). Propanoic acid, 2-[(trimethylsilyl) oxy]-,trimethylsilyl ester#Lactic acid di-TMS, Peak area %(1.24%). N-(trimethylsilyl) furan-2-carboxaldimine, Peak area %(0.40%). Glycerol-tri-TMS ether, Peak area %(6.93 %). Bis (trimethylsilyl) succinate, Peak area %(1.57%). D-pinitol, pentakis (trimethylsilyl) ether (**Figure 6c**), Peak area %(20.87%). Benzoic acid, 3, 4, 5-tris (trimethylsilyloxy)-,trimethylsilyl ester#Gallic-Acid-TMS (**Figure 5a**),Peak area %(6.86%). Linoleic acid TMS (**Figure 6a**), Peak area %(1.76%). Oleic acid, TMS (**Figure 6b**), Peak area %(1.59%). Silan,tri methyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy]#Farnesol-TMS (**Figure 5c**), Peak area %(1.31%). α-D-Glucopyranoside,1,3,4,6-tetra kis-O-(trimethylsilyl)-β-D-fructofuranosyl2,3,4,6-tetrakis-O-(trimethylsilyl) #Sucrose-octa -TMS, Peak area %(2.35%). Catechine, penta-TMS-ether (2RCis) (**Figure 5b**), Peak area %(4.97%). 12-methoxy-2-trimethylsilyloxy-19-nor-5- a'-podocarpa-1,3,8,11,13-pentaene, peak area %(3.80%). Trimethyl-13-

ethoxycarbonyl-3,7,12,14-tetramethyl-15-propylporphyrin-2,8,18-tripropionate, peak area % (0.40%).7-[3'-Chloro-1'-ethyl-6-methoxy-2-(p-methoxyphenyl)indol-5'-yl]nitrohept-1-ene, peak area %(1.94%).

These mass spectra can be identified from the data library and Biological activities of some bioactive compounds of AESBAS are based on Dr. Duke's Phytochemical and Ethnobotanical Databases [17] were tabulated in **Table 4**. Various bioactive compounds in the extract confirms the application of AESBAS for various diseases. The results of the GC-MS-TMS analysis can be used as an active medicinal plant tool for the identification of the plant.

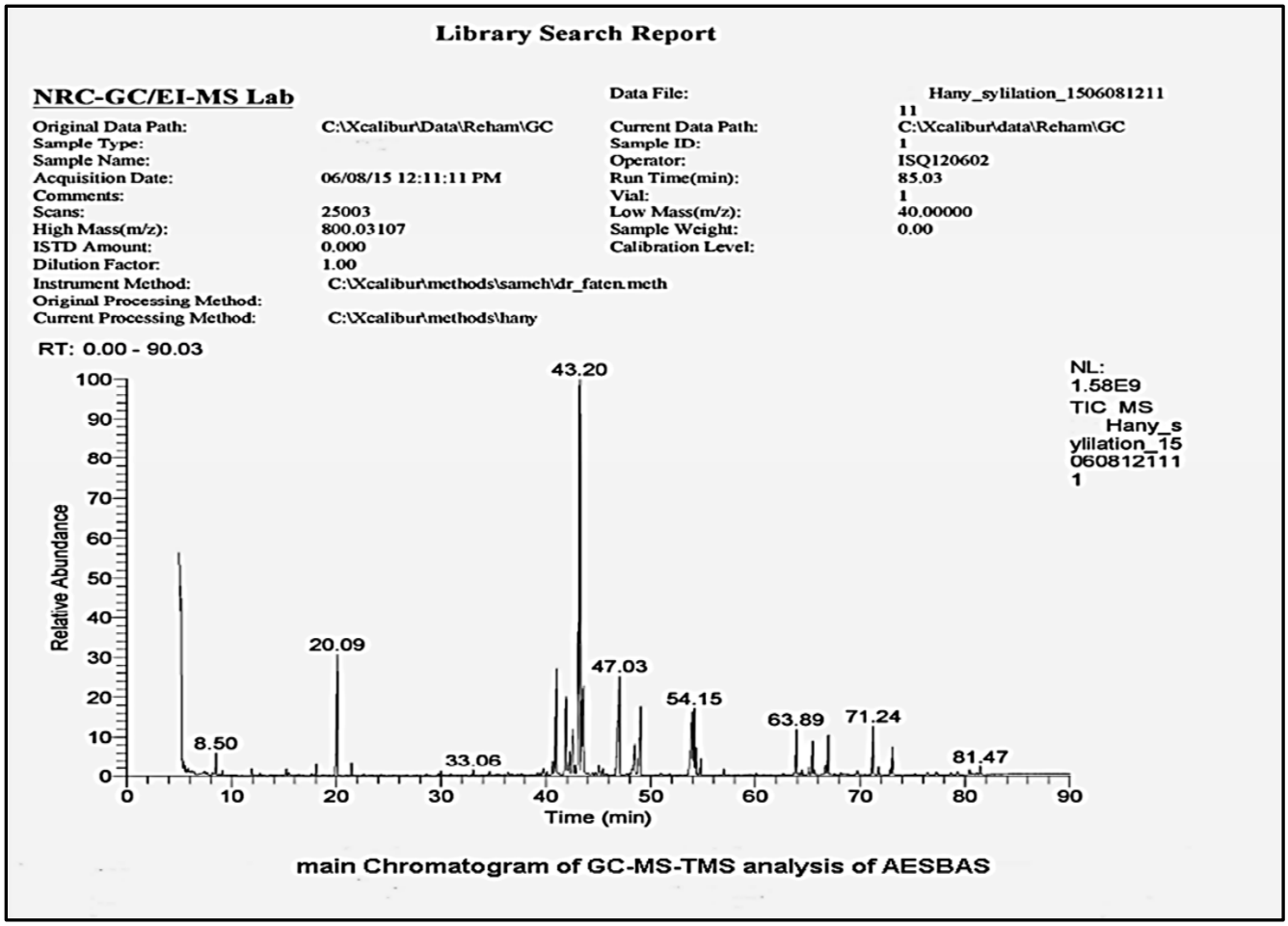


Figure 4: Main Chromatogram of GC-MS-TMS analysis of AESBAS These mass spectra can be identified from the data library.

Discussion

Effects of subchronic administration of AESBAS on some haematological Parameters in Albino Rats

There were no significant changes in the total White Blood Cells (WBC) which suggest that the immune system have not been compromised by the extract. A non-significant difference in platelet number could be an indication that it does not have the potential to stimulate thrombopoietin production [18].

There was no significant difference in the Haematological parameters which include WBC (**Figure 2b**), RBC (**Figure 2c**), Hb (**Figure 2d**), MCV (**Figure 3a**), MCH (**Figure 3b**), MCHC (**Figure 3c**), RDW (**Figure 3d**), PLT (**Figure 3e**) and PDW (**Figure 3f**) of control rats relative to the separate rats groups that were given varying doses.

The blood parameters MCHC, MCV and MCH have a particular importance in anaemia diagnosis [19]. From (**Table1**) the non-significant effects on these indices relating to RBC suggest that there was no effect on the average size of the RBC and also in the haemoglobin weight per RBC. AESBAS induced no haemolysis or bone marrow depression; hence there were no signs of anemia in the test rats. Which give indication of the safety of AESBAS in traditional medicine.

Haematological parameters	**Control**	**300mg/kg body weight**	**600 mg/kg body weight**	**1200mg/kg body weight**	**P value**	**Results**
WBC (X 10^9/L)	8.89 ± 0.73	9.91 ± 0.93	10.74 ± 0.76	9.15 ± 0.47	0.324864 (p>0.05)	no significant change
RBC (X 10^{12}/L)	8.19 ± 0.15	8.51 ± 0.06	8.68 ± 0.17	8.68 ± 0.10	0.058808 (p>0.05)	no significant change
Hb (g/dl)	13.57 ± 0.44	14.47 ± 0.17	14.3 ± 0.16	13.97 ± 0.12	0.119734 (p>0.05)	no significant change
MCV (fL)	52.21 ± 0.64	51.65 ± 0.28	52.47 ± 0.51	51.25 ± 0.49	0.383063 (p>0.05)	no significant change
MCH (pg)	17.05 ± 0.23	16.52 ± 0.25	16.85 ± 0.2	16.3**0** ± 0.21	0.148445 (p>0.05)	no significant change
MCHC (g/dl)	32.81 ± 0.47	32.54 ± 0.57	31.29 ± 0.46	31.45 ± 0.33	0.079385 (p>0.05)	no significant change
RDW (fL)	13.61 ± 0.40	12.82 ± 0.49	13.92 ± 0.38	13.55 ± 0.47	0.384293 (p>0.05)	no significant change
PLT (X 10^9/L)	415.5 ± 24.33	403.22 ± 37.07	491.2 ± 18.13	476.37 ± 20.58	0.065079 (p>0.05)	no significant change
PDW (fL)	15.12 ± 0.07	15.14 ± 0.08	15.15 ± 0.07	14.96 ± 0.06	0.349856 (p>0.05)	no significant change

Table 1: Effect of sub chronic administration of AESBAS on some Haematological parameters in albino rats.

All the values are Mean ± SEM (SEM: standard error of mean).

S. No	Tests	Observation
1	Tannins	+
2	Flavonoids	+
3	Saponins	+
4	Steroid/terpenes	+
5	Cardiac glycoside	+
6	Alkaloids	-

Table 2: The results of the phytochemical analysis of AESBAS.

Key: (+ = Present), (– = Absent).

Phytochemical screening

Phytochemical screening of AESBAS confirms the presence of various secondary metabolites like tannins, flavonoids, saponin, steroid, terpenes and glycosides (**Table 2**). The results suggest that these components have phytochemical properties for curing various ailments and possess potential antimicrobial, antioxidant and anti-diarrhea.

S.NO	RT	Name of the compound	Molecular formula	MW	Peak area%	Probability %
1.	5.18	2-Ethyl-3-methylnaphtho[2,3-b] Thiophene-4,9-dione	$C_{15}H_{12}O_2S$	256	10.61	58.33
2.	8.50	Propanoic acid,2-(trimethylsilyl)oxy] -, trimethylsilyl ester	$C_9H_{22}O_3Si_2$	234	1.24	60.78
3.	11.95	N-(trimethylsilyl)furan-2-carboxaldimine	$C_8H_{13}NOSi$	167	0.40	55.79
4.	15.22	Butane-1,3-diol,1-methylene-3-methyl-,bis(trimethylsilyl)ether	$C_{12}H_{28}O_2Si_2$	260	0.84	30.90
5.	18.05	diethyleneglycol,bis (trimethylsilyl)ether	$C_{10}H_{26}O_3Si_2$	250	1.19	64.73
6.	20.09	Glycerol-tri-TMS ether	$C_{12}H_{32}O_3Si_3$	308	6.93	81.70
7.	21.43	Bis(trimethylsilyl)succinate	$C_{10}H_{22}O_4Si_2$	262	1.57	74.38
8.	29.97	3,6,9,12-tetraoxa-2,1,3-disilatetradecane2,2,13,13-tetramethyl	$C_{12}H_{30}O_4Si_2$	294	0.41	36.84
9.	33.06	L-hreonicacid,tris(trimethylsilyl) ether, trimethylsilyl ester	$C_{16}H_{40}O_5Si_4$	424	0.36	36.43
10.	40.62	a'-D-alactopyranoside,methyl2,6-bis-o-(trimethylsilyl)- ,cyclicmethyl boronate	$C_{14}H_{31}BO_6Si_2$	362	0.57	22.21
11.	41.93	Dion colactone A	$C_{23}H_{21}NO_4$	375	3.37	74.84
12.	42.57	D-(-)-Fructofuranose,pentakis (trimethylsilyl)ether(isomer2)	$C_{21}H_{52}O_6Si_5$	540	3.95	51.53
13.	43.20	D-pinitol,pentakis(trimethylsilyl)ether	$C_{22}H_{54}O_6Si_5$	554	20.87	95.73
14.	45.03	Talose,2,3,4,5,6-pentakis-o-(trimethylsilyl)	$C_{21}H_{52}O_6Si_5$	540	0.82	8.78
15.	45.41	Glucopyranose,1,2,3,4,6-pentakis-o-(trimethylsilyl)	$C_{21}H_{52}O_6Si_5$	540	0.39	35.27

16.	46.82	Benzoicacid,3,4,5-tris(trimethylsilyloxy)-,trimethylsilyl ester	$C_{19}H_{38}O_5Si_4$	458	0.41	85.29
17.	47.03	Benzoicacid,3,4,5-tris(trimethylsilyloxy)-,trimethylsilyl ester# Gallic Acid-TMS	$C_{19}H_{38}O_5Si_4$	458	6.86	83.22
18.	48.51	3-cyano-6,7-dihydro-2-methyl-4-(methylthio)- 5H-benzocyclohepta[1,2-b] pyridine Ethyl	$C_{17}H_{16}N_2S$	280	2.65	25.33
19.	49.03	12-methoxy-2-trimethylsilyloxy-19-nor-5- a'-podocarpa-1,3,8,11,13-pentaene	$C_{20}H_{28}O_2Si$	328	3.80	64.86
20.	53.96	Linoleic acid trimethylsilyl ester	$C_{21}H_{40}O_2Si$	352	1.76	74.69
21.	54.15	Oleic acid, trimethylsilyl ester	$C_{21}H_{42}O_2Si$	354	1.59	15.17
22.	54.35	Silan,tri methyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy] #FarnesolTMS	$C_{18}H_{34}OSi$	294	1.31	27.70
23.	57.01	5a'-androstan-17-one,3a',11a'-bis(trimethylsiloxy)	$C_{25}H_{46}O_3\ Si_2$	450	0.29	8.87
24.	63.89	α-D-Glucopyranoside, 1,3,4,6-tetra kis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl) #Sucrose-octa TMS	$C_{36}H_{86}O_{11}Si_8$	918	2.35	45.47
25.	65.45	Sucrose-octa TMS	$C_{36}H_{86}O_{11}Si_8$	918	1.38	49.09
26.	71.24	Catechine,penta-TMS-ether(2RCis)	$C_{30}H_{54}O_6Si_5$	650	4.97	60.93
27.	72.88	Trimethyl-13-ethoxycarbonyl-3,7,12,14-tetramethyl-15-propylporphyrin-2,8,18-tripropionate	$C_{42}H_{50}N_4O_8$	738	0.40	77.50
28.	73.10	7-[3'-Chloro-1'-ethyl-6-methoxy-2-(p-methoxyphenyl)indol-5'-yl]nitrohept-1-ene	$C_{25}H_{29}CLN_2O_4$	456	1.94	34.05

Table 3: Activity of phytocomponents Identified in Acacia Sieberiana extract by GC-MS-Derivatization analysis. (RT: retention time, MW: Molecular weight)

RT	**Peak Area**%	**Name of the compound**	**Activity****
47.03	6.86	Benzoicacid,3,4,5-tris(trimethylsilyloxy)-,trimethylsilyl ester# (GALLIC-ACID TMS)	Antibacterial, Antioxidant, Anticancer, Antiseptic; Antiviral, Hepatoprotective
53.96	1.76	Linoleic acid trimethylsilyl ester	Antiinflammatory, Antimenorrhagic, Antiprostatitic, Cancer-Preventive, Hepatoprotective
54.15	1.59	Oleic acid, trimethylsilyl ester	Antiinflammatory, Allergenic, Anemiagenic,Antileukotriene-D4, Cancer-Preventive
54.35	1.31	Silan,trimethyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy] #(Farnesol-TMS)	Antiadenomic,Anticancer; Antileukemic; Antimelanomic;

			Antispasmodic,Sedative
63.89	2.35	α-D-Glucopyranoside, 1,3,4,6-tetra kis-O-(trimethylsilyl)-β-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-#(Sucrose-octa TMS)	Antiophthalmic; Antioxidant; Atherogenic; Collyrium; Demulcent; Flatugenic; Hypercholesterolemic
71.24	4.97	Catechine,penta-TMS-ether(2RCis)	Antioxidant, Antibacterial; Antiulcer, Antiviral,Antiinflammatory, Antiherpetic; Antihistaminic; Antiedemic; Antifeedant; Antiflu;

Table 4: Biological activities of some bioactive compounds of AESBAS.

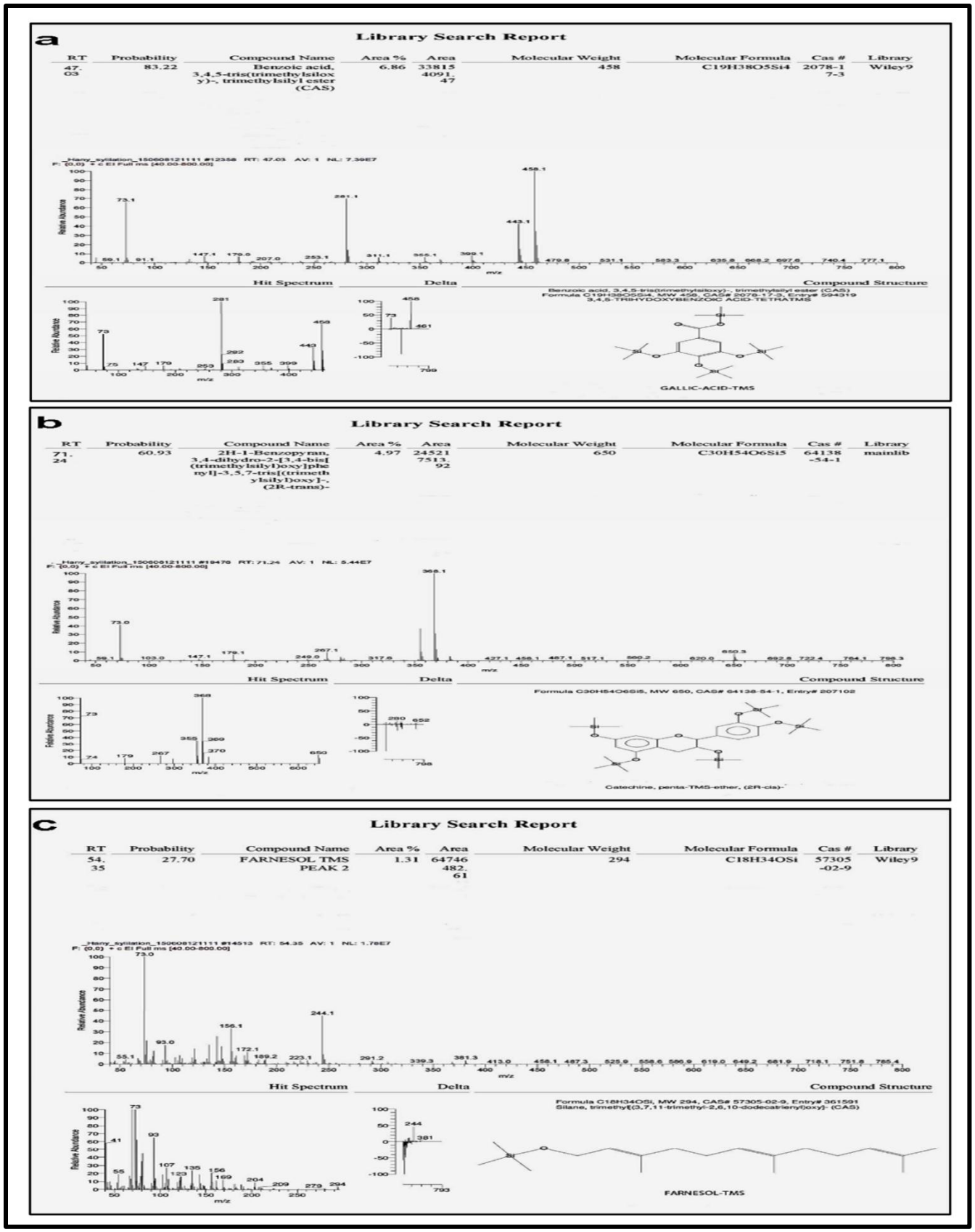
**Source: Dr.Duke's Phytochemical and Ethnobotanical Databases www.ars-gov/cgi-bin/duke/. 2013[Online database].

Gas Chromatography-Mass Spectrometry -Derivatization analysis (GC-MS -TMS)

Confirmation for the presence of tannins in AESBAS

Confirmation By (GC-MS -TMS) analysis as shown in **Table 3** and (**Figure 5a**) we found the presence of Benzoic acid, 3, 4, 5-tris (trimethylsilyloxy)-, trimethylsilyl ester (Gallic acid TMS) and Peak area (6.86%), which is an evidence for the presence of tannin in AESBAS which confirmed the Phytochemical Screening test. **Table 4** shows the biological activities of (Gallic acid TMS).

Figure 5 (a): GC-MS-TMS Chromatogram of Benzoic acid, 3, 4, 5-tris (trimethylsilyloxy)-, trimethylsilyl ester#Gallic-Acid-TMS (b) GC-MS-TMS Chromatogram of Catechine, penta-TMS-ether (2RCis). (C) GC-MS-TMS Chromatogram of silan, tri methyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy] #Farnesol-TMS.


a
Library Search Report
RT | Probability | Compound Name | Area % | Area | Molecular Weight | Molecular Formula | Cas # | Library
47.03 | 83.22 | Benzoic acid, 3,4,5-tris(trimethylsiloxy)-, trimethylsilyl ester (CAS) | 6.86 | 33815.47 | 458 | C19H38O5Si4 | 2078-17-3 | Wiley9
Hit Spectrum
Delta
Compound Structure
GALLIC-ACID-TMS
b
Library Search Report
RT | Probability | Compound Name | Area % | Area | Molecular Weight | Molecular Formula | Cas # | Library
71.24 | 60.93 | 2H-1-Benzopyran, 3,4-dihydro-2-[3,4-bis[(trimethylsilyl)oxy]phenyl]-3,5,7-tris[(trimethylsilyl)oxy]-, (2R-trans)- | 4.97 | 24521.92 | 650 | C30H54O6Si5 | 64138-54-1 | mainlib
Hit Spectrum
Delta
Compound Structure
Catechine, penta-TMS-ether, (2R-cis)-
c
Library Search Report
RT | Probability | Compound Name | Area % | Area | Molecular Weight | Molecular Formula | Cas # | Library
54.35 | 27.70 | FARNESOL TMS PEAK 2 | 1.31 | 64746.61 | 294 | C18H34OSi | 57305-02-9 | Wiley9
Hit Spectrum
Delta
Compound Structure
FARNESOL-TMS

Confirmation for the presence of Flavonoids in AESBAS

The antidiarrheal effect of the plant could be attributed to the phytochemicals such as flavonoids and tannins levels and have been reported to exhibit antidiarrheal activity through denaturing protein hence forming protein tannates which minimize the intestinal mucosa permeability [20].

Confirmation By (GC-MS -TMS) analysis, as shown in **Table 3** and **Figure 5b** the presence of Catechine (sub class of flavon-3-ols), penta-TMS-ether (2RCis) and Peak area (4.97%), is an evidence for the presence of flavonoids AESBAS which confirmed the Phytochemical Screening test. **Table 4** shows the biological activities of Catechine, penta-TMS-ether (2RCis).

Confirmation for the presence of Terpenoids in AESBAS

Terpenoids are a large group of natural compounds that can be found in numerous living organizms; especially plants, fungi, molds, and marine animals. Many of them have various biological activities, e.g. antitumor, antiviral, antibacterial, virostatic, antiulcerotic, anti-inflammatory and others [21].

From the **Table 3** and **Figure 5c** By (GC-MS -TMS) analysis, it reveals the presence of silane,trimethyl[(3,7,11-trimethyl-2,6,10-dodecatrienyl)oxy] #Farnesol TMS and Peak area (1.31%), which is an evidence for the presence of terpenoids in AESBAS which confirmed the Phytochemical Screening test. **Table 4** shows the biological activities of Farnesol TMS.

Confirmation for the presence of Saponins in AESBAS

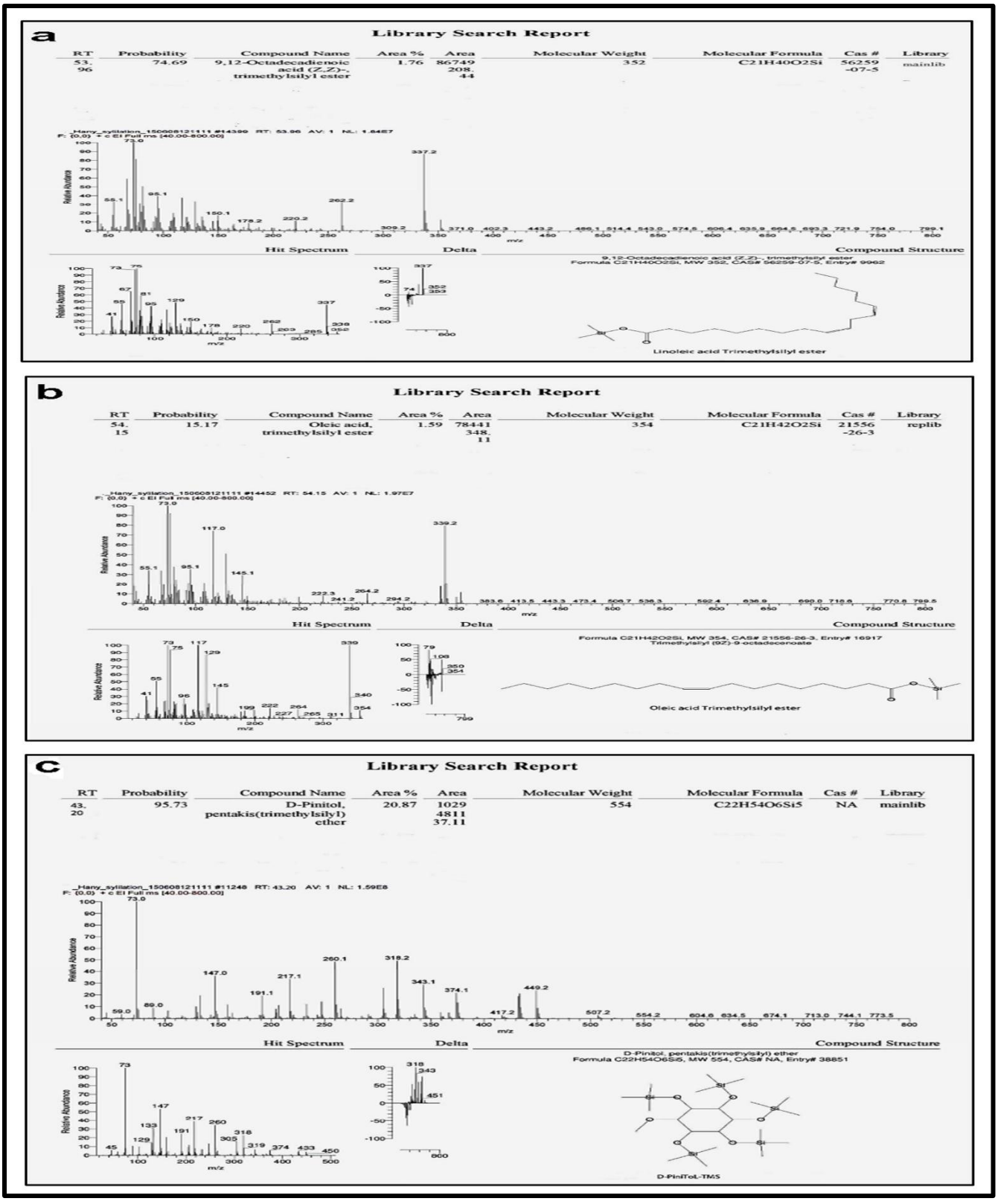
Saponin has antimicrobial activityis due to its ability to cause leakage of proteins and certain enzymes from the cell [22].

From **Table 3** By (GC-MS -TMS) analysis, it reveals the presence of fraction Glucopyranose,1,2,3,4,6-pentakis-o-(trimethylsilyl)and Peak area (0.39%)and 5a'-androstan-17-one,3a',11a'-bis(trimethylsiloxy) and Peak area (0.29%), Sucrose-octa TMS and Peak area (2.35%), which is an evidence for the presence of Saponins in AESBAS which confirmed the Phytochemical Screening test. **Table 4** shows the biological activities of Sucrose-octa TMS.

Confirmation for the presence of Cardiac glycosides in AESBAS

From the (**Table 3**) by (GC-MS -TMS) analysis, it reveals the presence of Sucrose-octa TMS and Peak area (2.35%),5a'-androstan-17-one,3a',11a'-bis(trimethylsiloxy) and Peakarea(0.29%),D-(-)-Fructofuranose, pentakis(trimethylsilyl) ether(isomer2)and Peak area (3.95%) which is an evidence for the presence of Cardiac glycosides in AESBAS which confirmed the Phytochemical Screening test.

Figure 6 (a): GC-MS-TMS Chromatogram of Linoleic acid -TMS.(b) GC-MS-TMS Chromatogram of Oleic acid-TMS. (c) GC-MS-TMS Chromatogram of D-pinitol-TMS.


a
Library Search Report
RT 53.96 | Probability 74.69 | Compound Name 9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester | Area % 1.76 | Area 86749 2088.44 | Molecular Weight 352 | Molecular Formula C21H40O2Si | Cas # 56259-07-5 | Library mainlib
Hit Spectrum
Delta
Compound Structure
Linoleic acid Trimethylsilyl ester
b
Library Search Report
RT 54.15 | Probability 15.17 | Compound Name Oleic acid, trimethylsilyl ester | Area % 1.59 | Area 78441 348.11 | Molecular Weight 354 | Molecular Formula C21H42O2Si | Cas # 21556-26-3 | Library replib
Hit Spectrum
Delta
Compound Structure
Oleic acid Trimethylsilyl ester
c
Library Search Report
RT 43.20 | Probability 95.73 | Compound Name D-Pinitol, pentakis(trimethylsilyl) ether | Area % 20.87 | Area 1029 4811 37.11 | Molecular Weight 554 | Molecular Formula C22H54O6Si5 | Cas # NA | Library mainlib
Hit Spectrum
Delta
Compound Structure
D-PiniToL-TMS

Confirmation for the presence of linoleic and Oleic acid in AESBAS

From the **Table 3**, (**Figure 6a**) and (**Figure 6b**) by (GC-MS -TMS) analysis reveals the presence of Linoleic acid trimethylsilyl ester and Peak area (1.76%), Oleic acid, trimethylsilyl ester and Peak area (1.59%). **Table 4** shows the biological activities of some bioactive compounds of AESBAS.

From the (**Table 3**), (**Figure 6c**) Gas Chromatography-Mass Spectrometry–sylilation (GC-MS -TMS) analysis reveals the presence of D-pinitol, pentakis (trimethylsilyl)ether and Peak area (20.87%) D-Pinitol, it has played a positive role in regulating insulin-mediated glucose uptake in the liver [23].

Conclusions

AESBAS was reported to have exhibited potent antidiarrheal. From Haematological study, AESBAS does not have haemotoxic activities that could limit its medical use as an anti-diarrhea. Active Plants, contain a secondary metabolite with interesting biological activities. From a Phytochemical Screening study of AESBAS which confirmed by Gas Chromatography-Mass Spectrometry (GC-MS) Derivatization analysis, separation, and identification of the existence of various active compounds with different chemical structures like Catechine penta-TMS, Farnesol-TMS, Oleic acid-TMS, Linoleic acid-TMS, Gallic-acid-TMS, D-pinitol-TMS. Which leads to many biological activities of plants like anti-microbial, antioxidant and antidiarrheal activities.

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