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GC-MS AND HPLC ANALYSIS OF THE MOST BIOACTIVE COMPONENTS OF STEM BARK EXTRACT OF *Adansonia digitata (Linn).*

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ABSTRACT
The present investigation was to quantify and characterize the chemical profile of bioactive components of stem bark of Adansonia digitata using GC-MS and HPLC analysis. The plant stem bark were extracte d using three solvents, the bioactive components of each solvent were characterize using Gas chromatogr aphy mass spectrometry (GC MS) analysis and High performance liquid chromatography (HPLC) analys is. The GC MS analysis revealed the chemical profile of the extract with different compounds with distinct peaks, retention time (RT), molecular formular, molecular weight, chemical structure and area%. Nineteen (19) components were identified in petroleum ether, Twenty two (22) components were identified in ethanol and twenty two (22) components were identified in aqueous. The HPLC analysis shows different compounds with distinct peaks and their retention time. The analysis reveal major phytochemicatcals like Triterpenoids, alkaloids, fatty acids, vitamins and etc. The presence of various bioactive compounds confirms the use of stem bark extract of Adansonia digitata for various ailments by Herbal system of medicine. Further study is recommended in order to isolate, identify and purified each of the bioactive constituents present in the stem bark of Adansonia digitata to yield a pure compounds that can be packaged in to orthodox drugs.

Keywords: *Adansonia digitata, GC-MS and HPLC analysis, Bioactive Components, Traditional medicine and extract.*

INTRODUCTION

Medicinal plants have bioactive compounds, which help to treat various ailments caused by microorganisms. These compounds may have evolved in plants as self-defense against pests and pathogens to help plants to establish themselves in their environment (Sukumaran *et al*., 2011). Main concern is shifting towards traditional medicinal plants to tap their unexplored bioactive potential, as nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably from plant origin, many based on their use in traditional medicine (Cowan, 1999). Herbal therapy medicine uses plant extracts for their therapeutic value. It is the oldest form of healthcare known to mankind (Manzoor and Maksuda, 2000) and remains an important element of human and livestock healthcare systems in many developing countries (Lambert *et al.*, 2005). *Adansonia digitata* Commonly known as "Baobab" is a deciduous tree and belongs to the plant family called Bombaceae. The tree is mostly known for its exceptional height, and may live for several hundred years. The trunk tends to be bottle-shaped and can reach an impressive diameter. The branches are thick, wide, and stout compared to the trunk, and can be spread evenly across the height of the tree. The bark tends to be smooth, ranging in colour from reddish brown to grey, with the rare exception of being rough and wrinkly like elephant skin.

Adansonia digitata stem bark extract is been used for the remedy of stomach disorder, diarrhoea, dysentery, antioxidant, antimaleria, antiinflammation. A semi-fluid gum obtained from baobab bark is used to treat sore throat (FAO, 1988). The bark produces strong fibers used in making ropes, mats, bag and hats. The smooth fibers of the inner aspect of the bark are extra important than the outer bark for weaving (Igboeldi *et al.,* 1997). The timber is whitish, spongy and light air dried and is used particularly for fuel (Venter and Venter, 1996). A water storage capacity varies from 1000 to 9000 litres per tree (Craig, 1991). Baobab consists of a number of materials typically used for the treatment of numerous diseases in the African traditional medicine and for that reason it is also named "the small pharmacy" (Obizoba and Anyika, 1994). The present investigation was to quantify and characterize the chemical profile of bioactive components of stem bark of *Adansonia digitata* using GC MS and HPLC analysis.

MATERIALS AND METHODS

Collection, Identification and authentification of the plant materials

The plant material (stem bark) of *Adansonia digitata* was collected by scraping the tree bark using sterile knife during the dry season in the month of February 2015. Identification and
Authentication was comfirmed by a taxonomist (Mal.Bahaud deen) in the Department of Plant Biology, Bayero Universit y, Kano and a voucher specimen number was provided as Ac cession Number (NO.BUKHAN 0036), from their herbarium.

Preparation of powder and extract

The plant stem bark was air-dried at room temperature in the laboratory for two weeks. After drying the stem bark was pounded into powder form using clean mortar and pestle and stored till use following the method of Mukhtar and Okafor, (2002). Required quantity of powdered of stem bark of *Adansonia digitata* were weighed and percolated with petroleum ether, ethanol for two weeks and aqueous for one week with shaking at regular intervals. The mixture was then filtered through a clean muslin cloth followed by filtration with whatman No.1 filter paper and the filtrate was allowed to evaporate at ambient temperature (37^{OC}). While for the aqueous the filtrate was allowed to evaporate using water bath at 45^{0}C. The crude extracts were kept under refrigerated condition at 4^{0}C until required for further use (Betoni *et al*., 2006).

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The Gas chromatography Mass spectrometry (GC-MS) analysis of petroleum ether, ethanol and aqueous fractions of the active stem bark of *Adansonia digitata* was carried out at NARICT, Zaria-Nigeria using GC-MS (Model, QP 2010 PLUS, Shimadzu, Japan). Equipped with a VF-5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. The column oven temperature was programmed from 80°C to 280°C for 2°C min^{-1}. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The temperature of the injector was fixed to 250°C and one of the detector to 200°C. Helium (99.9995% purity) was the carrier gas fixed with a flow rate of 1.5 ml min^{-1}. The mass range from 40-1000 m/z was scanned at a rate of 3.0 scans/s. One micro liter (1.0µl) of the extracts samples was injected with a Hamilton syringe to the GC-MS manually for total ion chromatographic analysis (TIC) analysis in split injection technique. Total running time of G C MS is 27min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of component

The identification of the bioactive compounds in the petroleum ether, ethanol and aqueous fractions of *Adansonia digitata* was carried out by GC-MS based on the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library (i.e. The Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST Library) and the Interpretation of mass spectrum GC-MS was conducted using data base of National Institute of Standards Technology (NIST) and Fatty Acid Methyl Esters Library version 1.0 (FAME library) sources were used for matching the identified components in the extract. The molecular weight, molecular formula and the number of hits used to identify the name of the compound from NIST Libraries were recorded.

High performance liquid chromatography (HPLC) Analysis

The HPLC analysis of Petroleum ether, ethanol and aqueous crude fractions was carried out with Chromatographic system (HPLC Agilent Technology) at Chemistry Department, Bayero University, Kano. Consists of auto sampler with 1-100µl fixed loop capacity and an UV-Visible detector (diode array detector) and the column as 250mm×4.6 mm. The mobile phase consist of Acetonitrite: water, Acetonitrite: Methanol: water gradient system. Separation was performed by using isocratic mode, elution performed at a flow rate of 1ml/min. with injection of 20µl of the samples. The samples were run for 15min and detection was done at 254nm by UV detector (diode detector). All chromatographic data were recorded.

RESULTS AND DISCUSSION

The results of Phytochemical components identified from petroluem ether fraction by GC-MS Analysis showed the retention time (RT), molecular formular, molecular weight (MW), area percentage (%) and compound names. It revealed nineteen (19) compounds (Table 1). The chromatogram of petroleum ether fraction shows nineteen peaks indicating different compounds (Figure 1), Phytochemical components identified from ethanol fraction by GC-MS analysis showed the retention time (RT), molecular formular, molecular weight (MW), area percentage (%) and compound name. The GC-MS analysis revealed tweenty two compounds (Table 2). The chromatogram of ethanol fraction shows twenty-two peaks indicating different compounds (Figure 2). And aqueous fraction GC-MS Analysis showed retention time (RT), molecular formular, molecular weight (MW), area percentage (%) and compound name. The analysis revealed nineteen compounds (Table 3). Aqueous fraction reveals nineteen peaks indicating different compounds (Figure 3).

The high performance liquid chromatography (HPLC) analysis result of petroleum ether, ethanol and aqueous fractions shows that. The petroleum ether fraction reveal a total number of eight chromatograms with different retention times, with 14.460 and 13.239 as the highest and lowest peaks observed respectively (Figure 4). The ethanol fraction reveals a total number of eighteen chromatograms at different retention times having the highest peak with a retention time of 2.102 and 13.740 been the lowest (Figure 5). The aqueous fraction extract also revealed a total number of six chromatograms with different retention time. It shows a prominate peak with a retention time of 2.098 and 6.357 as the lowest peak observed (Figure 6).

The GC-MS analysis of *A. digitata* stem bark revealed the presence of nineteen compounds in petroleum ether, twenty-two compounds in ethanol fraction (Table 2), and twenty-two compounds in aqueous fraction. The identified compounds possess

many biological properties. For instance, Linolenic acid poss esses anti-inflammatory, cancer preventive, hepatoprotective, antihistaminic, antieczemic and antiandrogenic, n-Hexadecanoic acid - palmitic acid can be an antioxidant, hypocholesterolemic, nematicide and lubricant activities. Phytol- Diterpene is an antimicrobial, anticancer, anti-inflammatory and diuretic agent Praveen, K. P, *et al*., (2010) which is similar with the study of this research finding. Similarly Maria *et al.* (2011) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al*. (2011) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008). This study also agreed with work on Phytol- Diterpene. Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al*., 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al,* 2002) and *Melissa officinalis* and Sharafzadeh, 2011). Parasuraman *et al*. (2009) identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus*. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid Siddig Ibrahim *et al*. (2009). n-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 - Octadecadienoic acid, 9, 12, 15-Octadecatrienoic acid and Squalene were identified in the ethanol leaf extract of *Aloe Vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen, K.P, *et al*., 2010). Squalene is used in cosmetics as a natural moisturizer. Devi *et al.* (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid. These reports were in accordance with the result of this study. It also concurred with Shukla *et al.,* (2001) who reported that, Chemicals that have been isolated and characterized from Adansonia *digitata* plant usually belong to the classes of terpenoids, steroids, vitamins, carbohydrates and lipid. Chromatogram showed the relative

concentration of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyses the compound eluted at different times to identify the nature and structure of the compounds.

Table 1: Phytochemicals identified from Petroleum ether fraction extract of stem bark of *A. digitata* by GC-MS Analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area%
1	3.860	Benzene, 1,2,3-trimethyl	C_9H_{12}	120	1.91
2	4.251	Heptane, 5-ethyl-2-methyl	$C_{10}H_{22}$	142	4.96
3	5.549	Undecane	$C_{11}H_{24}$	156	2.04
4	8.624	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	0.64
5	11.211	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	2.09
6	11.950	Undecanoic acid	$C_{11}H_{22}O_2$	186	6.81
7	13.124	Undecenoic acid, octyl ester	$C_{19}H_{36}O_2$	296	0.92
8	13.607	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	3.04
9	14.317	Decanoic acid	$C_{10}H_{20}O_2$	172	2.99
10	16.191	Valeric acid, 2-pentadecyl ester	$C_{20}H_{40}O_2$	312	9.36
11	16.938	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	5.66
12	18.016	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.25
13	19.951	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	8.91
14	20.066	Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	8.89
15	20.383	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	2.85
16	20.836	Oleic Acid	$C_{18}H_{34}O_2$	282	13.30
17	21.067	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.05
18	22.492	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	4.85
19	22.809	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.79
20	24.325	9-Octadecenal	$C_{18}H_{34}O$	266	8.24
21	26.191	9-Octadecanone	$C_{18}H_{36}O$	268	1.63
22	27.221	1-Octyn-3-ol, 4-ethyl-	$C_{10}H_{18}O$	154	1.83

Table 2: Phytochemicals from ethanol fraction extract of *A. digitata* by GC-MS Analysis.

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area%
1	5.854	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	0.31
2	8.623	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.36
3	11.212	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228	1.04
4	11.997	Undecanoic acid	$C_{11}H_{22}O_2$	186	8.05
5	13.131	Decanoic acid, 2-ethylhexyl	$C_{18}H_{36}O_2$	284	0.57
6	13.612	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	1.88
7	14.361	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.44
8	16.195	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	5.05
9	16.953	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	4.08
10	18.076	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.13
11	18.261	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.93
12	19.947	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	4.84
13	20.078	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	8.14
14	20.395	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	2.33
15	20.885	Oleic Acid	$C_{18}H_{34}O_2$	282	16.19
16	21.217	Octadecanoic acid	$C_{18}H_{36}O_2$	284	3.83
17	22.511	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	5.62
18	24.337	10-Undecenal	$C_{11}H_{20}O$	168	9.40
19	24.772	Docosanoic acid, methyl ester	$C_{23}H_{46}O$	354	10.40
20	25.673	3-Bromooctane	$C_8H_{17}Br$	192	2.49
21	26.210	9-Octadecanone	$C_{18}H_{36}O$	268	2.91
22	27.235	11-Tridecenyl propionate	$C_{16}H_{30}O_2$	254	2.02

Table 3: Phytochemicals from aqueous fraction extract of *A. digitata* by GC-MS Analysis

S/N	Retention time	Compound name	Molecular formula	Molecular weight	Area%
1	5.869	Octanoic acid, methyl ester	$C_9H_{18}O_2$	156	0.33
2	8.633	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.37
3	11.220	Lauric acid, methyl ester	$C_{13}H_{26}O_2$	214	1.28
4	11.998	Undecanoic acid	$C_{11}H_{22}O_2$	186	8.87
5	13.137	Decanoic acid, 2-ethylhexyl ester	$C_{18}H_{36}O_2$	284	0.43
6	13.619	Methyl tetradecanoate	$C_{15}H_{30}O_2$	142	2.14
7	14.372	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.67
8	16.200	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	5.10
9	16.963	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	4.30
10	18.087	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.14
11	19.953	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	5.03
12	20.080	5-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	6.28
13	20.399	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	2.34
14	20.898	Oleic Acid	$C_{18}H_{34}O_2$	282	19.78
15	22.516	Decane, 1-fluoro-	$C_{10}H_{21}F$	160	5.43
16	24.343	E-2-Octadecadecen-1-ol	$C_{18}H_{36}O$	268	10.62
17	24.785	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	11.28
18	26.219	9-Octadecanone	$C_{18}H_{36}O$	268	4.44
19	27.243	11-Tridecenyl propionate	$C_{16}H_{30}O_2$	254	1.17

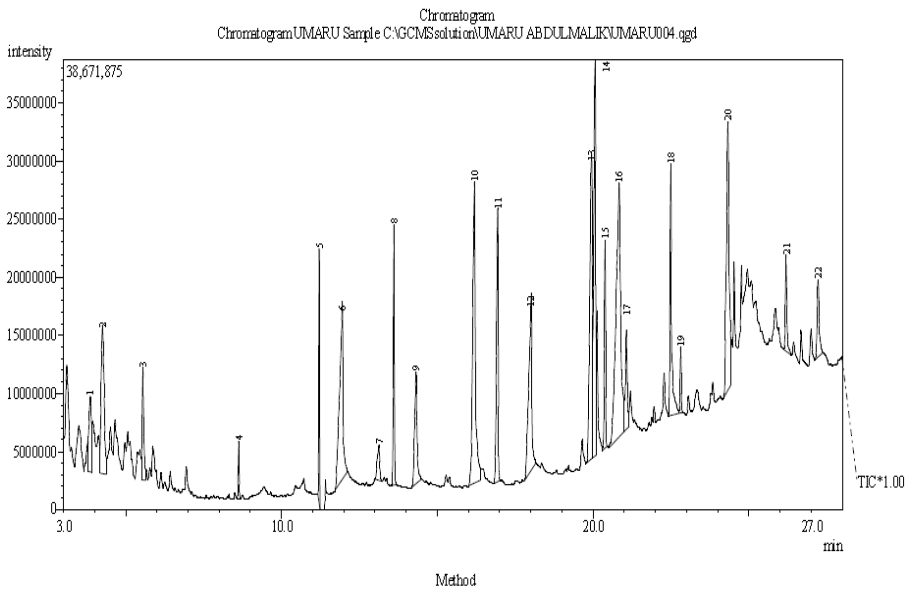

Figure 1. GC-MS Chromatograms of petroleum ether fraction of A. digitata.

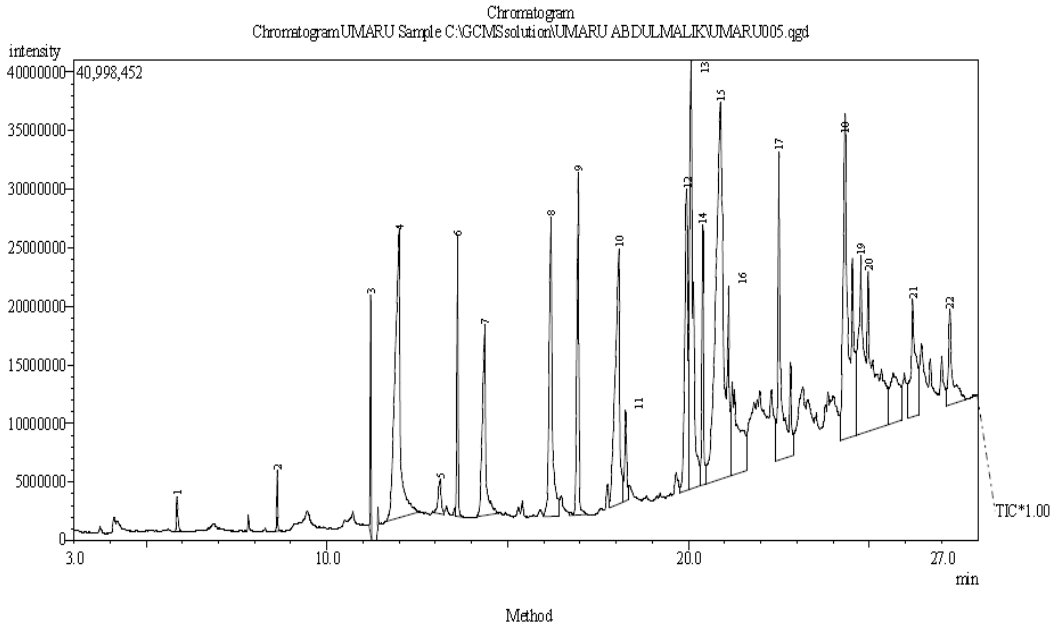

Figure 2. GC-MS Chromatograms of ethanol fraction of A. digitata.

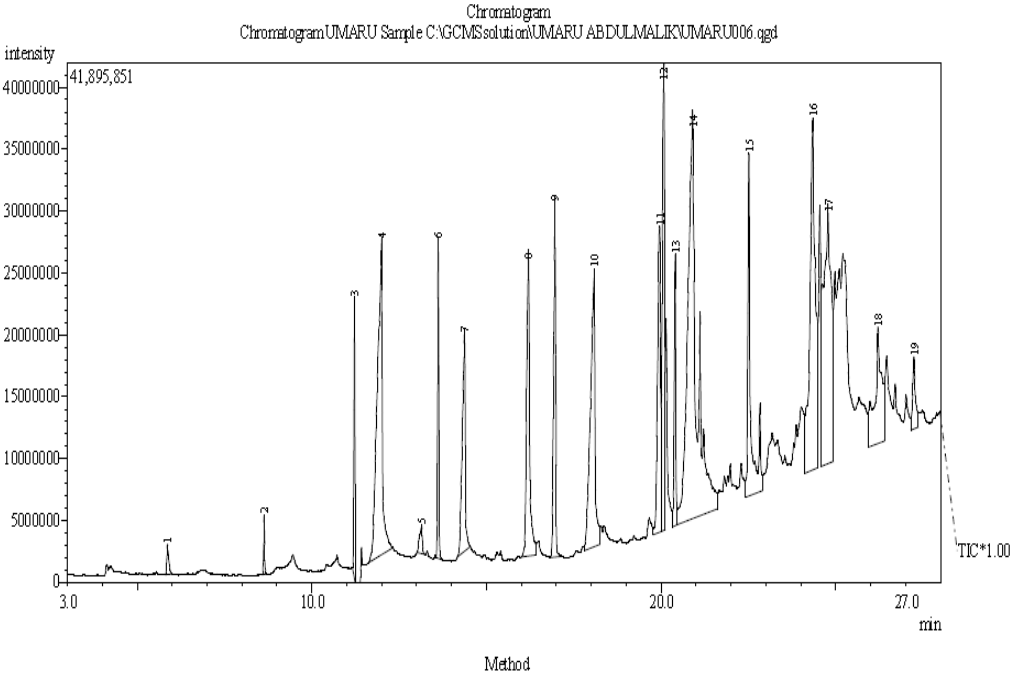

Figure 3. GC-MS Chromatograms of aqueous fraction of A. digitata.

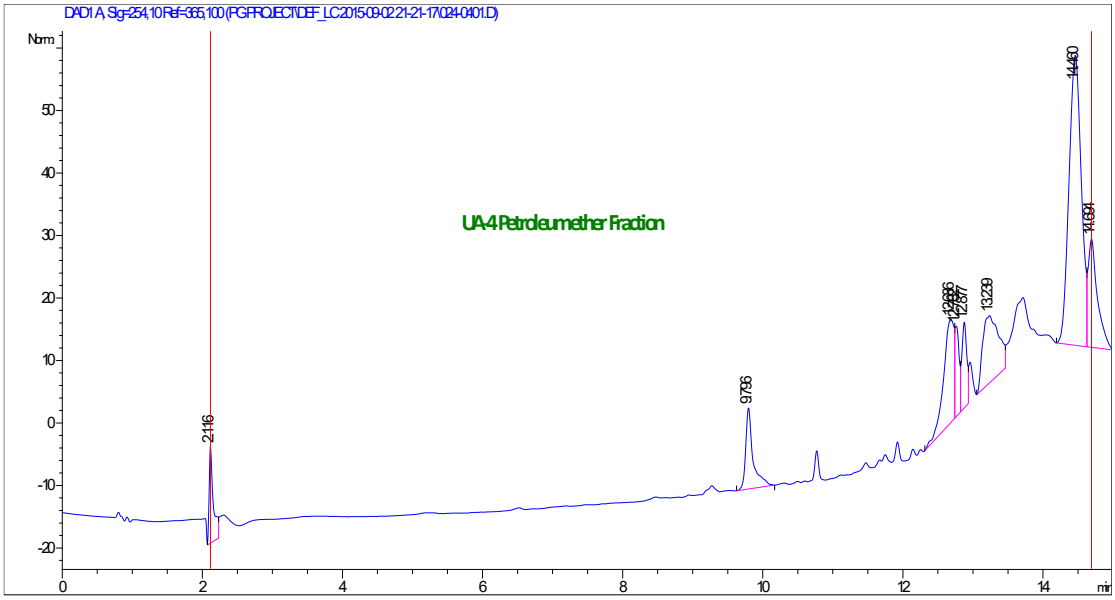

Figure 4. HPLC chromatograms of Petroleum ether fraction of extract of A. digitata.

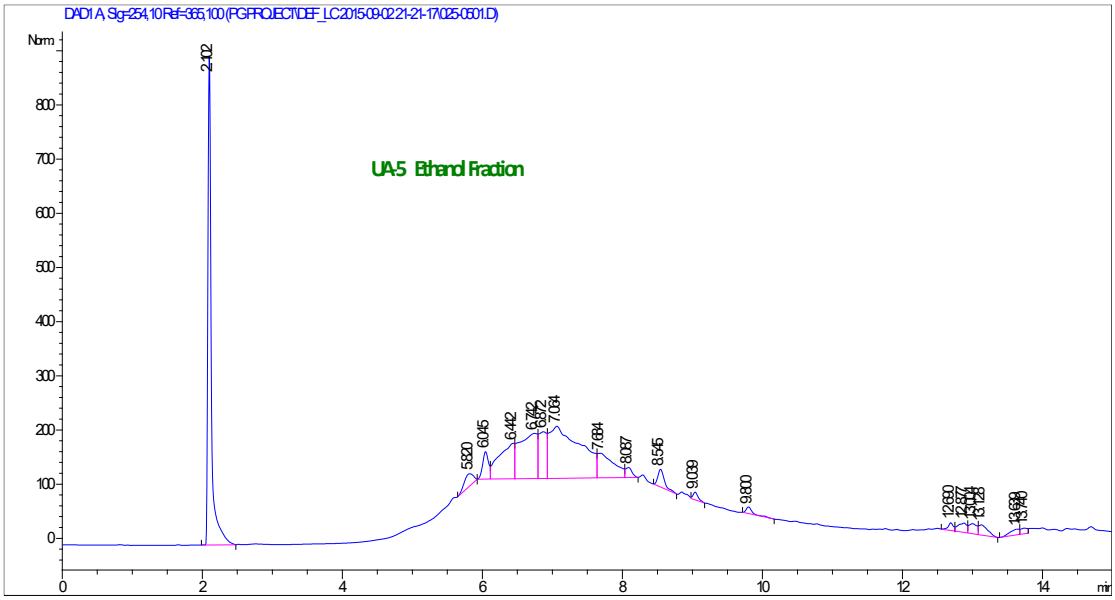

Figure 5. HPLC chromatograms of ethanol fraction of extract of A. digitata.

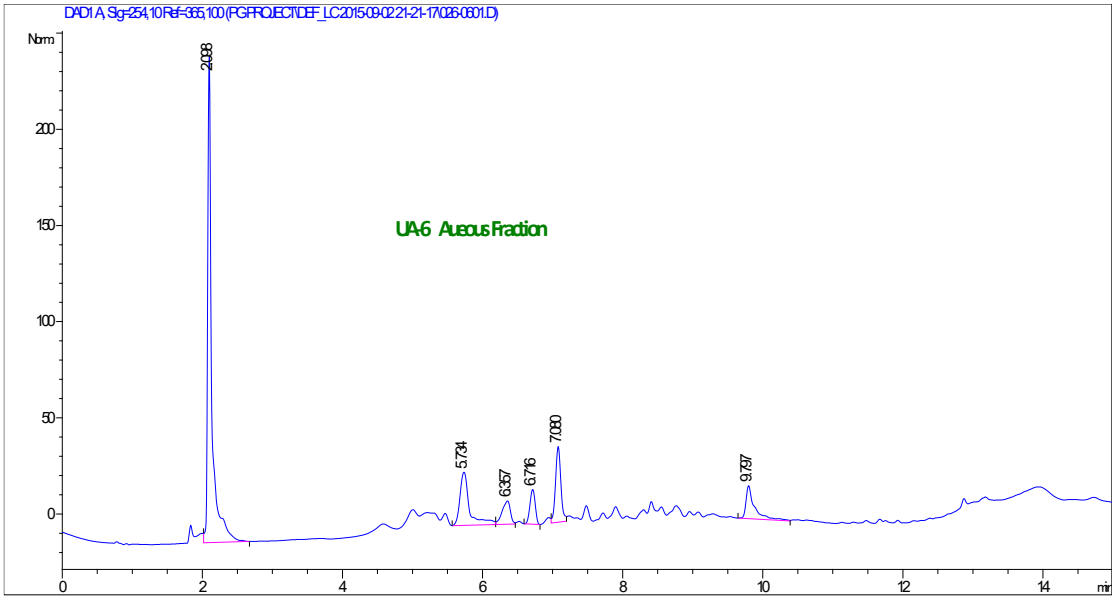

Figure 6. HPLC chromatograms of aqueous fraction of extract of A. digitata.

CONCLUSION

In the present study, twenty-two components each from petroleum ether, ethanol and nineteen from aqueous stem bark of were identified by GC-MS analysis. The presences of various bioactive compounds justify the use of this plant for the treatment of various ailments in Herbal medicine. It is recommended as a plant of phytopharmaceutical importance.

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