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# GC-MS AND HPLC ANALYSIS OF CRUDE EXTRACTS OF STEM BARK OF *Adansonia digitata*

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***ABSTRACT***
***The present investigation was carried out to characterize the chemical profile of the crude extra ct of stem bark of Adansonia digitata using GC-MS and HPLC analysis The plant stem bark were extracted using three solvents, the crude extracts of each solvent were characterized using Gas chromatography mass spectrometry (GC MS) analysis \High performance liquid chromatography (HPLC) analysis. GC-MS analysis revealed the chemical profile of the extract with different compound, distinct peak, retention time (RT), molecular formular, molecular weight (MW), and ch emical structure and area%. With fifteen (15) components identified in pet. Ether, twenty two (2 2) components were identified in ethanol and Eight (8) components were identified in aqueous extracts. The HPLC analysis shows different compounds with distinct peaks and their retention times. The presence of various compounds confirms the use of stem bark extract of Adansonia digitata for the treatment of various ailments by traditional system of medicine.***
***Keywords: Adansonia digitata, Gc-Ms analysis, HPLC analysis, Stem bark and extract.***

## INTRODUCTION

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. Medicinal plants have bioactive compounds which help to treat various ailments caused by microorganisms. These compounds may have evolved in plants as self defence against pests and pathogens to help plants to establish themselves in their environment (Sukumaran *et al.*, 2011). Our 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* Linn Commonly known as "Baobab" is a deciduous tree and belongs to the plant family called Bombacacea. 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 bark being rough and wrinkly like elephant skin.

*Adansonia digitata* stem bark extract is been used in the treatment of stomach upset, 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, bags and hats. The smooth fibers of the inner side of the bark are more important than the outer bark for weaving (Igboeldi *et al.*, 1997). Baobab contains a number of substances usually employed 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).

## 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 from Bayero University, campus (Old Site) Kano, Nigeria during dry season in the month of February, 2015. Identification and authentification was comfirmed by a Taxonomist at the Department of Plant Biology, Bayero University, Kano and a voucher specimen number was provided as Accession Number ( BUKHAN 0036 ), at the herbarium.

### Preparation of powder and extract

The plant stem bark was air-dried at room temperature in the laboratory for two weeks, and pounded into a powdered form using clean mortar and pestle according to Mukhtar and Okafor, (2002). Twenty gram (20g) of powdered of the stem bark of *Adansonia digitata* were weighed and separately percolated with One hundered miles (100ml) of petroleum ether and 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 Whitman's No.1 filter paper and the filtrate was allowed to evaporate at ambient temperature. While for the aqueous the filtrate was allowed to evaporate using water bath at $45^0$C. The crude extracts were kept 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 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 2ºC $min^{-1}$. 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 GC MS is 27min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

### Identification of component

The identity of the bioactive compounds in the petroleum ether, ethanol and aqueous 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 luquid chromatography ( HPLC) Analysis

The HPLC analysis of Petroleum ether, ethanol and aqueous crude extract was carried out with Chromatographic system (HPLC Agilent Technology) at Chemistry Department, Bayero University, Kano. The mobile phase consist of Acetonitrite: water, Acetonitrite: Methanol: water gradient system and 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).

## RESULTS AND DISCUSSION

The result of Phytochemical components identified from petroluem ether extract by GC-MS Analysis showed the retention time (RT), molecular formular, molecular weight (MW), area percentage (%) and compound names. It revealed fifteen (15) compounds (Table 1). Phytochemical components identified from ethanol extract by GC-MS Analysis showed the retention time (RT), molecular formular, molecular weight (MW), area percentag (%) and compound name. The GC-MS analysis revealed tweenty two compounds (Table 2). And aqueous extract GC-MS Analysis showed retention time (RT), molecular formular, molecular weight (MW), area percentage (%) and compound name. The analysis revealed eight (8) compounds (Table 3).

The high performance liquid chromatography (HPLC) analysis result of petroleum ether, ethanol and aqueous extracts shows that the petroleum ether extract reveals fifteen (15) chromatograms with different retention times, with 14.460 and 13.239 as the highest and lowest peaks observed respectively (Figure 1). The ethanol extract reveals twenty two (22) chromatograms at different retention times having the highest peak with a retention time of 2.102 and 13.740 been the lowest (Figure 2). The aqueous extract also reveals eight (8) 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 3). The high performance liquid chromatography (HPLC) analysis machine (HPLC Agilent Technology) used for this analysis does not enable one to have a separate collection of different compounds. Rather, it shows chromatogram peaks representing compounds with their distinct retention time.

The identified compounds possess many biologica l properties. For instance, Linolenic acid possesse s antiinflammatory, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic and antiandrogenic, n-Hexadecanoic acid - palmitic acid can be an antioxidant, hypocholesterolemic, nematicide, pesticide and lubricant activities. Phytol-Diterpene is an antimicrobial, anticancer, anti-inflammatory and diuretic agent (Praveen *et al*., 2010). 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 and Manimegalai, 2008). 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, Phenol, 2, 4-bis (1-phenylethyl) which all have medicinal properties. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.

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* (Sharafzadeh *et al*., 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 (Siddiq *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 *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 and 9, 12-Octadecadienoic acid. These reports are in accordance with the result of this study.

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. The large compound fragments into small compounds giving rise to appearance of peaks at different M/Z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. In addition to this the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The HPLC analysis separate and identified different peaks representing compounds with distinct retention times.

**Table 1: Phytochemical components identified from Petroleum ether extract of stem bark from *Adansonia digitata* by GC-MS Analysis**

| S/N | Retention time | Compound name | Molecular formula | Molecular weight | Area % |
|---|---|---|---|---|---|
| 1 | 3.119 | n-Nonane | $C_9H_{20}$ | 128 | 17.38 |
| 2 | 3.259 | Cyclohexanemethanol,2-methyl- | $C_8H_{16}O$ | 128 | 7.07 |
| 3 | 3.408 | 1H-Indene,octahydro-trans- | $C_9H_{16}$ | 124 | 5.50 |
| 4 | 3.503 | Cyclohexane,propyl- | $C_9H_{18}$ | 126 | 12.08 |
| 5 | 3.837 | Benzene,1,2,4-trimethyl | $C_9H_{12}$ | 120 | 14.77 |
| 6 | 4.252 | n-decane | $C_{10}H_{22}$ | 142 | 14.53 |
| 7 | 4.531 | Decane,4-methyl- | $C_{11}H_{24}$ | 156 | 7.06 |
| 8 | 4.605 | Benzene,1,2,3-trimethyl | $C_9H_{12}$ | 120 | 4.43 |
| 9 | 5.557 | n-Undecane | $C_{11}H_{24}$ | 156 | 9.31 |
| 10 | 5.861 | 7,7-Dimethyl-4-methylenebicyclo | $C_{10}H_{16}O$ | 152 | 1.88 |
| 11 | 6.098 | Nephthalene,decahydro-2-methyl- | $C_{11}H_{20}$ | 152 | 1.70 |
| 12 | 6.923 | n-dodecane | $C_{12}H_{26}$ | 170 | 0.63 |
| 13 | 17.573 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 1.68 |
| 14 | 20.479 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 1.63 |
| 15 | 20.756 | Nonadecanoic acid | $C_{19}H_{38}O_2$ | 298 | 0.34 |

**Table 2: Phytochemical components from Ethanol crude extract of *Adansonia digitata* by GC-MS Analysis.**

| S/N | Retention time | Compound name | Molecular formula | Molecular weight | Area% |
|---|---|---|---|---|---|
| 1 | 7.819 | Nonanoic acid | $C_9H_{18}O_2$ | 158 | 0.30 |
| 2 | 11.076 | Phenol,2,6-bis (1,1-dimethylethyl) | $C_{14}H_{22}O$ | 206 | 0.72 |
| 3 | 12.012 | 1-Tetradecene | $C_{14}H_{28}$ | 196 | 0.28 |
| 4 | 15.218 | 1-OCtadecyne | $C_{18}H_{34}$ | 250 | 0.73 |
| 5 | 17.623 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 15.47 |
| 6 | 18.126 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 284 | 6.57 |
| 7 | 20.089 | Phytol | $C_{20}H_{40}O$ | 296 | 3.32 |
| 8 | 20.517 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 27.32 |
| 9 | 20.732 | 9-Octadecynoic acid | $C_{18}H_{32}O_2$ | 280 | 6.05 |
| 10 | 20.815 | (E)-9-Octadecenoic acid ethyl ester | $C_2H_{38}O_2$ | 310 | 12.63 |
| 11 | 21.139 | Octadecanoic acid, ethyl ester | $C_{20}H_{40}O_2$ | 312 | 3.94 |
| 12 | 21.516 | 9-Octadecenoic acid | $C_{18}H_{32}O_2$ | 280 | 1.39 |
| 13 | 22.336 | Hexadecanoic acid, 2,3-dihydroxypropyl ester | $C_{19}H_{38}O_4$ | 330 | 1.68 |
| 14 | 22.405 | n-Centane | $C_{16}H_{34}$ | 226 | 1.48 |
| 15 | 23.369 | Docosanoic acid | $C_{24}H_{48}O_2$ | 368 | 0.78 |
| 16 | 23.444 | Nonadecane | $C_{19}H_{40}$ | 268 | 1.02 |
| 17 | 24.158 | 2-Methly-Z,Z-3,13-octadecadienol | $C_{19}H_{36}O$ | 280 | 2.16 |
| 18 | 24.391 | Hexadecane | $C_{16}H_{34}$ | 226 | 4.34 |
| 19 | 24.821 | Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester | $C_{26}H_{42}O_4$ | 418 | 2.06 |
| 20 | 25.207 | Docosanoic acid, ethyl ester | $C_{24}H_{48}O_2$ | 368 | 1.79 |
| 21 | 26.213 | Eicosane, 2-methyl- | $C_{21}H_{44}$ | 296 | 4.83 |
| 22 | 27.694 | Squalene | $C_{30}H_50$ | 410 | 1.16 |

**Table 3: Phytochemical components from aqueous crude extract of *Adansonia digitata* by GC-MS Analysis.**

| S/N | Compound name | Molecular formula | Molecular weight | Retention Time | Area% |
|---|---|---|---|---|---|
| 1 | 2-Hexanone, 3,3-dimethyl- | $C_8H_{16}O$ | 128 | 3.129 | 35.93 |
| 2 | Pentanal,2,2-dimethyl- | $C_7H_{14}O$ | 114 | 3.317 | 9.64 |
| 3 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 17.586 | 16.16 |
| 4 | Oleic acid | $C_{18}H_{34}O_2$ | 282 | 20.493 | 25.22 |
| 5 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 20.765 | 4.46 |
| 6 | Decane,1-fluoro- | $C_{10}H_{21}F$ | 160 | 22.329 | 1.66 |
| 7 | 9-Octadecenal | $C_{18}H_{34}O$ | 266 | 24.152 | 2.52 |
| 8 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 24.928 | 4.40 |

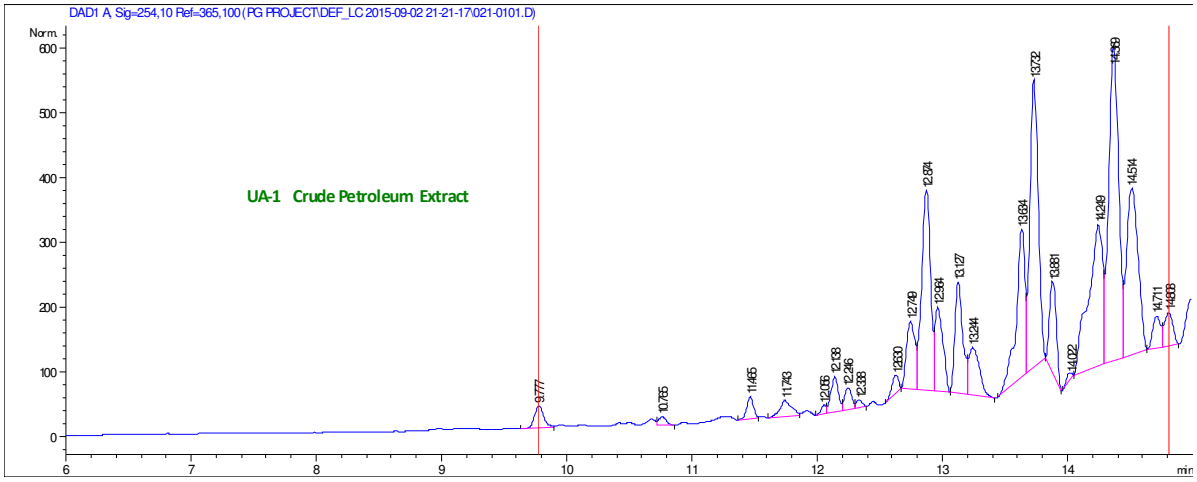


Figure 1: HPLC chromatograms of petroleum ether crude extract of stem bark of *Adansonia digitata*.

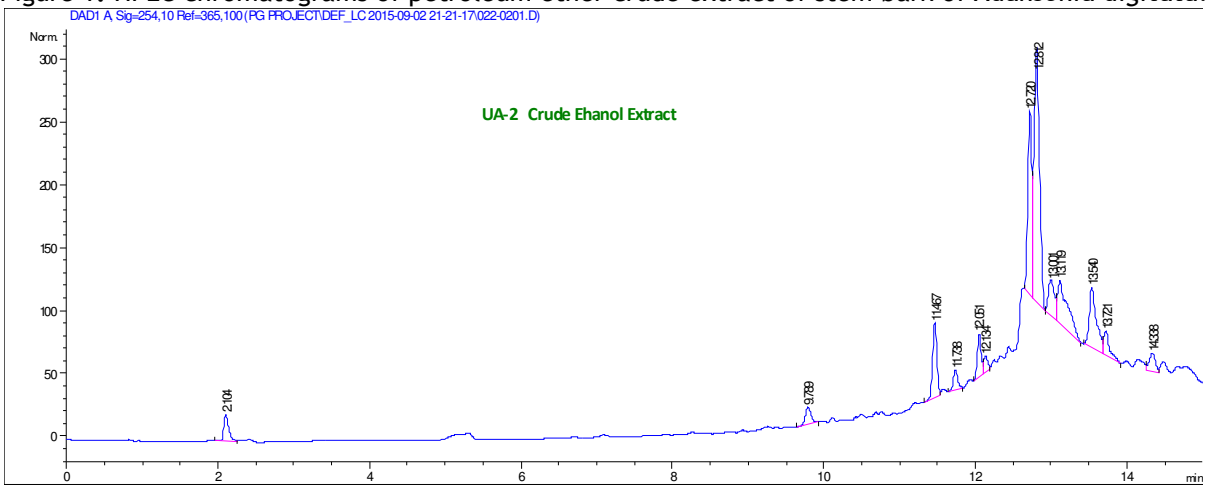


Figure 2: HPLC chromatograms of ethanol crude extract of stem bark of *Adansonia digitata*.

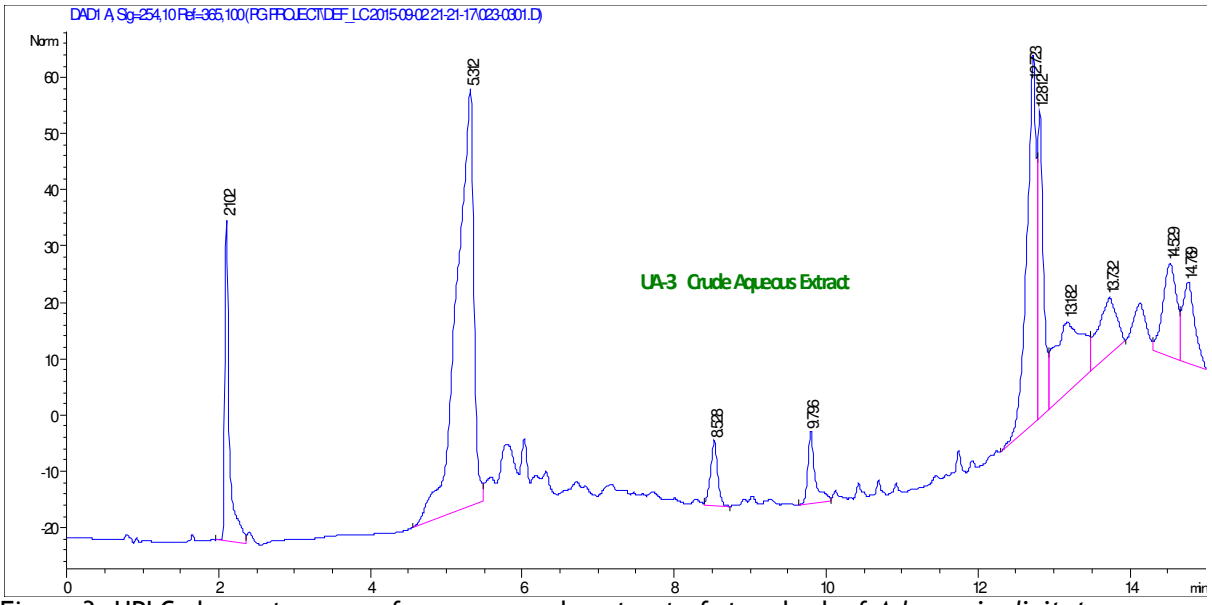


Figure 3: HPLC chromatograms of aqueous crude extract of stem bark of *Adansonia digitata*.

## CONCLUSION

In the present study, twenty two components each from petroleum ether, ethanol and nineteen from aqueous stem bark of *Adansonia digitata* were identified by GC-MS analysis. The presences of various bioactive compounds justify the use of this plant for various ailments in traditional medicine.

## Recommendation

Further studies on toxicity profile of the extract to assess the significant effect on the function of liver, kidney and intestine,

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