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GC-MS ANALYSIS OF ETHYL ACETATE EXTRACT OF *Ficus sycomorus* ROOT LINN. (MORACEAE)

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ABSTRACT
Gas Chromatography-Mass Spectrometry (GC-MS) analysis of a fraction obtained from the column chromatography of the ethyl acetate extract of Ficus sycomorus Linn. root revealed the presence of two compounds. The mass spectral data shows that all the compounds have molecular ion peak and base peak with M/Z 468 and 218 respectively. The tentative identification was achieved by comparing their mass spectra with the data system library and other published spectra. The compounds are suggested to be pentacyclic triterpenoid isomers, and this include; 12-Oleanen-3-yl, acetate (3.alpha) and Urs-12-en-3-ol, acetate (3.beta). The presence of these compounds in F. sycomorus suggests that the plant conforms to other members of the genus Ficus. The result of this study shows that pentacyclic triterpenoid isomers can be useful in the chemotaxonomy of the Moraceae Family if the investigation is extended to other genera and species. Further studies should be carried out to isolate the compounds in their pure forms and detail spectroscopic studies should also be carried out to fully elucidate their structures.
Keywords: Chemotaxonomy, column chromatography, Ficus sycomorus, GC-MS analysis and Moraceae.

INTRODUCTION

The use of natural products as medicines has been described throughout history in the form of traditional medicines, remedies, potions and oils with many of these bioactive natural products still being unidentified. The dominant source of knowledge of natural product uses from medicinal plants is as a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases (Kinghorn *et al*., 2011). Current trends in drug development process are focused on natural sources, especially sources of plant origin due to some proven correlation between folkloric medicinal uses of some of these plants to biological activity (Kunle *et al*., 2003).

Ficus species contain flavanoid glycosides, alkaloids, phenolic acids, steroids, saponinscoumarins, tannins, oleanolic acid, rusolic acid, α-hydroxyursolic acid, protocatechuic acid, maslinic acid. The enzymatic constituents present are ascorbate oxidase, ascorbate peroxidase, catalase, peroxidase, and the phenolic compounds present are gallic acid and ellagic acid (Subramanian and Misra, 1978).

Triterpinoid constituents such as rhoiptelenol, 3-α-hydroxyisohop-22(29)-en-24-oic acid were isolated from the methanolic extracts of fresh leaves and stems of *F. thumbergii*. This species also contains lupenyl acetate, α-amyrin acetate, β-amyrin acetate, lupeol, β-amyrin, α-amyrin, glutinol, ursolic acid, betulinic acid in its leaves and stems. Besides the leaves, bark and fruits of *F. benjamina* contains cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol (Junichi *et al*., 1994).

Two new Pentacyclic triterpenes 8, 26-cyclo-urs-21-en-3β,20β-diol and 3β-acetoxy-8,26-cyclo-ursan-20β-ol and also 3-friedelanone, oleanolic acid, betulinic acid, lupeol acetate, α and β amyrine, 3,5,7,4'-tetra hydroxyl flavones, 3,5,7,3',4'-pentahydroxy flavanate were reported from the stem bark of *F. cordata* (Herve, *et al*., 2008).

Gallic acid, quercetin, rutin, β-Sitosterol-3-*O*-β-D-glucopyranoside, isoquercitrin and quercetin-3-*O*-β-D-galactopyranosyl (1→6) glucopyranoside are some of the compounds isolated from the leaves of *F. sycomorus. (*Mortada *et al.,* 2010). Also, steroids, terpenoids, flavonoids, tannins, saponins, reducing compounds and alkaloids were reported to be present in the leaves and stem bark of the plant (Sofowara, 1993).

The leaves of *F. sycomorus* has been reported to have antidiabetic and antioxidant (70% methanol extract) properties. It also exhibit anti tumour activity and anti bacterial activity, but no anti fungal activity (Mousa, 1994). Aqueous extract of stembark exhibits sedative, anticonvulsant and muscular activities (Sandabe *et al*., 2003; Sandabe *et al*., 2006).

The *in vitro* antimicrobial screening of the methanol root bark extract of *F. sycomorus* revealed that the extract exhibited varying activity against *Enterococcus faecalis, Escherichia coli, Salmonella typhi, Shigella dysenteriae and Candida albicans* (Abubakar *et al.,* 2015). However, the chemistry of the root of *F. sycomorus* is still unknown, therefore, this study was carried out to identify and/or isolate some of the chemical constituents of the root of this important medicinal plant.

MATERIALS AND METHODS

Plant Collection, Identification and Preparation

The plant was collected from Basawa, Zaria, Kaduna State, Nigeria. It was identified in the Herbarium of the Department of Biological Sciences, Ahmadu Bello University Zaria. A reference sample (voucher specimen number, 1466) has been deposited in the Herbarium. The roots were dried and powdered using pestle and mortar. The resultant powder was subsequently referred to as the powdered plant material.

Extraction of the Powdered Plant Material

The powdered plant material (500 g) was macerated with ethyl acetate (1L) for 48 hours. The extract was then filtered and concentrated over water bath at a temperature of 45^{o}C.

Preliminary Phytochemical Screening

The presence of some basic secondary metabolites in the powdered plant material was determined using standards methods (Safowora, 2008; Evans, 1996).

Column Chromatography

The sample (3 g of ethyl acetate extract) was chromatographed on 100 g of Silica gel using gradient elution, starting with pure hexane to hexane: ethyl acetate (90: 10). Eluates of 20 ml were collected, and fractions with the same Thin Layer Chromatography (TLC) profiles were combined and labeled as combined fraction A.

Combined fraction A was then eluted with hexane and a total number of 30 fractions of 5 ml volumes each were collected in which fraction 20 afforded two spots. One of the spots observed on fraction 20 was isolated through preparative TLC using Hexane: Ethyl acetate (7:3) as the solvent system.

Identification of the Components Using GC-MS

GC-MS analysis was performed on Agilent GC-MS (6890N, 5975) at the MS Unit of Stellenbosch University, Matieland, South Africa.

RESULTS

Preliminary Phytochemical Screening

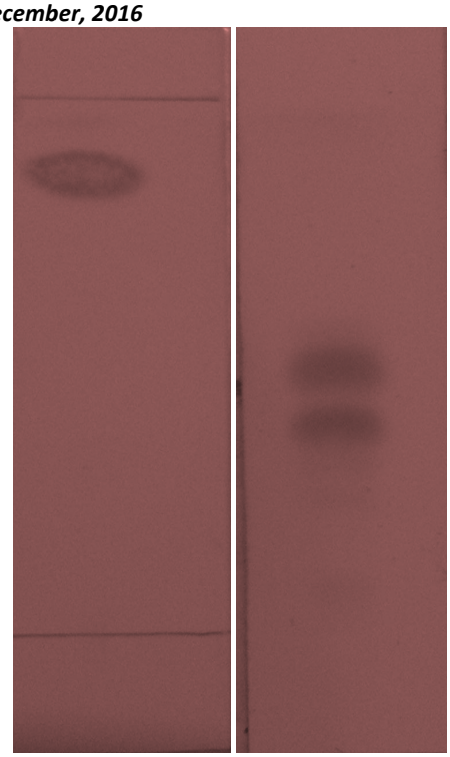
The preliminary phytochemical screening of the ethyl acetate extract revealed the presence of triterpenes/steroids, flavonoids, tannins and alkaloids. The result is summarized in the table below;

Table 1: Phytochemical Constituents of Ethyl Acetate Extract of *F. sycomorus* Root

Constituent Tested	Inference
Anthraquinones:	
Borntrager's test	Absent
Saponins:	
Frothing test	Absent
Cardiac Glycosides:	
Keller-keliani test	Absent
Kedde's test	Absent
Triterpenoids and/or Steroids:	
Liebermann-Burchard test	Present
Salkowski's test	Present
Flavonoids	
Shinoda test	Present
Sodium hydroxide	Present
Tannins Ferric chloride test	Present
Alkaloids	
Mayer's Reagent	Present
Dragendorff's Reagent	Present
Wagner's Reagent	Present

Column Chromatography

One of the spots observed on fraction 20 was isolated through preparative TLC, it revealed a single spot (Hexane: Ethyl acetate 9:1) and two spots (Hexane: Chloroform 7:3). This shows that it was not a single compound rather a mixture of two compounds. The mixture was labelled as fraction DDK.



Hexane: Ethyl acetate (9:1), Hexane: Chloroform (7:3)

Plate I and II: TLC Profile of Fraction DDK

GC-MS Analysis of Fraction DDK

GC-MS analysis of fraction DDK revealed the presence of two compounds which peaks were observed at 17.881 and 18. 531 minutes of retention, suggesting the fraction to be a two compounds mixture. The compounds were labelled as C_1 and C_2 respectively. Electron Impact Mass Spectrometry (EIMS) was used to obtain the mass spectra of the compounds. A characteristic molecular ion $[M]^+$ peak was observed at M/Z 468 for all the two compounds, suggesting that the compounds are isomers.

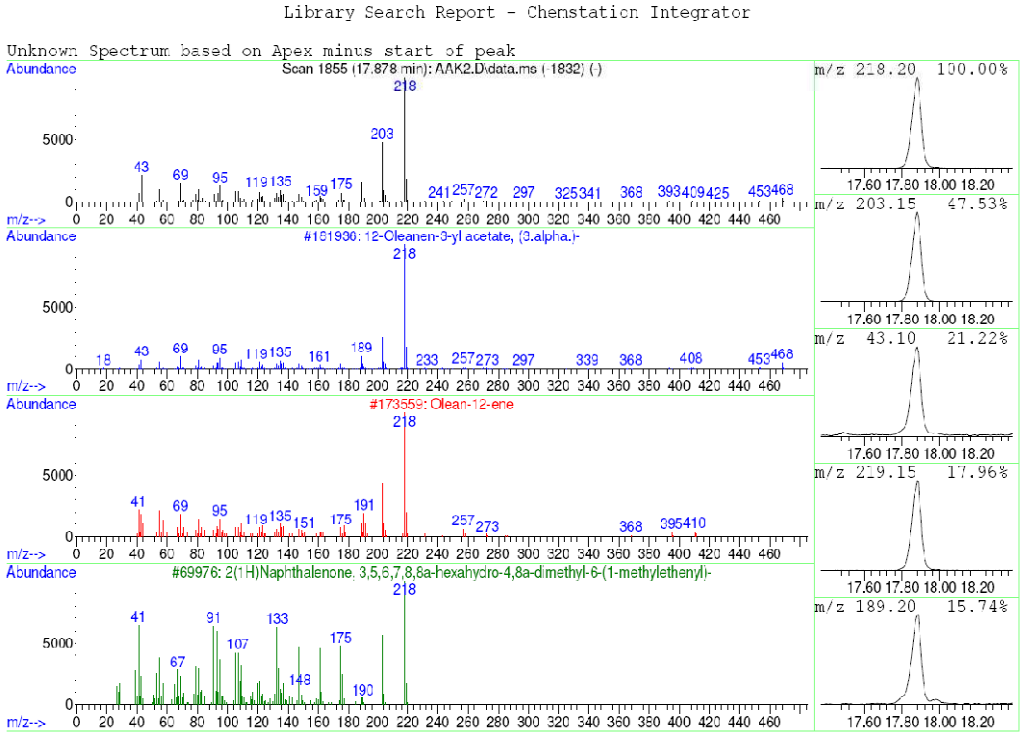
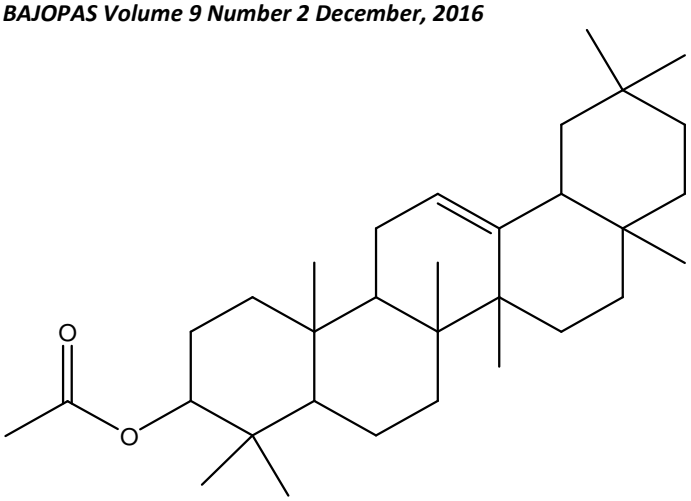


Fig. 1 Mass Spectra of Compound C_1 with the Possible GC-MS Library Match



12-Oleanen-3-yl, acetate (3.alpha)

Fig. 2 Suggested Structure of Compound C_1

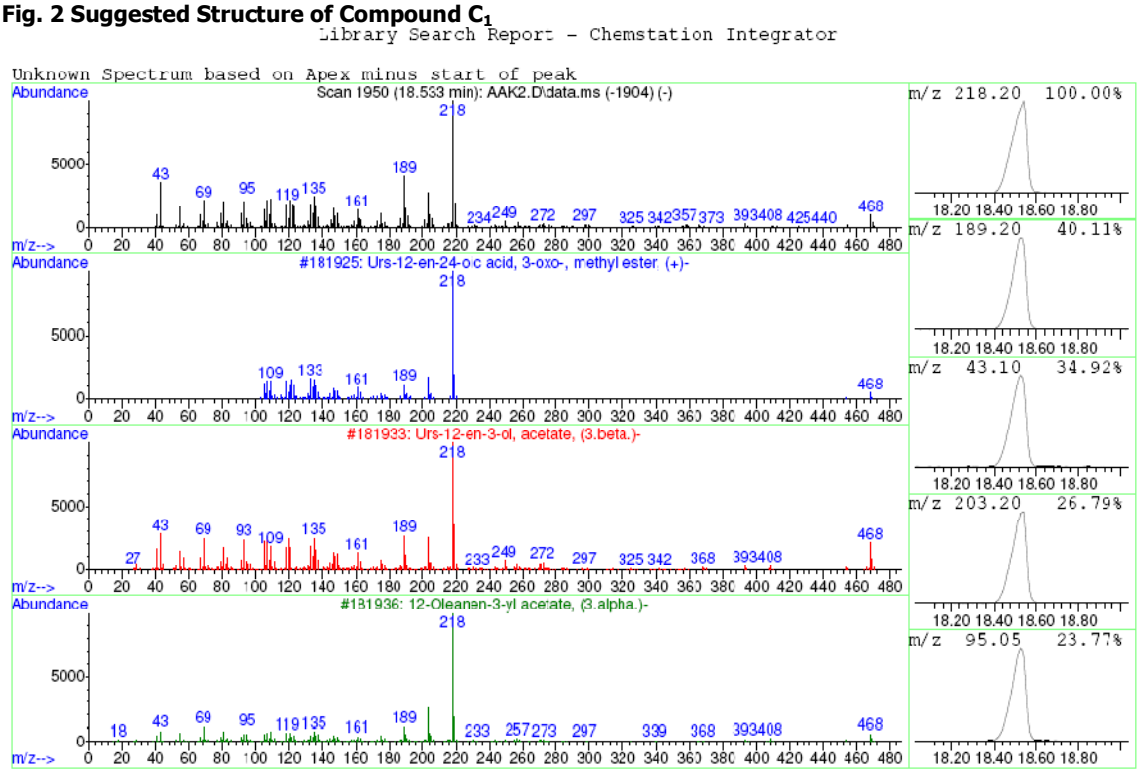
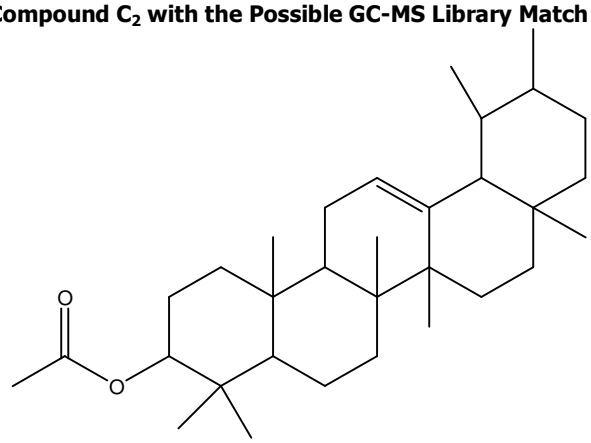


Fig. 3 Mass Spectra of Compound C_2 with the Possible GC-MS Library Match



Urs-12-en-3-ol, acetate (3.beta)

Fig. 4 Suggested Structure of Compound C_2

DISCUSSION

Two compounds were found to be present in fraction DDK, and the tentative identification was achieved by comparing their mass spectra with the data system library. The mass spectral data shows that all the two compounds have molecular ion peak with M/Z 468 and a base peak with M/Z 218. The compounds are suggested to be pentacyclic triterpenoid isomers, and this include; 12-Oleanen-3-yl, acetate (3.alpha) and Urs-12-en-3-ol, acetate (3.beta). The compounds are also known as β-amyrin acetate and α-amyrin acetate respectively (Janete *et al*., 1997).

Pentacyclic triterpenoid isomers generally coexist and as such very difficult to separate. The resolution of these isomers could be improved through coordination chromatography (Kai *et al*., 2013). These isomers could be differentiated by examination of the relative intensities of the peaks at M/Z 189 and 203; β-amyrin acetate has an M/Z 203 peak around twice the intensity of the M/Z 189 peak, while α-amyrin acetate shows both peaks with similar intensity (Janete *et al*., 1997).

Some of the relevant mass spectral data of the compounds are presented below;

Compound Fragments, M/Z (relative abundance)

(1) α-amyrin acetate 468 (M^+, 11), 408 ([M-HOAc]$^+$, 41),249([$C_{16}H_{25}O_2$]$^+$,4), 218 (RDA, 100), 203 ([218- CH_3]$^+$,27)

(2) β-amyrin acetate 468 (M^+, 10), 408 ([M-HOAc]$^+$, 19),249([$C_{16}H_{25}O_2$]$^+$, 10), 218 (RDA, 100), 203 ([218- CH_3]$^+$,48)

RDA= Retro-Diels Alders reaction which is responsible for the prominent fragmentation of such type structure of terpenes (McLafferty and Turecel, 1993).

It is important to mention that both alpha-amyrin acetate and beta-amyrin acetate have already been reported from several species of the genus *Ficus*, and this include; *F. racemosa*, *F. sur, F. Palmata, F. cordata* and *F. thumbergii* (Subramanian and Misra 1978; Sisay and Abeba, 2005; Herve, *et al*., 2008; Junichi *et al*., 1994). The presence of these compounds in *F. sycomorus* suggests that the plant conforms to other members of the genus.

CONCLUSION

It is evident that the compounds identified have not been isolated in their pure forms. It is therefore recommended that further studies should be carried out to isolate the compounds in their pure forms using coordination chromatography, and detail spectroscopic studies should also be carried out to fully elucidate their structures. However, the result of this study can be very useful in the chemotaxonomy (i.e application of chemistry to systematics) of the Moraceae family if the investigation is extended to other genera and species.

Contribution of Authors

Author Abubakar, U. S. performed the laboratory work, results interpretation and wrote the first draft of the manuscript. Author Danmalam, U. H. designed, supervised the development of the work and helped in results interpretation, while authors Ahmed, A., Abdullahi, S. and Abba, A. performed the literature search, collected and prepared the plant material. Finally, author Rukayya, N. made extensive corrections to the manuscript, and all the authors approved the final version of the manuscript before submission.

Conflict of Interest

The authors declare no conflict of interest.

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