Research Article

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Isolation and identification of compounds in the leaf *Ficus sycomorus Linn* Moraceae by Gas Chromatography-Mass Spectrometry, Infra-red and Ultraviolet Spectroscopy

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ABSTRACT

This study was aimed at evaluating phytochemical constituents and characterization of the active principles using UV, FTIR and GC-MS spectroscopic techniques. The leaves of *Ficus sycomorus* were collected from Alau-Dam, Jere Local Government Area of Borno State, Nigeria. Seven hundred grams (700 g) of dry pulverized *Ficus sycomorus* leaves were extracted with 95% methanol using soxhlet extractor and a gummy dark green mass of 124.8 g crude extract was obtained, given a percentage yield of 17.83% $^{w}/_{w}$. Eighty grams (80 g) of crude methanol extract was fractionated through column chromatography and twenty-two (22) eluents of 100 mL aliquot were obtained. Similar fractions were then pooled on the basis of their R_f values on thin layer chromatography (TLC) and four (4) pooled fractions were obtained, coded as F_A, F_B, F_C and F_D. The preliminary phytochemical evaluation investigations revealed the presence of alkaloids, carbohydrates, tannins, cardiac glycoside, cardinolides, saponins, terpenoids and flavonoids. Anthraquinones and combine anthraquinones were absent. Preparative thin layer chromatography (PTLC) of column fraction F_C yielded four sub-fractions (coded C_1, C_2, C_3 and C_4). The interpretation of the UV spectra of sub-fraction C_1 revealed that, fraction C_1 consist of absorption ƛmax at 650.60 nm and 503.00 nm which are similar to ƛmax of alkaloids. Also, the UV spectra of sub-fraction C_3 revealed absorption $ƛ_{max}$ at 657.20 nm, 602.80 nm and 503.20 nm which are also similar to $ƛ_{max}$ of alkaloids. These observations were supported by the major functional groups present in their FTIR spectra, having bands at 3333.1 cm^{-1} which corresponds to N-H stretch in secondary amine, 1790 cm^{-1} corresponding to C=O stretch of ring carbonyl, 1427.37 cm^{-1} corresponding to C=C stretch of aromatic compounds and 2962.76 cm^{-1} corresponding to C-H stretch methyl group. These sub-fractions were also subjected to Gas Chromatography-Mass Spectrometry (GC-MS) and the analysis of the result compared with NIST library revealed similar compounds. The compounds were, 2-Acetyl-3-methylaminocyclopentenone, 9-anthracenyltrimethylsilane, 6,13-bis(2,5-dimethylphenyl)-Dibenzo[C,H]diazecine, 4'-dimethylamino-2'-(trimethylsilyl)acetanilide, 5-Methyl-4-hydroxybenzoylhydrazonefurfurole, 4-(3,4-dimethoxyphenyl)-5-methyl-2-thiazolamine and Cyclobarbital.

1. Introduction

Medicinal plants are plants that possess some natural constituents that produce a definite physiological action on human or animal systems. Plant base natural constituents can be derived from any part of the plant like barks, leaves, flowers, roots, fruits and seeds [1] .(Collectively, plants produce diverse arrays, of low

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molecular mass natural products known as secondary metabolites. These natural products may include alkaloids, flavonoids, terpenes, saponins, tannins, steroids or phenols. *Ficus sycomorus* belongs to moraceae, a family that is reputable for its medicinal value and consist of about 40 genera and over 1,400 species of trees, shrubs, vine and herbs, often with milky latex juices [2]. *Ficus sycomorus* is reported to have many traditional medicine uses in the treatment of snake bites, jaundice, chest pain, dysentery, cool, coughs and throat infections [3]. The objective of the research is to evaluate the phytochemical constituents of methanol leaf extract of *Ficus sycomorus*, isolate and purify the possible bioactive constituents and characterize the active components by using spectroscopic techniques.

Plate 1: Leaf of *Ficus sycomorus*

2. Results and Discussion

2.1 Extraction Profile of Ficus sycomorus

Table 1 showed the masses, colour, texture and percentage yields obtained from the crude extract and pooled column fractions of *Ficus sycomorus* leaf. Extraction of 700 g powdered leaves with methanol yield 124.8 g of crude with percentage yield of 17.83%. Column chromatography of 80 g of crude extract yield pooled fractions A to D with masses 3.25g, 3.50, 1.0, and 4.52 g respectively. While their percentage yields were 4.07, 4.38, 1.25 and 5.65%. The color of crude extract and pooled fraction portions (A to D) were dark army green, dark green, dark green, light brown, and brown respectively. The texture of crude extract was stick and gummy, while that of the pooled column fractions (A to D) were waxy, gummy, sticky and sticky respectively.

Table 1. Mass, percentage yield, colour and texture of crude extract and pooled column fractions obtained from *Ficus sycomorus* leaves.

Entry	Extract fractions	Mass (g)	Yield (%)	Colour	Texture
1	Crude extract	124.8	17.83	Dark army green	Gummy
2	*A*	4.25	7.08	Dark green	Waxy
3	B	4.5	7.5	Dark green	Gummy
4	C	1.0	1.25	Light brown	Sticky
5	D	5.52	9.2	Brown	Sticky

Table 2 showed the masses, colour, texture and percentage yield of the sub-fractions obtained from 1.00g of pooled column fraction C. The masses of the sub-fractions (C_1 to C_3) were 25,18, and 22 mg respectively. While their percentage yields were 2.5, 1.8 and 2.2% respectively. The colours of the fractions range from colourless for C_1, yellow for C_2 and C_3. Fractions C_1, C_2 and C_3 were crystals.

Table 2. masses, colour, texture and percentage yield of the sub-fractions

Sub-fraction	Mass(mg)	% yield	Colour	Texture
C1	25	2.5	Colourless	Crystal
C2	18	1.8	Yellow	Crystal
C3	22	2.2	Yellow	Crystal

The results for the preliminary phytochemical evaluation of crude methanol extract obtained from *Ficus sycomorus* are shown in **Table 3**. The crude methanol extract revealed the presence of carbohydrates, tannins, cardiac glycosides, cardinolides, saponins, alkaloids, terpenoids and flavonoids. Anthraquinones and combine anthraquinones were absent. Table 4. shows the phytochemical constituents of column fractions (A to D).

Table 3. Phytochemical evaluation for crude methanol extract of *Ficus sycomorus*

Phytoconstituent	**Test**	**Result**
Alkaloids	Dragendorff's	+
	Mayer's	+
Anthraquinones		
Free- anthraquinones	Borntrager's	-
Combine anthraquinones	Borntrager's	-
Carbohydrates		
General test	Molisch's	+
Monosaccharide	Barfoed's	+
Free reducing sugar	Fehling's	+
Combine reducing sugar	Fehling's	+
Ketoses	Salivanoff's	+
Soluble starch		+
Cardenolides	Keller-Kalian's	+
Cardiac glycosides		
Steroidal nucleus	Salkowsi's	+
Steroidal nucleus	Liebermann-Buchard's	+
Terpenoids		+
Flavonoids	Lead acetate	+
	Ferric Chloride	+
	Shinoda's	+
	Sodium Hydroxide	+
Saponins glycosides	Frothing	+
Tannins	Ferric Chloride	+
	Lead acetate	+
Phlobatannins		+

Key: + = present, - = absent

Table 4. Phytochemical evaluation of column fractions of *F. sycomorus* methanol leaf extract

Phytochemical	Test	FA	FB	FC	FD
Cardiac glycoside	Salkowski's	+	+	+	-
	Liebermann Burchard's	+	+	+	-
Saponin glycosides	Frothing	+	+	-	+
Tannin	Ferric chloride	+	-	+	-

Cardenolide	keller killanin	+	-	+	-
Alkaloid	Dragendorff's	-	+	+	-
Carbohydrate	Molisch's	+	+	-	+
Free reducing sugar	Fehling's	+	-	-	+
combine reducing sugar	Fehling's	+	-	-	+
Ketoses	Salivanoff's	-	+	+	-
Flavanols/phenol	Ferric chloride	-	+	+	-

2.2 Structural Elucidation/Analysis

The results of UV and FTIR spectroscopic data of sub-fractions C1 and C3 have revealed the conjugation peaks and the multiple functional groups present in the compounds. The data is presented in Table 5, 6, 7 and 8.

Table 5. UV Spectroscopic Data of Sub-fraction C_1

S/No	λ_{max} (nm)	Absorbance
1	650.60	0.294
2	503.00	0.566
3	430.20	3.663
4	426.00	3.726

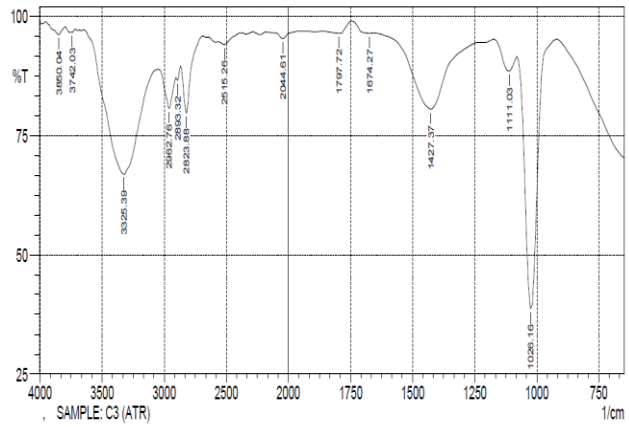


Fig 4. IR Spectra Analysis of Sub-fraction C_3

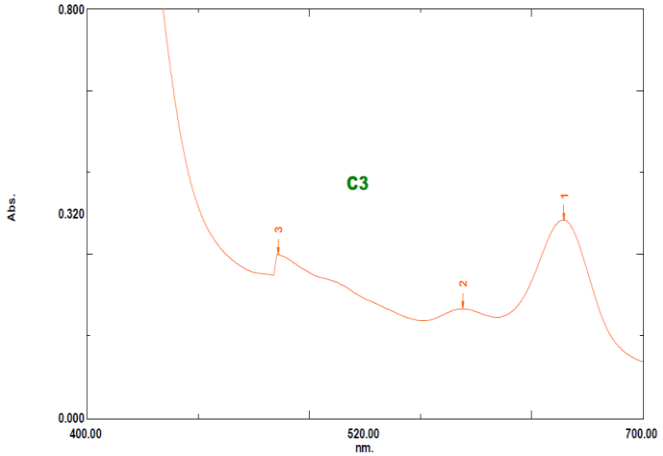


Fig 5. UV Spectra Analysis of Sub-fraction C_3

Table 6. IR Spectroscopic Data of Sub-fraction C_3

S/N	Group	Vibration	Absorption frequency (cm^{-1})
1	Secondary amine	N-H stretch	3325.39
2	Methyl	C-H asymmetric stretch	2962.76
3	Methyl	C-H symmetric stretch	2893.32
4	N-CH_3 methyl	C-H stretch	2823.88
5	Thiol	S-H stretch	2515.26
6	Isothiocyanate	H-NCS stretch	2044.61
7	Carbonyl	C=O stretch	1797.72
8	Amine	C=N stretch	1674.27
9	Aromatic	C=C stretch	1427.37
10	Alkyl Fluoride	C-F stretch	1111.03
11	Amine	C-N stretch	1026.16

Table 7. UV Spectroscopic Data of Sub-fraction C_3

S/No	λ_{max} (nm)	Absorbance
1	657.20	0.387
2	602.80	0.214
3	503.20	0.320

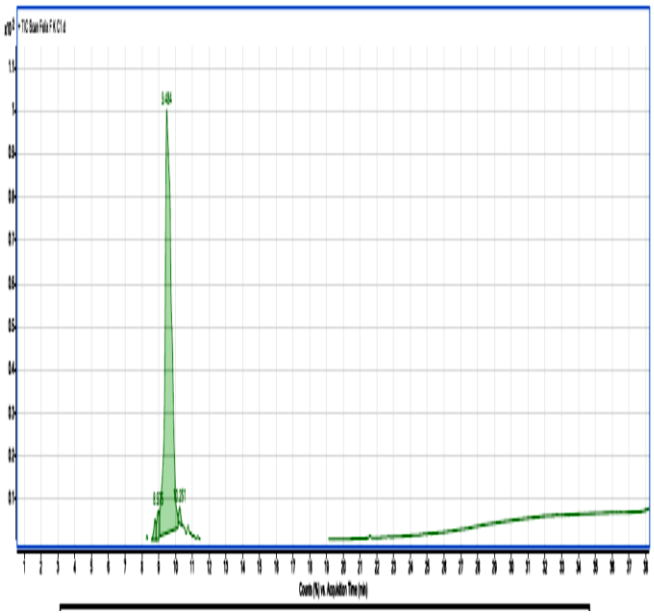


Fig.6. GC-MS Chromatogram of sub-fraction C_1 obtained from F_c

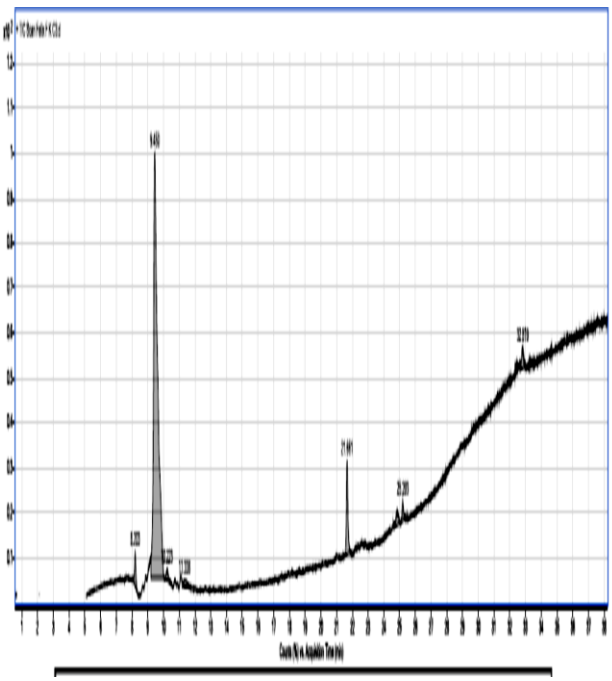


Fig.7. GC-MS Chromatogram of sub-fraction C_3

Table 8 Peaks, Rate time, Molecular weight and Bioactivity of NIST Library of Compounds similar to GC-MS Spectra of Sub-fraction C_1.

Peak	Rate time (min)	% Area	Compound	Mw ($gmol^{-1}$)
1	8.975	4543847.61	O-(2-methylpropyl)-Hydroxylamine	89
2	9.484	20779600.1	Cyclobarbital	236
3	10.251	5.52	6,13-bis(2,5-dimethylphenyl)-Dibenzo[C,H] diazecine	502

Table 9. Peaks, Rate time, Molecular weight and Bioactivity of NIST Library of Compounds similar to GC-MS Spectra of Sub-fraction C_3.

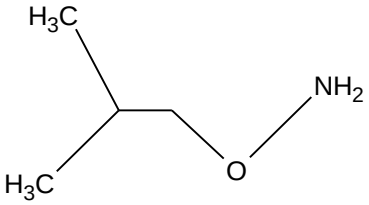
Peak	Rate time (min)	% Area	Compound	Mw ($gmol^{-1}$)
1	8.203	289655.52	9-Anthracenyltrimethylsilane	250
2	9.450	11414197.62	4’-Dimethylamino-2’-(trimethylsilyl)acetanilide	250
3	10.223	150615.98	2-Acetyl-3-methylamino cyclopentenone	153
4	11.338	165260.87	5-Methyl-4-hydroxybenzoylhydrazone furfurole	244
5	21.660	934938.85	Chloroacetic acid	220
6	25.203	169413.76	5-methyl-4-hydroxy-2-benzylidene-coumaran-3-one	250
7	32.819	272099.05	4-(3,4-dimethoxyphenyl)-5-methyl-2-Thiazolamine	250

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Compound C_1 and C_3

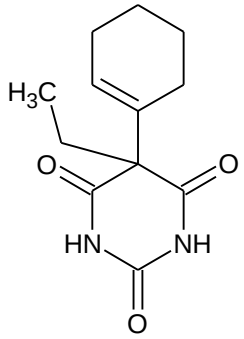
The information obtained from the GC-MS spectra data of sub-fraction C_1 and C_3 compared with the NIST library of compounds revealed compounds that are similar to sub-fraction C_1 and C_3 base on the similarity of their spectra data (Figures 5 and 6). The C_1 Compounds are: O-(2-methylpropyl)-Hydroxylamine, Cyclobarbital and

6,13-bis(2,5-dimethylphenyl)-Dibenzo[C,H] diazecine. The structures of the compounds are shown below in figure 7.

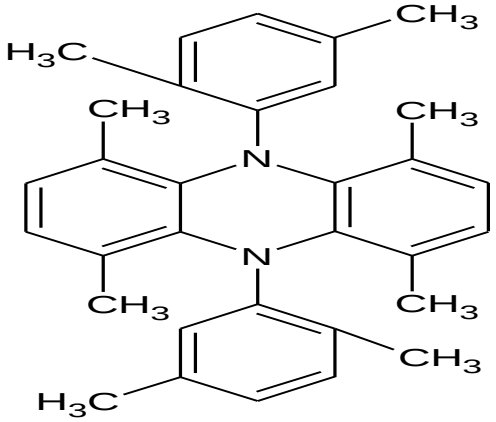
The C_3 Compounds as revealed by the GCMS analysis and NIST library of Compunds are: 9-anthracenyltrimethylsilane, 4'-dimethylamino-2'-(trimethylsilyl)acetanilide, 2-Acetyl-3-methylaminocyclopentenone, 5-Methyl-4-hydroxybenzoylhydrazonefurfurol, 4-(3,4-dimethoxyphenyl)-5-methyl-2-thiazolamine,(Chloroacetic acid), 5-methyl-4-hydroxyl-2-benzylidene-coumaran-3-one, and O-(2-methylpropyl)-Hydroxylamine. The structures of the compounds are shown below in figure 7.



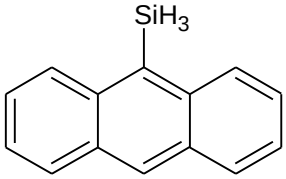
(I) O-(2-methylpropyl)-Hydroxylamine



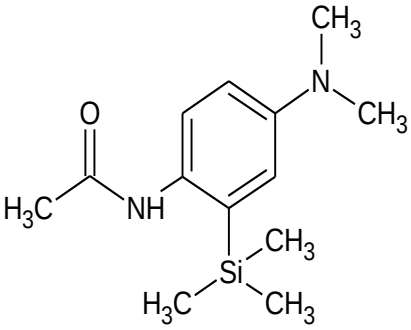
(II) Cyclobarbital



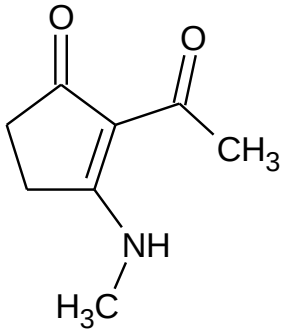
(III) 6,13-bis(2,5-dimethylphenyl)-dibenzo[C,H]diazecine



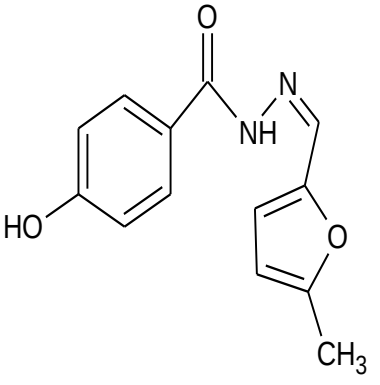
(IV) 9-anthracenyltrimethylsilane



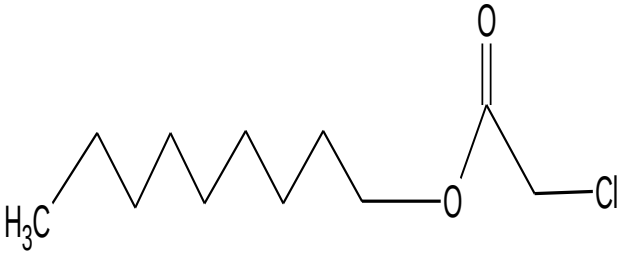
(V) 4'-dimethylamino-2'-(trimethylsilyl)acetanilide



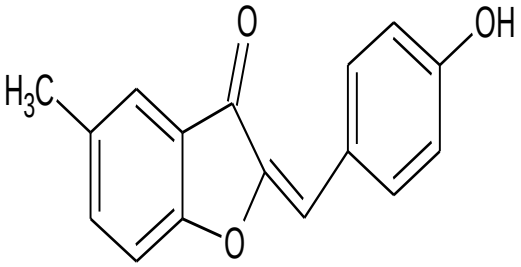
(VI) 2-Acetyl-3-methylaminocyclopentenone



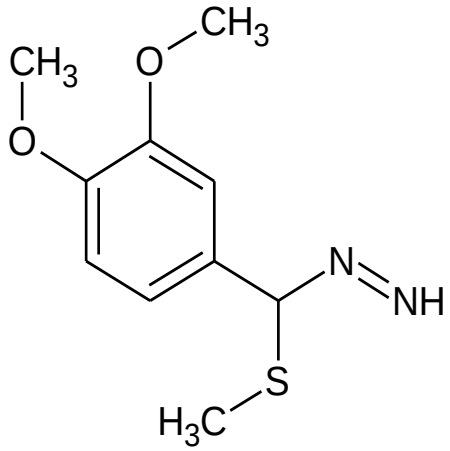
(VII) 5-Methyl-4-hydroxybenzoylhydrazonefurfurol



(VIII) Chloroacetic acid



(IX) 5-methyl-4-hydroxyl-2-benzylidene-coumaran-3-one



(X) 4-(3,4-dimethoxyphenyl)-5-methyl-2-thiazolamine

Figure 8. NIST library of compounds similar to GC-MS spectra of sub-fraction C_1

2.4 Discussion

Medicinal plants which form the backbone of traditional medicine have in the last few decades been the subject of pharmacological studies [7]. Phytochemical analysis of *Ficus sycomorus* leaf extract revealed the presence of some secondary metabolites such as, saponins, flavonoids, cardiac glycosides, carbohydrates, tannins, terpenes, alkaloids and steroids. There was no anthraquinone present. The chemical constituents present in the extract have been reported to possess many therapeutic values. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also have anti-microbial, anti-fungi, anti-parasite, anti-viral, anti-allergenic, anti-spasmodic, anti-hyperglycemic, anti-inflammatory and immuno-modulatory properties [8].

Quercetin, a well known flavonoid, inhibits oxidation and cytotoxicity of low-density lipoprotein and can reduce risk for heart diseases or cancer [9] [10].

Recent *in-vitro* studies have established that the health benefits such as hypocholesterolemic (cholesterol lowering) effect, anti-carcinogenic, anti-oxidative, anti-tumor, anti-virus, anti-hepatic, anti-diabetic and hepato-protective properties of food legumes are due to presence of saponins [10]. Several alkaloids isolated from natural herbs exhibits anti-proliferation, anti-bacteria, anti-viral, insecticidal and anti-metastatic effect on various types of cancers both *in-vitro* and *in-vivo* [11].

In this research, the powdered leaves of *Ficus sycomorus* were extracted with methanol. The crude methanol extract was subjected to column chromatography and four pooled fractions were obtained, coded as F_A, F_B, F_C and F_D. The pooling was done base on the similarity of R_f values of the eluents. The PTLC of fraction F_C revealed the presence of three compounds (corresponding to 3 spots) having Rf values of 0.55, 0.63 and 0.72 respectively.

The solvent phase used in the separation was chloroform, ethylacetate and methanol in the ratio of (3:2:1). These sub-fractions were then collected as pure compounds. The pooled column fraction coded F_C after subjecting to further purification using preparative thin layer chromatography (PTLC) produces three sub-fractions were coded C_1, C_2 and C_3.with each one of them with single spots. The two sub-fractions two (C_1 and C_3) were analyzed using spectroscopic techniques such as FTIR, UV and GCMS.

In using spectroscopy for structure determination, peaks in the region of 1600-4000 cm^{-1} are usually emphasized because this is the region in which the vibrational characteristics of particular functional groups are found [12, 13].

The relative intensities of the bands are variable, but their sharpness, broad band and the presence of needle-like peak is characteristic of a particular functional group present. According to related research journals [12, 13]. Research have reported that bands of IR spectra ranging from 3345-3325, 2975-2950, 2825-2780, 2600-2550, 2150-1990 and 1790-1700cm^{-1} are due to N-H aliphatic secondary amine stretching, C-H methyl asymmetric stretching, C-H methyl symmetric stretching, N-CH_3 (C-

H stretching), S-H stretching (Thiols), –NCS vibration (isothiocyanate) and cyclic ketones respectively.

Also, absorptions at 1680-1620, 1480-1420, 1150-1000 and 1090-1020cm^{-1} are due to C=N stretching, C=C aromatic stretching, C-F alkyl fluorides and C-N stretching respectively. The IR spectra of sub-fraction C_1 showed absorption band at 3333.10cm^{-1} indicating N-H aliphatic secondary amine stretching, it also has another band at 2962.76cm^{-1} which indicates a C-H methyl asymmetric stretching as reported by Coates (2000).

In a similar vein, then band displayed at 2823.88cm^{-1} is indicating C-H stretching (N-CH3). The band shown at 2522.98cm^{-1} is indicating a thiol S-H stretching and the band at 2044.61cm^{-1} indicates isothiocyanate –NCS vibrations.

The absorption band at 1790.00cm^{-1} is indicating the carbonyl functionality especially for cyclic ketones, this is in agreement with the findings of [14].

Furthermore, the absorption band at 1427.37cm^{-1} indicates aromatic C=C stretching, while that which appeared at 1111.03cm^{-1} indicates C-F alkyl fluoride vibrations.

Also, the absorption band at 1026.66cm^{-1} is indicating C-N stretching. The bands at 3850.04cm^{-1} and 3981.21cm^{-1} are indicating monomeric alcohol or intramolecular hydrogen bond effect.

The UV spectra of C_1 showed two distinct absorption bands at 650.60nm and 503.00nm. The infrared (IR) spectra of sub-fraction C_3 showed absorption peak at 3325.39 cm^{-1} indicating N-H aliphatic secondary amine stretching, another band at 2962.76 cm^{-1} indicating C-H methyl asymmetric stretching and the one at 2893.32 cm^{-1} indicates C-H methyl symmetric stretching.

Furthermore, the absorption band at 2823.88 cm^{-1} indicates C-H stretching of (N-CH_3) and the band at 2515.26 cm^{-1} indicates Thiols S-H vibrations. In a similar manner, the absorption band at 2044.61 cm^{-1} is indicating isothiocyanate vibrations and which is at 1797.72 cm^{-1} indicates carbonyl functionality.

The absorption band at 1674.27 cm^{-1} indicates C=N stretching, while that which appeared at 1427.37 cm^{-1} indicates aromatic C=C stretching. The vibrational band at 1111.03 cm^{-1} is indicating a C-F alkyl fluoride vibration, while that appears at 1026.16 cm^{-1} indicates C-N stretching vibrations.

The absorption bands at 3850.04 and 3742.03 cm^{-1} are indicating monomeric alcohol or intramolecular hydrogen bond effect. The UV spectra of C_3 showed three distinct absorption bands at 657.20 nm, 602.80 nm and 503.20 nm.

The GC-MS spectra data of sub-fraction C_1 and C_3 compared with the NIST library of compounds revealed compounds that are similar to sub-fraction C_1 and C_3 base on the similarity of their spectra data (Figures 5 and 6). These compounds are; (i) 2-Acetyl-3-methylaminocyclopentenone (ii) 9-anthracenyltrimethylsilane (iii) 6,13-bis(2,5-dimethylphenyl)-dibenzo[C,H]diazecine (iv) 4'-dimethylamino-2'-(trimethylsilyl)acetanilide (v) 5-Methyl-4-hydroxybenzoylhydrazonefurfurole (vi) 4-(3,4-dimethoxyphenyl)-5-methyl-2-thiazolamine (vii) Cyclobarbital (viii) Chloroacetic acid (ix) 5-methyl-4-hydroxyl-2-benzylidene-coumaran-3-one (x) O-(2-methylpropyl)-Hydroxylamine.

3. Experimental

3.1 Sample Collection, Identification, Preparation and Extraction

Fresh leaves of *Ficus sycomorus* were collected from Alau-Dam, Jere Local Government Area of Borno State, Nigeria,West Africa**.** The herbarium specimen was identified by Professor S.S. Sanusi, a plant taxonomist from the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria.

Specimen voucher number 8012B was allocated to the plant material and deposited at the research laboratory of the Chemistry Department of University of Maiduguri, Nigeria.

The plant leaves were air-dried under shade and care was taken to render it free of foreign materials through manual picking. The dried leaves were pulverized using wooden mortar and pestle.

Seven hundred grams (700 g) of the pulverized sample material were extracted with absolute methanol using soxhlet extractor.

The crude extract was concentrated over a water-bath and then exposed to air at 25^0C to dryness. The dried extract was weighed, labeled and stored in a desiccator subject to further analysis.

3.2 Phytochemical Evaluation and Purification of the methanol leaf extract of Ficus sycomorus

The methanol extract was subjected to qualitative phytochemical screening using standard procedures described. [4, 5, 6].

3.3. Column Chromatography and Thin Layer Chromatography

The crude extract of *Ficus sycomorus* was subjected to column chromatography for separation of fractions.

The column was packed with silica gel (stationary phase) and the extract was applied on top of the column using a little of cotton wool to separate the silica gel and the extract. Eluting solvents (mobile phase) were applied from top through the column, starting with less polar (100% ethylacetate) to most polar solvent (100% butanol) mixture ratios.

The mobile phase dripped down by gravity and different components in the mixture having different interactions with the stationary and mobile phases at varying degrees were clearly separated.

The separated components were collected sequentially and carefully labeled for further analysis.

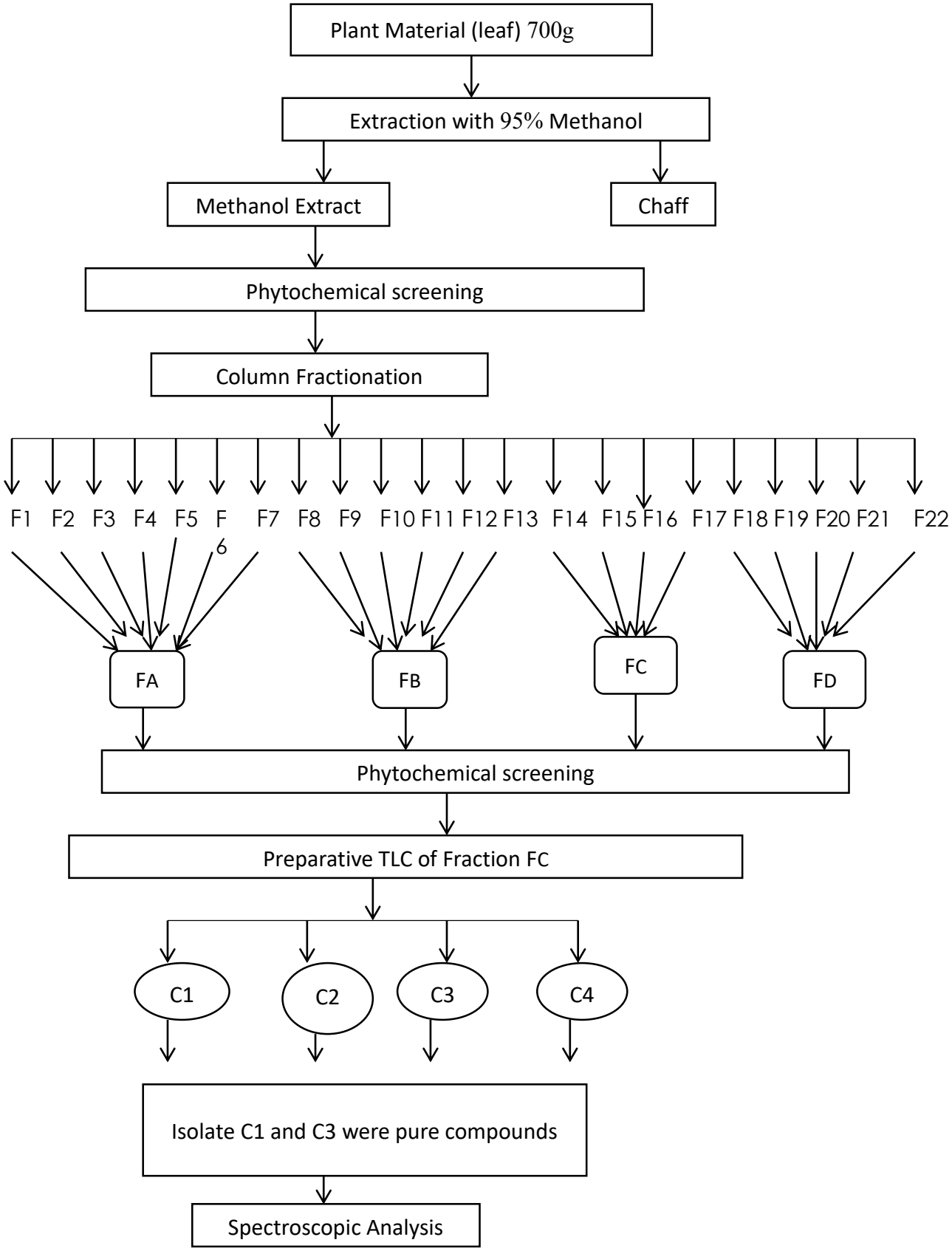


Figure 1. Extraction , Purification scheme of the leaf extract of *Ficus sycomorus*

Purification of the pure compounds isolated through bioassay guided procedure was carried out using thin layer chromatography. Chromatographic plate was obtained and prepared by spotting it with extract solution at about 2 cm above the lower edge of the plate using a micro-pipette. The plates were then developed by placing the lower edge of the plate in a chromatographic tank containing a mixture of chloroform, ethylacetate and methanol (in the ratio of 3:2:1) as solvent system and letting the spotted area stay just above the solvent surface. The solvent was allowed to move up the plate through capillary action, and the sample components moved up the plate at different rate, depending on their solubility and degree of retention by the stationary phase. Isolation was identified after drying the plate and exposing it to iodine vapor.

3.4 Characterization and Structural Elucidation

The pure isolates were subjected to spectroscopic techniques for identification of the possible structure of the compounds. The type of spectroscopy used are the UV-Visible Spectroscopy machine (UV-2500PC series), Fourier Transform Infra-red spectroscopy machine(Shimadzu FTIR-8400S Series) and Gas Chromatography-Mass Spectroscopy machine (GC 7890B, MSD 5977A, Agilent Technology).

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