Journal of Biological Sciences and Bioconservation
Volume 11, Number 3, 2019
ISSN: 2277-0143

Purification and Analysis of bioactive compounds from *Citrus aurantifiolia* Linn stem bark by Gas-Chromatography-Mass spectrometry

Mustapha A. Tijjani[1]* Fanna I. Abdulrahman[1],Sherrif Umar1, Fatima M. Dungus[2]

[1]Department of Chemistry, University of Maiduguri, Borno State, Nigeria

[2]Departement of Clinical Pharmacy, University of Maiduguri, Borno State, Nigeria

Email: mustaphatijjani22@yahoo.com

ABSTRACT

The objectives of this research are to extract the air-dried stem bark (500g) of *citrus aurantifolia* with methanol using cold infusion (maceration) technique, partition the extract with solvents of graded polarities (n-hexane, ethyl acetate, n-butanol suspended in water) and phytochemically screened and fractionate and purify the n-butanol partitioned portion using a combination of column and thin layer chromatography and finally subject the possible pure fraction(s) to gas chromatography and mass spectrometry (GC-MS) analysis. The methanol crude extract yield 14.90% $^{w}/_{w}$ dark green in colour, gummy in texture. the n-hexane partitioned portion yield 0.44% $^{w}/_{w}$ light green in colour oily paste texture, ethylacetate partitioned portion yield 5.32% $^{w}/_{w}$ dark brown in colour, gummy in texture, while n-butanol yield 21.04%$^{w}/_{w}$, brown in colour, gummy in texture and finally aqueous partitioned portion yield 52.08% $^{w}/_{w}$ brown in colour power in texture respectively. The presence of metabolites such as carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins were recorded in the methanol crude extract while, anthraquinones, alkaloids and saponins

were not detected in methanol crude stem bark extract. Whereas cardiac-glycosides, terpeniods and flavonoids were present in n-hexane portion and n-butanol portion but carbohydrates, anthraquinones, tannins, saponins, phlobatannins and alkaloids were not found in both of the portions.. The purification of compounds was done by using a combination of column and thin layer chromatography techniques. The n-butanol partitioned portion was subjected column chromatography after, rerunning, recombination and pooling four compounds, coded C_{a1}, C_{a2}, C_{a3} and C_{a4} were obtained. The melting points were sharp and uncorrected. In gas chromatography and mass spectrometry (GC-MS) analysis of compound of sample C_{a1} fourteen compounds were identified by comparison with the library of NIST. Among the compounds, were found to be 3, 5, 9-Trioza-5-Phosphaheptacos-18-en-1-aminium, Pentacosanoic acid, Oleic acid, 7, 8-Epoxylanostan-11-ol. Analysis of Compound of sample C_{a2} shows that only one compound was identified by comparison with the library of NIST. The compound was found to be 3H-Cycloocta[c]pyran-3-one. Analysis of Compound of sample C_{a3} shows eight compounds were identified by comparison with the library of NIST. Among the compounds were found to be 1, 3-Dioxane, Pregn-5-en-20-one, 9-Octadecenoic acid, Heptadecanoic acid, Epoxylanostan-11-ol. Analysis of Compound of sample C_{a4} shows that eight compounds were identified by comparison with the library of NIST. Among the compounds were found to be Glycidol stearate, Andrast-4-ene-3-one, Octadecanoic acid, Dihydromorphine.. The dihydromorphine which believed to be a reduced formed of morphine with a molecular formula of $C_{17}H_{21}NO_3$. It has been reported that the 7, 8-double bond of morphine also is not required for analgesic activity as

indicated by the relative analgesic potency of dihydromorphine. Also, oxidation of the 6-OH of dihydromorphine to yield hydromorphone further increases activity.

Keywords: Citrus aurantifolia, purity, column chromatography, Mass spectrometry

Introduction

Natural substances of botanical origin have been used throughout the world for human and animal health care [1-2 especially in Africa. The lack of apparent resistance development by pathogens to the phytochemicals present in herbal preparations as well as their good absorbance and to sugar (glycon) moiety which is absorbed and distributed in the system without problem thus carrying the active phytochemicals (aglycone) to the infected distribution to the area of infection perhaps due to the fact that apart from alkaloids, they are mostly compounds associated areas, make these compounds even more attractive [3-4].Knowledge of the chemical constituent of plants is desirable because such information will be of value for the synthesis of complex chemical substances[5] . Medicinal plants contain physiologically active constituents, which over the years have been exploited in traditional medical practice for the treatment of various ailments [6]. Researchers are currently being conducted on medicinal plants/extracts to isolate and purify the active fractions for preparation of drugs from natural sources [7] due to their less toxic effects and affordability [8]. The active principles isolated from plants appeared to be one of the important alternatives, when compared to many sub-standard orthodox synthetic

medicines, because of their less or no side effects and better bio-availability [9-10].

Citrus aurantifolia was used traditionally as laxative, appetizer, stomachic, digestive, anthelmintic, dyspepsia, flatulence and helmenthiasis [11]. *Citrus aurantifolia* was also used for cold fevers, sore throats, sinusitis and bronchitis, as well as helping asthma. Its oil is mainly used as antidepressant because it promoted refreshment to the tide mind. It can be helpful for rheumatism arthritis, obesity and has an astringent and toning action to clear oily skin and acne, in the treatment of herpes, cuts and insect bites.[12-13]. The objectives of this research are to extract the air-dried stem bark (500g) of *Citrus aurantifolia* with methanol using cold infusion (maceration) technique, partition the extract with solvents of graded polarities (n-hexane, ethyl acetate, n-butanol suspended in water) and phytochemically screened and fractionate and purify the n-butanol partitioned portion using a combination of column and thin layer chromatography and finally subject the possible pure fraction(s) to gas chromatography and mass spectrometry (GC-MS) analysis.

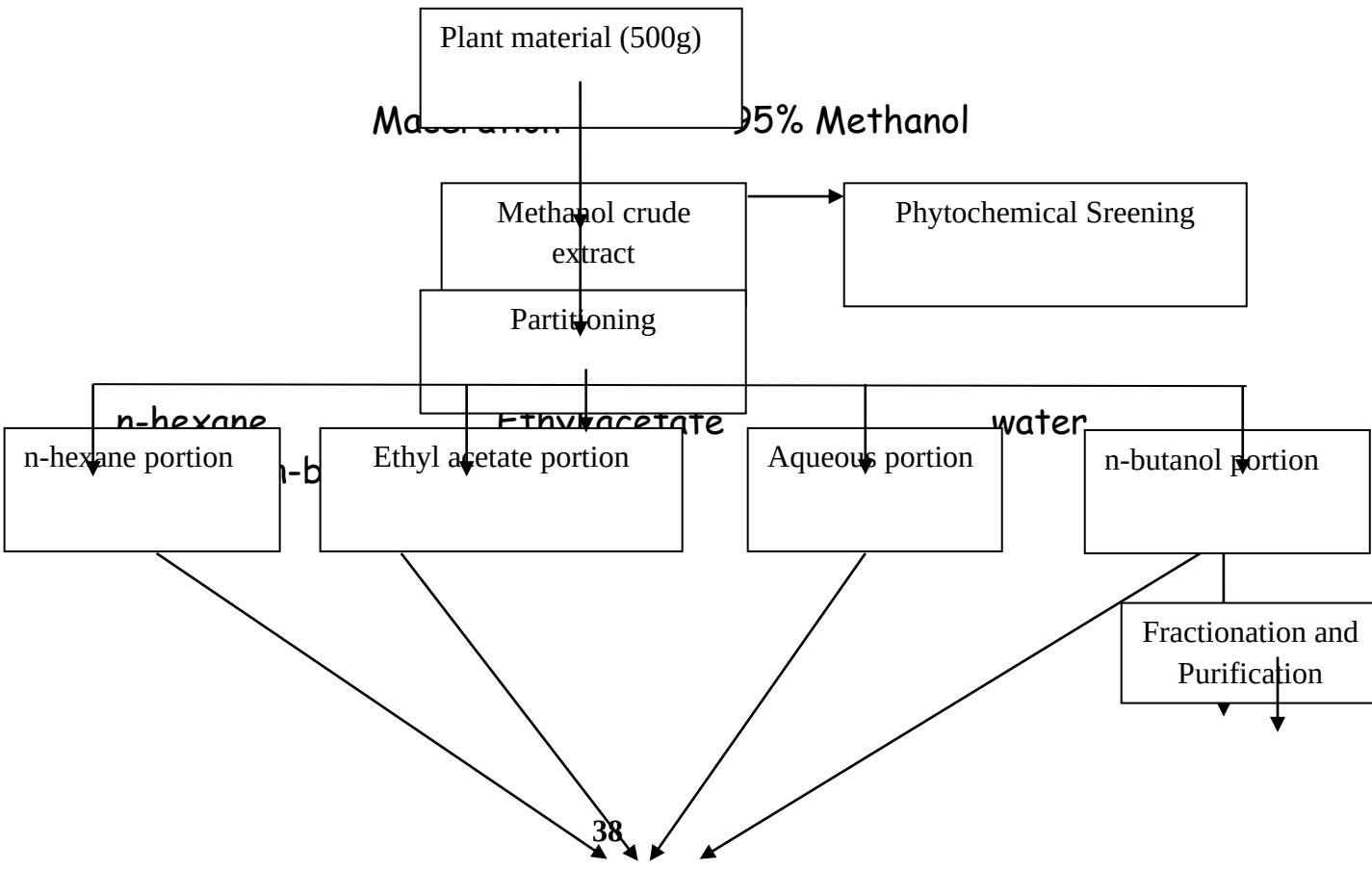
EXPERIMENTATION

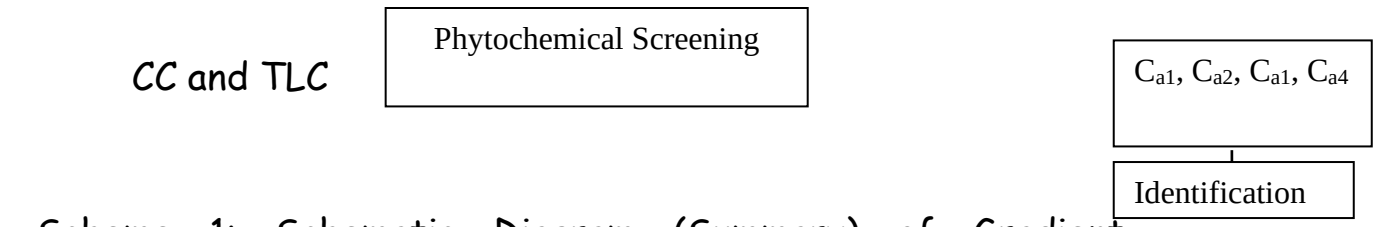
Sample Collection and Identification

The stem bark of *Citrus aurantifolia* was collected from Damboa road near Mashidimami water ventures area at Maiduguri, Borno State, Nigeria and identified by a plant Taxonomist at the Department of Biological Sciences, University of Maiduguri. It was air-dried under laboratory condition and pulverized into fine powder with voucher number 323b and deposited in the research laboratory, Chemistry Department, University of Maiduguri.

Extraction and Preliminary Phytochemical Screening

The powdered air dried material (500g) was extracted in methanol using cold infusion (maceration) technique; it was filtered and the filtrate was concentrated in vacuo using rotary evaporator at reduced pressure and the extract concentrate was estimated.The extract was subjected to Phytochemicals evaluation using standard procedures described by many authors [14-15].

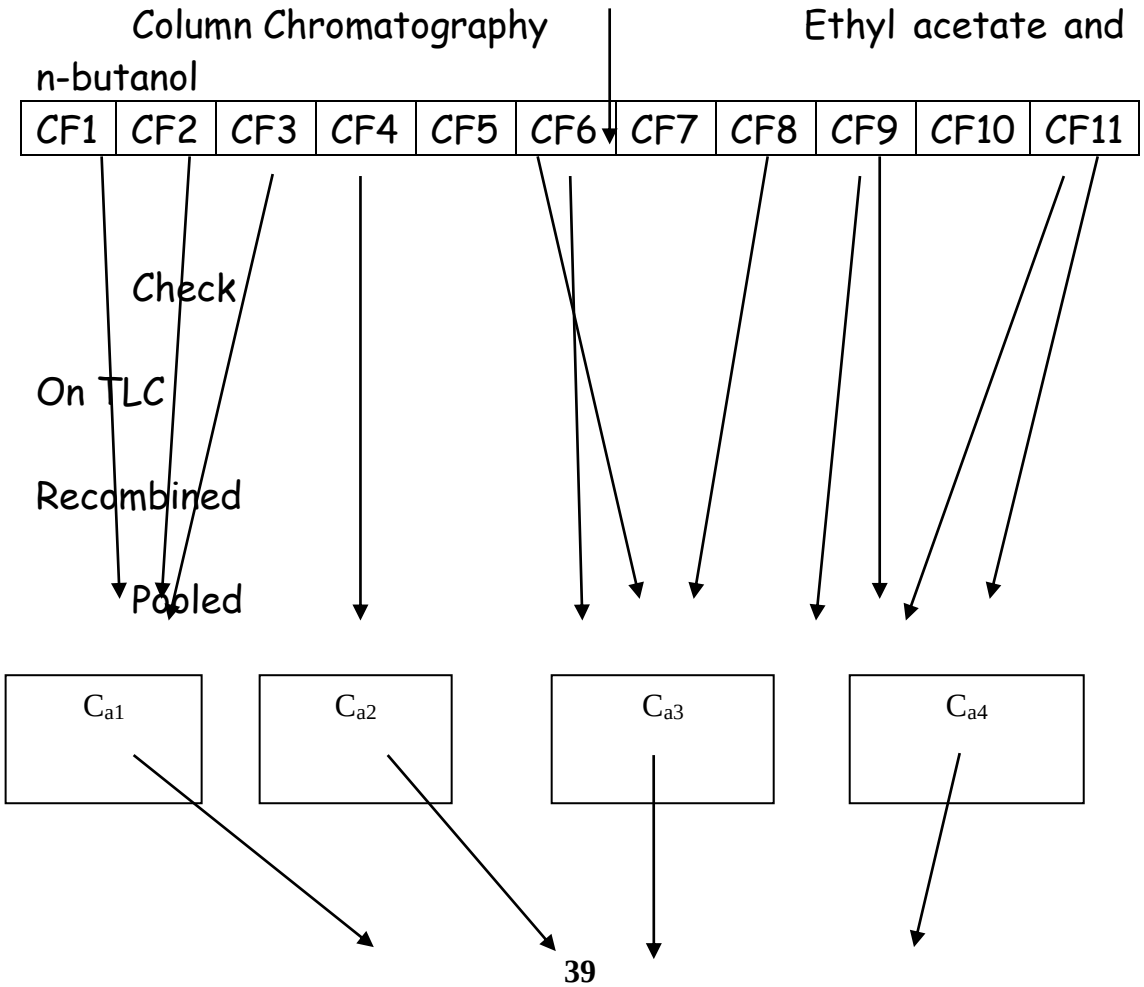




Scheme 1: Schematic Diagram (Summary) of Gradient Extraction of Stem bark of *Citrus aurantifolia* Maceration (cold infusion) techniques.

Fractionation and Purification

Methanol extract of *Citrus aurantifolia* was partitioned using solvents of graded polarities. Dry partitioning was used, where the crude extract was partitioned sequentially with n-hexane, ethyl acetate and the residue later suspended in distilled water for further partitioning with n-butanol using separating funnel.



GC-MS

Identification of Compounds

Scheme [illegible] and Purification of *Citrus aurantifolia*

Structural identification using Gas Chromatography- Mass Spectrometry (GC-MS)

The gas chromatography system, fitted with a 30m × 250µm x 0.25µm Rtx-5MS capillary column, maximum temperature was 325°C, coupled to Agilent 5977A MSD was used for futher purification and identification of compounds. The sample fractions were diluted with appropriate methanol (1/100, V/V) and filtered and 1µL were injected into injector. All data were obtained by collecting the full-scan mass spectra within the scan range. The percentage composition of the pure isolate constituents was expressed as a percentage by peak area. The identification and characterization of chemical compounds in the sample was based on GC retention time. The mass spectra were computer matching with those of standards available in mass spectrum libraries that is National Institute of Standards and Technology.

RESULTS AND DISCUSSION

Table 1: The weight, percentage yield, colour and texture of the methanol crude and partitioned portions of stem bark extract of *Citrus aurantifolia* Linn.

Parameters	MCE	NHP	EAP	NBP	AQP
Colour	Dark green	Light green	Dark brown	Brown	Brown
Texture	Gummy	Oily	Gummy	Gummy	powder

		paste			
Weight(g)	74.50	0.11	1.33	5.26	13.02
Percentage yield(%)$^{w}/_{w}$	14.90	0.44	5.32	21.04	52.08

Key: MCE= Methanol crude extract, NHP= n-Hexane extract, EAP= Ethyl acetate extract,
NBP= n-Butanol extract, AQP= Aqueous portion.

The table 1 showed the results of extraction of stem bark of *citrus aurantifolia*. The methanol crude extract yield 14.90% $^{w}/_{w}$ dark green in colour, gummy in texture. The n-hexane partitioned portion yield 0.44% $^{w}/_{w}$ light green in colour oily paste texture, ethylacetate partitioned portion yield 5.32% $^{w}/_{w}$ dark brown in colour, gummy in texture, while n-butanol yield 21.04%$^{w}/_{w}$, brown in colour, gummy in texture and finally aqueous partitioned portion yield 52.08% $^{w}/_{w}$ brown in colour power in texture respectively.

Table 2: Preliminary Phytochemical Screening of crude methanol and partitioned portions of stem bark extract of *Citrus aurantifolia* Linn.

S/No	Test	MCE	NHP	EAP	NBP	AQP
1	Test for Carbohydrates					
I	General test-molish test					
Ii	Test for monosaccharide-Barfoed test	+		-	-	+
Iii	Test for Free reducing sugars-Fhlings	+		-	-	+
Iv	Test for Combined reducing sugars	+	-	-	-	+
V	Test for Pentoses	+	-	-	-	-
Vi	Test for Ketoses	+	-	+	-	-
2	Test for soluble Starch	+	-	-	-	+
3i	Test for Anthraquinones	-	-	-	-	-
Ii	Test for Combined Anthraquinones	-	-	-	-	-
4	Test for Cardiac-glycosides					
I	Salkowski's Test	+	+	-	+	-
Ii	Liebermann-Burchadr's test	+	+	+	+	+

5	Test for Terpenoids	+	+	+	+	-
6	Test for Flavonoids					
I	Shinoda's test	+	+	+	+	-
ii.	Ferric Chloride test	+		+	+	+
iii.	Lead acetate test	-		-	-	-
iv.	Sodium hydroxide test	+		-	-	-
7	Test for Saponins					
i.	Fronthing test	-		-	-	-
8	Test for Phlobatannins	+		-	-	-
9	Test for tannins	-		-	-	-
i.	Ferric Chloride Test	+				
ii.	Lead acetate test	-		-	-	-
10	Test for Alkaloids					
I	Drangendroff's reagent	-	-	-	-	-
ii.	Mayer's reagent	-	-	-	-	-

Key: MCE = Methanol crude extract, NHP = n-Hexane portion, EAP = Ethyl acetate portion,

NBP = n-Butanol portion, AQP = Aqueous portion, (+) = Present

(-) = Absent.

Table 2 showed results of preliminary phytochemical screening of the methanol crude stem bark extract together with its partition portions of *Citrus aurantifolia*. The presence of metabolites such as carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins were recorded in the methanol crude extract while, anthraquinones, alkaloids and saponins were not detected in methanol crude stem bark extract. Whereas cardiac-glycosides, terpeniods and flavonoids were present in n-hexane portion and n-butanol portion but carbohydrates, anthraquinones, tannins, saponins,

phlobatannins and alkaloids were not found in both of the portions. The presence of carbohydrates, terpeniods, cardiac-glycosides and flavonoids were found in ethyl acetate portion however, anthraquinones, saponins, tannins, phlobatannins and alkaloids were not found. The aqueous portion revealed the presence of cardiac-glycosides and carbohydrates but flavonoids, anthraquinones, saponins, tannins, phlobatannins and alkaloids were absent.

Table 3: Determination of Melting point and Retention factor of compounds obtained in *Citrus aurantifolia*

S/No	Compounds	R_f Value	Melting Point(°C)
1	C_{a1}	0.22	286.00-287.33
2	C_{a2}	0.67	290.00-291.00
3	C_{a3}	0.27	277.00-279.00
4	C_{a4}	0.25	262.00-264.00

Table 3 above showed the results of melting point and retention factor of compounds C_{a1}, C_{a2}, C_{a3} and C_{a4} obtained from stem bark extract of *Citrus aurantifolia*. Compound C_{a1} had melting point of (286.00-287.33 °C) with R_f value of (0.22). The melting point of compound C_{a2} was found to be (290.00-291.00 °C) with R_f value of (0.67). The melting point of compound C_{a3} was also found to be (277.00-279.00 °C) with R_f value of (0.27). The compound C_{a4} was found to had melting point of (262.00-264.00 °C) with value of R_f (0.25) respectively.

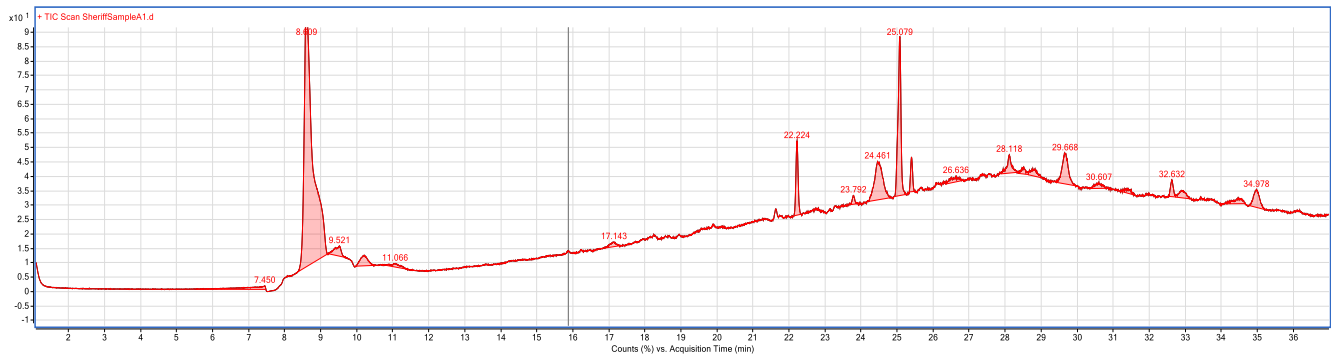


Fig.1: Chromatogram of Compound C_{a1}

Table 4: Gas chromatography and Mass spectrometry analysis of Compound C_{a1}

Peak no.	RT	Area	Height	Width	Name
1	7.45	2028596.68	50700.71	1.918	
2	8.609	61651725.23	3711485.52	0.446	
3	9.521	1831020.08	143966.32	0.446	
4	10.208	2202431.32	147431.25	0.479	
5	11.066	859725.32	48528.32	0.562	
6	17.14	805464.9	65260.2	0.39	9α-Floro-11β,17β-

	3	8	9	6	diol-17a-methyl-5a-androstan-3-one
7	22.224	4531454.89	1058072.72	0.19	Pentadecanoic acid
8	23.792	623651.83	125573.09	0.22	
9	24.461	8769302.49	548885.97	0.599	1,2,3-propanetryl ester
10	25.079	12974177.84	2268474.5	0.312	3,5,9-Trioza-5-phosphaheptacos-18-en-1-aminium,
11	25.4	1926283.96	489495.11	0.145	Pentacosanoic acid
12	26.636	12`18939.36	83858.42	0.452	
13	28.118	2332068.17	261295.48	0.446	3,19:14,15-diepoxypregnan-20-one
14	28.507	861278.79	96231.17	0.292	Dodecanoic acid
15	28.77	1256079.6	99415.8	0.355	7,8-Epoxylanostan-11-ol
16	29.668	5327476.59	437782.15	0.544	Oleic acid
17	30.607	1042524.58	77834.9	0.412	
18	31.402	740855.46	62331.82	0.309	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol
19	32.6	1312368.6	236497.	0.19	Preg-5-en-20-one

	32	5	69	3	
20	32.59	142196.51	104528.44	0.383	
21	34.486	1357943.29	80137.6	0.515	7,8-Epoxylanostan-11-ol
22	34.978	2960591.45	251046.88	0.416	5H-Cyclopropa(3,4)benz(1,2-e)azulen-5-one

The table 4 above showed the results gas chromatography mass spectrometry analysis of compound (C_{a1}). Fourteen compounds were identified by comparison with the library of NIST. Peak number five (5) had a retention time of 11.066 min. also peak number seven (7) had a retention time of 23.792 min. The remaining peaks had the following retention times respectively as shown in table 4. But other compounds could not be identified from the library.

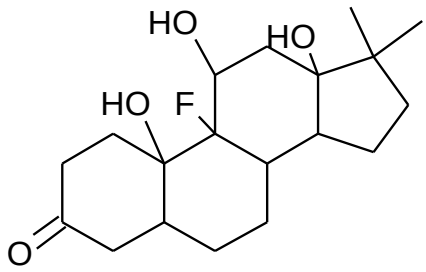


Fig.2.: 9a-Flouro-11β-diol-17a-methyl-5a-andrastan-3-one

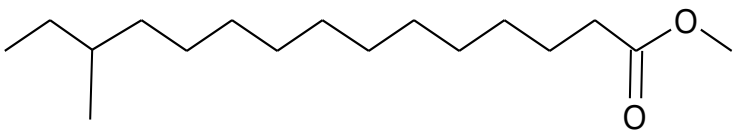


Fig.3: Pentadecanoic acid

Chromatogram of Compound C_{a2}

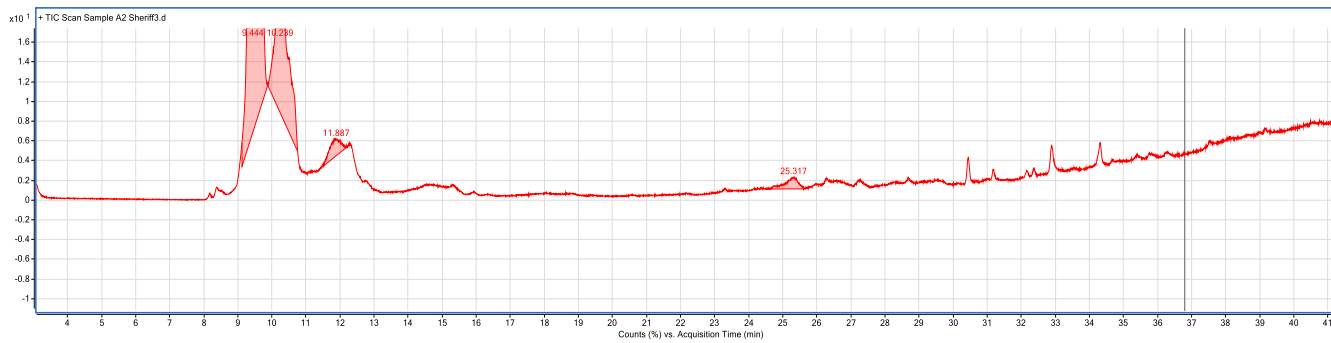


Fig.3: Chromatogram of Compound C_{a2}

Table 4: Gas chromatography and Mass spectrometry of Compound C_{a2}

Peak no.	RT	Area	Height	Width	Name
1	9.444	38317171.04	1803377.83	0.767	
2	10.239	7924311.53	363764.02	0.868	
3	11.887	789172.02	32922.65	0.687	
4	25.317	619268.07	23135.7	0.973	3H-Cycloocta[c]pyran-3-one

The above showed the results Compound C_{a2}. Only one compound with peak number (4) had a retention time of 25.317 min. It was identified by comparison with the library of National Institute of Standards and Technology (NIST). The remaining peaks had the following retention times respectively as shown in table 4. But other compounds could not be identified from the library.

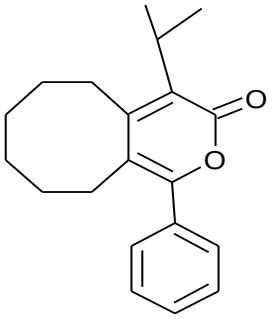


Fig.4: 3H-Cycloocta[c] Pyran-3-one

Chromatogram of Compound C_{a3}

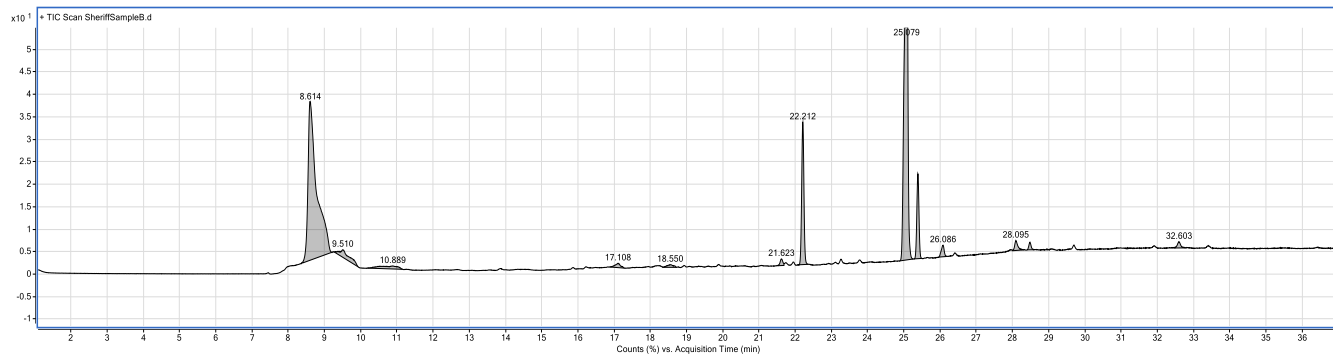


Fig.5: Chromatogram of Compound C_{a3}

Table 5: Gas chromatography and Mass spectrometry of Compound C_{a3}

Peak no.	RT	Area	Height	Width	Name
1	8.614	60641839.89	3601898.79	0.795	
2	9.51	3451029.82	1743.74.27	0.661	
3	10.889	2380074.82	68627.73	0.952	
4	17.108	1011121.64	97691.3	0.389	
5	18.55	74719.38	60689.24	0.389	1,3-Dioxane
6	81.683	788881.44	151796.9	0.189	Pregn-5-en-20-one
7	22.212	14013385.57	3249660.87	0.234	Hexadecanoic acid
8	25.079	59951252.6	9941428.8	0.365	9-Octadecenoic acid
9	25.388	7999572.23	1939082.76	0.2	Heptadecanoic acid
10	26.086	1482558.33	263582.69	0.224	7,8-Epoxylanostan-11-ol
11	28.09	1340421.12	226252.47	0.303	Docosanoic

	4				acid
12	28.484	740541.22	174734.2	0.165	Docosanoic acid
13	32.603	912328.48	140401.08	0.325	

The above showed the results Compound (C_{a3}). Eight compounds were identified by comparison with the library of National Institute of Standards and Technology (NIST). Peak number five (5) had a retention time of 13.824 min. also peak number seventeen (17) had a retention time of 27.139 min. The remaining peaks had the following retention times respectively as shown in table 4. but, other compounds could not be identified from the library.

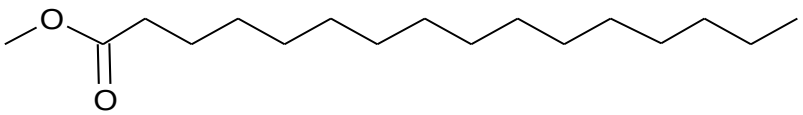


Fig. 6: Hexadecanoic acid

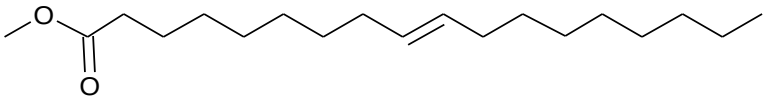


Fig.7: 9-Octadecenoic acid

Chromatogram of Compound C_{a4}

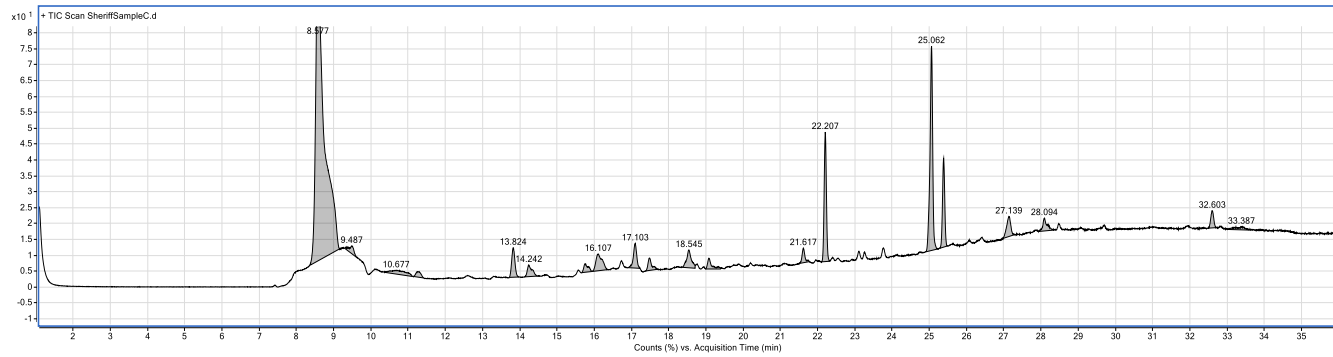


Fig.8: Chromatogram of Compound C_{a4}

Table 6: Gas chromatography and Mass spectrometry of Compound C_{a4}

Peak no.	RT	Area	Height	Width	Name
1	8.577	56878974.09	345628 3.8	0.772	
2	9.487	832520.94	92937.58	0.309	
3	10.677	1380822.81	44585.36	0.75	
4	11.26	610675.28	65630.14	0.263	4H-cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-B]oxiren-4-one
5	13.824	2092952.91	350010	0.246	1-(p-methoxyphenyl)-3-phenyl-3-(2-oxocyclohexyl)-1-propanone
6	14.242	1227627.87	138423 .73	0.346	Glycidol stearate
7	15.752	822539.41	101272.22	0.259	
8	16.107	2353589.79	199612.84	0.399	
9	17.103	1830642.51	292699 .62	0.292	Androst-4-en-3-one
10	17.48	1032296.35	145430 .43	0.303	1H2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecane-

					11-1-one
11	18.544	1883667.35	210955.76	0.341	
12	19.077	995760.40	127479.33	0.395	
13	21.617	902040.09	173724.80	0.258	
14	22.207	6449043.45	153559 3.58	0.210	Methyl-18-methylicosanoate
15	25.062	12132023.59	242210 5.06	0.292	10-Octadecanoic acid
16	25.388	4348461.06	105020 9.33	0.199	
17	27.139	1813519.54	237870.30	0.290	Dihydromorphine
18	28.094	1217287.93	152597.13	0.383	
19	32.603	1110441.91	203924.89	0.188	
20	33.387	683394.33	37546.01	0.578	

The above showed the results of compounds of Sample (C_{a4}) eight compounds were identified by comparison with the library of NIST. Peak number five (5) had a retention time of 13.824 min. also peak number seventeen (17) had a retention time of 27.139 min. The remaining peaks had the following retention times respectively as shown in table 6. But, other compounds could not be identified from the library. But, compounds could not be identified from the library of NIST.

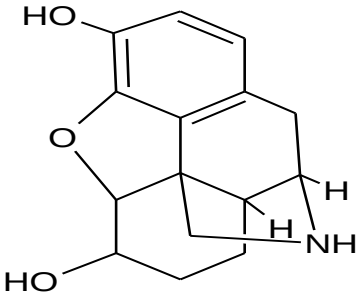


Fig.8: Dihydromorphine.

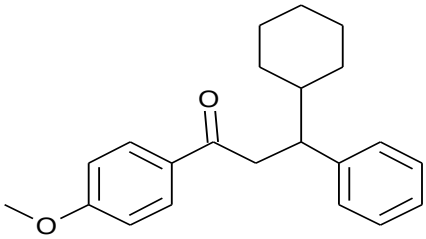


Fig.9: 1-(-P-methoxyphenyl)-3-phenyl-3-(2-oxocyclohexyl)-1-propanone

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavours, aroma, colours, biopesticides and food additives. *Citrus* contained phytochemicals that were beneficial for healthIn extraction and partition of stem bark of *citrus aurantifolia*, methanol crude extract yield 14.90% $^{w}/_{w}$ dark green in colour, gummy in texture

The results of preliminary phytochemical screening of the methanol crude stem bark extract of *Citrus aurantifolia* together with its partitioned portions indicates that the plant

contain many secondary metabolites. The presence of secondary metabolites such as carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins were observed in the extracts . These phytochemicals have been reported to influence physiological activities of the body . The absence of alkaloids and other constituents might be due to topographical variations. Phytochemicals found are implicated to have much medicinal importance [15].The phytochemical studies of the methanol crude stem bark extract of *Citrus aurantifolia* revealed presence of some useful chemical compounds such as flavonoids, cardiac-glycosides, tannins, saponins, and terpenoids. These compounds have been known to exert pharmacological and antagonistic effects and still some are capable of protecting the active ingredient in herbs from decomposing either chemically or physiologically [16]. Flavonoids exhibit several biological effects such as antihepatotoxic, anti-inflammatory and antiulcer activity[18-19]. Tannins are polyphenols that are obtained from various parts of different plants [20]. In addition to its use in leather processing industries, tannins have shown potential antiviral and antibacterial. [21-24]

The successful separation of biomolecules by the chromatographic technique depends upon suitable solvent system which needs an ideal range of partition coefficient (K) for each target compound . The purification of compounds was done by using a combination of column and thin layer chromatography techniques. The n-butanol partitioned portion was subjected column chromatography after, rerunning, recombination and pooling four compounds.

. In gas chromatography and mass spectrometry (GC-MS) analysis of compound of sample C_{a1} fourteen compounds were identified by comparison with the library of NIST. Among the

compounds, were found to be 3, 5, 9-Trioza-5-Phosphaheptacos-18-en-1-aminium, Pentacosanoic acid, Oleic acid, 7, 8-Epoxylanostan-11-ol. Other compound could not be identified from the library. Analysis of Compound of sample C_{a2} shows that only one compound was identified by comparison with the library of NIST. Among the compound was found to be 3H-Cycloocta[c]pyran-3-one. Other compounds could not be identified from the library. Analysis of Compound of sample C_{a3} eight compounds were identified by comparison with the library of NIST. Among the compounds were found to be 1, 3-Dioxane, Pregn-5-en-20-one, 9-Octadecenoic acid, Heptadecanoic acid, Epoxylanostan-11-ol. Other compounds could not be identified from the library. Analysis of Compound of sample C_{a4} shows that eight compounds were identified by comparison with the library of NIST. Among the compounds were found to be Glycidol stearate, Andrast-4-ene-3-one, Octadecanoic acid, Dihydromorphine. Other compounds could not be identified from the library. The dihydromorphine which believed to be a reduced formed of morphine with a molecular formula of $C_{17}H_{21}NO_3$. The 7, 8-double bond of morphine also is not required for analgesic activity as indicated by the relative analgesic potency of dihydromorphine. Also, oxidation of the 6-OH of dihydromorphine to yield hydromorphone further increases activity.

Acknowledgement

We wish to acknowledge Tertiary Education Trust Fund **(TETFUND)** for sponsoring this research and Chemistry Department, University of Maiduguri for usage of the Research laboratory.

REFERENCES

1. Sexana, J. M. (2003). Relevance of Herbs in Improving Health Index of Livestock Animals. *Nat. Veter. Med. Ass. Conf.: Ibadan, Nigeira.*

2. Enzo, A.P. (2006). Review article: Phytochemicals from Traditional Medicinal Plants Used in the Treatment of Diarrhea:Modes of Action and Effects on Intestinal Function, Res.20:717-724.

3. Odama, L. E., Shok, M. and Olurinola, P. E. (1997). The Preliminary Phytochemical Investigation of the Bark of *Cieba pentandra* (a traditional wound healing herb) and the Evaluation of Antibacterial Effect of the Isolated Component (Bub). *J. Pharm. Res. Dev.* 2:56-60.

4. Olutimeayin, *G. O.*, Oladosu, P. and Ibrahim, K. (2001). Preliminary Antimicrobial Screening of Aqueous and Methanolic Extracts of *Parkia clappertoniana* (Keay) Root Bark. *J. Phytomed.*

5. Savithramma, N. Linga, M. and Suhrulatha, D. (2011). Screening of Medicinal Plants for Secondary Metabolites. Middle-East *J. of Sci. Res..*;8(3): 579-584.

6. Okigbo, R. N. and Igwe, D. I. (2007). The Antimicrobial Effects of *Piper guineense* 'uziza' and *Phyllantus amarus* 'ebe-benizo' on *Candida albicans* and *Streptococcus faecalis. J. Immun. Microb.* 54 (4):353-366.
7. El-mahmood A. M. and Amey, J. M. (2007). *In vitro* Antibacterial Activity of Parkia Biglobosa (Jacq) Root

Bark Extract Against Some Microorganisms Associated with Urinary Infections. *Afr. J. Biotechnol.* 6(11): 1272-1275.

8. Mohammad, A., Shohreh, S., Amirhossein, A., Maryam, G. and Mohammad, S. (2010). Anti- diabetic Effects of Aqueous Fruits Extract of *Diospyros lotus L.* on Streptozotocin-Induced Diabetic Rats and the Possible Morphologic Changes in the Liver, Kidney and Heart. *J. Pharmacognosy. Phytother.* 2(2): 10-16.

9. Scazzochio, F., Cometa, M., Tomassin, L. and Palmery, M. (2001). Antibacterial Activity of Hydrastis Canadensis Extract and its Major Isolated Alkaloids. *Panta.Med.* 67: 561-564.

10. Joshi, B. Sah, G. B. Basnet, B. B. Bhatt, M. Sharma, R. Subedi, K., Pandey, J. and Malla, R. (2011). Phytochemical Extraction and Antimicrobial Properties of Different Medicinal Plants: Ocimum sanctum (Tutsi), *Eugenia caryophyllata* (Clove), Achyranthes bidentata (Datiwan) and *Azadirachta indica* (Neem). *J. Microbiol. Amtimicrob. 3(1),1-7.*

11. Liu, Y.Q., Heying, E. and Sherry, A. (2012). History, Global distribution and Nutritional Importance of Citrus fruits. *Comprehensive Reviews in Foo Science and Food Safety;* 11(6): 530-545.

12. Scora, R.W. (1975). On the History and Origin of *Citrus*. *Bull. Torrey Bot. Club* 102:369–375.

13. Srinivasan, D., Ramasamy, S. and Sengottuvelu, S. (2008) Protective Effect of Polyherbal Formulation on Experimentally induced ulcer in rats. *Pharmacologyonline;*1: 331-350.

14. Brain, K. R. and Tuner, T. D. (1975). The Practical Evaluation of Pharmaceutical Weight Science Technical Bristol, Britain.140-144; 152-154.

15. Sofowora, A. (1993). Screening Plants for Bioactive Agents". In: *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd., Sunshine House: Ibadan. Nigeria. 134-156.

16. Tijjani, M. A., Abdulrahman, F. I., Khan, I. Z. and Sandabe, U. K. (2012). The Effects of Ethanol Extact of Vitex Doniana Stem Bark on Peripheral and Central Nervous System of Laboratory Animals. *Bulletin Pure and Applie Sciences*. 29C (2): 2010: 153-160

17. Bolon, B, and St. Omar, V. E. V. (1989). Behavioral and development effects in sucking mice following material exposure to the mycotoxin Secaconic acid. *J. Pharmaco., and Biochem. of Behav*, 34:229-231.

18. Bors, W., Heller, W., Michel, C. and Saran, M. (1990). Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods in Enzymology*, 186:343-355.

19. Colerige Smith, P. O., Thomas, P., Scurr, J. H. and Dormandy, J. A. (1980). Causes of various ulceration, a new hypothesis, *Brit. Med. J.*, 296:1726-1727.

20. Gajendiran, N. and Mahadevan, A. (1990). Utilization of catechin by Rhizobium spp. *Plant Soil*, 108: 263-266.

21. Lin, L. U., Shu-wen, L., Shi-bo, J. and Shu-guang, W. (2004). Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharmacologica Sinica*, 25(2): 213-218.

22. Akiyama, H., Kazuyasu, F., Yamasaki, O., Oono, T. and Iwatsuki, K. (2001) Antibacterial action of several tannins against *Staphylococcus aureus*. *J. Antimicr. Chemother.*, 48(48): 487-491.

23. Funatogawa, K., Hayashi, S., Shimomura, H., Yoshida, T., Hatano, T., Ito, H. and Iría, Y. (2004) Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. *Microbiology and Immunology*, 48 (4), 251-261.

24. Yang, C. L. and Kun-Ying Y. (2000). Induction of apoptosis by hydrolyzable tannins from *Eugenia jambos* L. on human leukemia cells. *Cancer Letters*, **157**: 65-75.

Reference to this paper should be made as follows: Mustapha A. Tijjani et al., (2019), Purification and Analysis of bioactive compounds from *Citrus aurantifiolia* Linn stem bark by Gas- Chromatography-Mass spectrometry. *J. of Biological Science and Bioconservation,* Vol. 11, No. 3, Pp. 34-63