WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 1, 129-138. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL SCREENING, ISOLATION AND STRUCTURAL ELUCIDATION OF BIOACTIVE COMPOUND FROM METHANOL BARK EXTRACTS OF *FICUS POLITA*

Zannah Zakariya Goni* and Musa Maikudi Idriss

Bayero University Kano, Department of Pure and Industrial Chemistry, P.M.B. 3011 Kano, Kano State, Nigeria.

Article Received on
27 Oct. 2019,
Revised on 17 Nov. 2019,
Accepted on 08 Dec. 2019
DOI: 10.20959/wjpr20201-16442

***Corresponding Author**
Zannah Zakariya Goni
Bayero University Kano, Department of Pure and Industrial Chemistry, P.M.B. 3011 Kano, Kano State, Nigeria.

ABSTRACT

The aim of this study was to screen the phytochemical constituents, isolate and elucidate the structure of bioactive compound from methanol extracts from the bark of *Ficus polita*. The qualitative phytochemical analysis of the bark extract of *Ficus polita* was done following standard procedures and the tests revealed the presence of saponins, tannins, steroids, triterpenoids and lipids. The methanol extract of the bark led to the isolation of one compound, E5. Structure determination was accomplished by means of spectroscopic methods (FT-IR, ^{13}C, ^{1}H, COSY, and HSQC NMR). The spectral data of the isolated compound E5 is identical with spectral data of literature study, the spectral interpretation of the compound E5 shows that it is a cardiolipin derivative, is a kind of disphosphatidylglycerol lipid in which all four of the phosphatidyl acyl groups are specified as oleoyl, it has two phosphatidic acid moieties connected to glycerol backbone in the center and potentially carries two negative charges, and is elucidated to be Tetraoleoylcardiolipin ($C_{81}H_{150}O_{17}P_2$) which is reported for the first time from *Ficus polita*.

KEYWORDS: *Ficus polita*, Methanol extract, Bark, Cardiolipin, Tetraoleoylcardiolipin.

INTRODUCTION

Ficus polita belongs to the well-known family of edible plants, Moraceae, it is an edible plant growing up to 15 and sometimes to 40 meters tall in coastal and dry forest (East and Southern Africa), lowland rainforest and gallery forest (West and Central Africa). The edible fruits are

chewed for dyspepsia, while leaves, bark and roots are used in the treatment of infectious diseases, cough, abdominal pains and diarrhea.[1][2] The bark, leave and branch is used in treatment of all pregnancy disorders and breast milk booster enhancing tonic.[3] The leaves, roots and bark are used by traditional people in Kano for the management of diabetes.[4] The methanol bark extract shows high antidiabetic activity, and effective use of the extract could lead to the cure of the diabetic problems around the world.[5] In the previous studies, researchers reported the isolation of euphol-*3-O*-cinnamate, lupeol, taraxar-14-ene, ursolic acid, ß-sitosterol, betulinic acid, sitosterol 3-O-ß-D-glucopyranoside and (E)-3,5,4'-trihydroxy-stilbene-3,5-O-b-D-diglucopyranoside and politamide from roots and stem bark parts of plant.[2][6] In the present study, preliminary phytochemical analysis, isolation and structural elucidation of methanol extract of the plant bark has been done.

MATERIALS AND METHODS

Collection of The Plant Samples And Preparation

The plant was identified and authenticated in herbarium of Bayero University Kano and a voucher specimen was deposited BUKHAN 104. The branches of *Ficus polita* were harvested and the barks peeled off while still fresh, cut into small portions, and dried at room temperature under shade for one month. The dried plant barks were grounded, and the powdered was kept at room temperature away from direct sunlight.[5]

Extraction and Isolation of *Ficus Polita* Bark

The powdered sample (500g) was macerated using Ethanol (2L) for 7 days with occasional agitation. The solvent was evaporated using rotary evaporator at 40^0C. The crude ethanol extract was macerated sequentially using n-hexane, chloroform, ethyl acetate (EtOAc) and methanol (MeOH). The fractions of these solvents were dried by exposing them to air at room temperature.[5] The methanol extract (5g) was subjected to column chromatography, the column was eluted with n-hexane, EtOAc and MeOH.[7] This gave different fractions and each fraction was analyzed on TLC and similar fractions were combined according to their TLC patterns which were pooled together. Pooled fraction with single spot was subjected to spectroscopic analysis. The structure of isolated pooled component (E5) was elucidated by NMR (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC) and IR techniques. The resulting spectral data obtained for E5 was interpreted by comparing with identical literature spectral data, FIDs of both the literature data and the experimental data were viewed, processed and compared using Spinworks, and the structure was elucidated.[8]

Phytochemical Screening

Ficus polita bark crude ethanol extract and fractions were screened for the presence of phytochemicals such as alkaloids, flavonoids, tannins, saponins, triterpenoids, steroids, carbohydrates, anthraquinones and lipids using standard phytochemical procedures.[9][10]

RESULTS AND DISCUSSION

RESULTS

Table 1: Phytochemical screening results of *Ficus polita* bark extracts.

Phytochemical constituent	Fractions				
	Crude Extract	n-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	+	-	-	-	-
Flavonoids	+	-	-	+	-
Tannins	+	+	+	+	+
Triterpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Saponin	+	+	+	+	+
Anthraquinones	+	+	+	+	-
Carbohydrates	+	+	+	+	-
Lipids	+	+	+	+	+

Key: + = Presence - = Absence

Table 2: Column Chromatography of Methanol Extract of *Ficus Polita* Bark.

Solvent System	Volume	50ml Label Portion Collected				
		1st	2nd	3rd	4th	Weight (g)
n-Hexane	100% (200ml)	A_1	A_2	A_3	A_4	0.0037
n-Hexane : Ethyl acetate	95:5% (190:10ml)	A_5	A_6	A_7	A_8	0.0040
n-Hexane : Ethyl acetate	80:20% (160:40ml)	B_1	B_2	B_3	B_4	0.0153
n-Hexane : Ethyl acetate	70:30% (140:60ml)	B_5	B_6	B_7	B_8	0.0102
n-Hexane : Ethyl acetate	50:50% (100:100ml)	C_1	C_2	C_3	C_4	0.0598
n-Hexane : Ethyl acetate	30:70% (60:140ml)	C_5	C_6	C_7	C_8	0.0536
n-Hexane : Ethyl acetate	10:90% (20:1800ml)	D_1	D_2	D_3	D_4	0.0579
Ethyl acetate	100% (200ml)	E_1	E_2	E_3	E_4	0.0535
Ethyl acetate : Methanol	95:5% (190:10ml)	E_5	E_6	E_7	E_8	0.0530
Ethyl acetate: Methanol	90:10% (180:20ml)	F_1	F_2	F_3	F_4	0.0930
Ethyl acetate : Methanol	85:15% (170:30ml)	F_5	F_6	F_7	F_8	0.3412
Ethyl acetate : Methanol	80:20% (160:40ml)	G_1	G_2	G_3	G_4	0.3848
Ethyl acetate : Methanol	75:25% (150:50ml)	G_5	G_6	G_7	G_8	0.3096
Ethyl acetate : Methanol	70:30% (140:60ml)	H_1	H_2	H_3	H_4	0.3758
Ethyl acetate : Methanol	65:35% (130:70ml)	H_5	H_6	H_7	H_8	0.3806
Ethyl acetate : Methanol	60:40% (120:80ml)	I_1	I_2	I_3	I_4	0.3881
Ethyl acetate : Methanol	50:50% (100:100ml)	I_5	I_6	I_7	I_8	0.3432
Ethyl acetate : Methanol	40:60% (80:120ml)	J_1	J_2	J_3	J_4	0.3526
Ethyl acetate : Methanol	30:70% (60:140ml)	J_5	J_6	J_7	J_8	0.3859
Ethyl acetate : Methanol	20:80% (40:160ml)	K_1	K_2	K_3	K_4	0.0828

Ethyl acetate : Methanol	15:85%	(30:170ml)	K_5	K_6	K_7	K_8	0.0271
Ethyl acetate : Methanol	10:90%	(20:180ml)	L_1	L_2	L_3	L_4	0.0125
Ethyl acetate : Methanol	5:95%	(10:190ml)	L_5	L_6	L_7	L_8	0.0121
Methanol	100%	(200ml)	M_1	M_2	M_3	M_4	0.0750

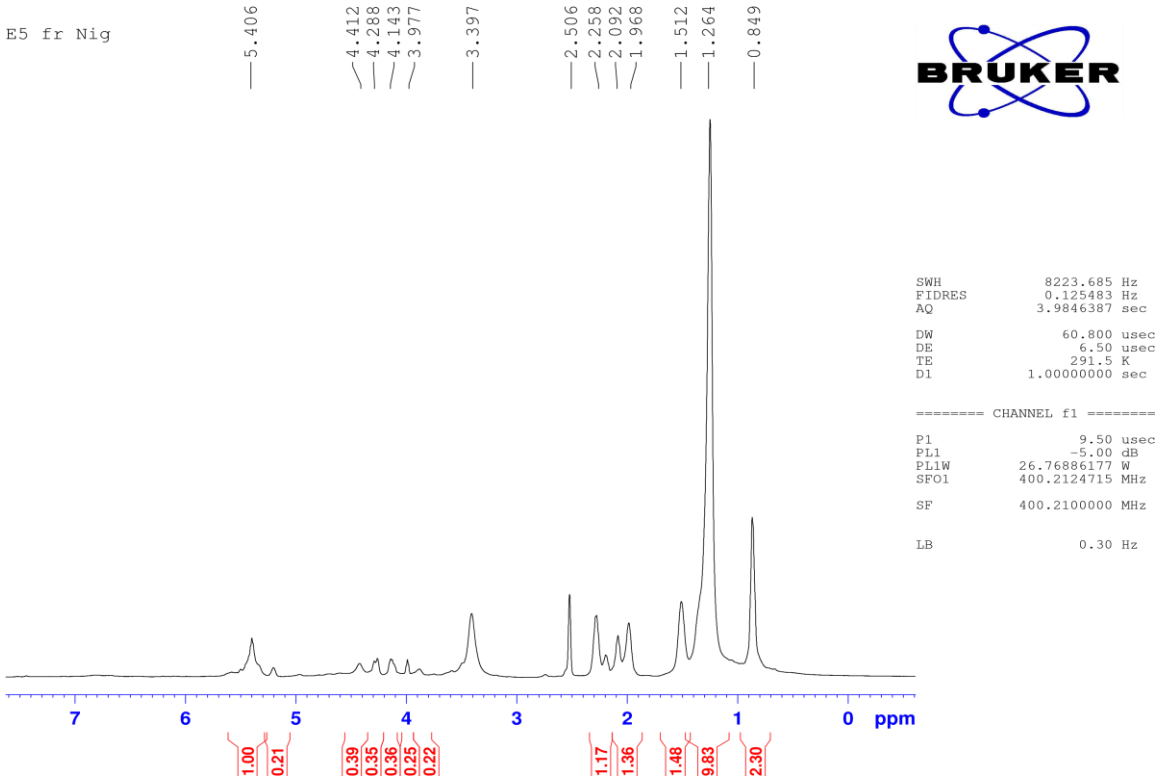

Fig. 1: ^{1}H NMR of compound E5 in DMSO-d_6.

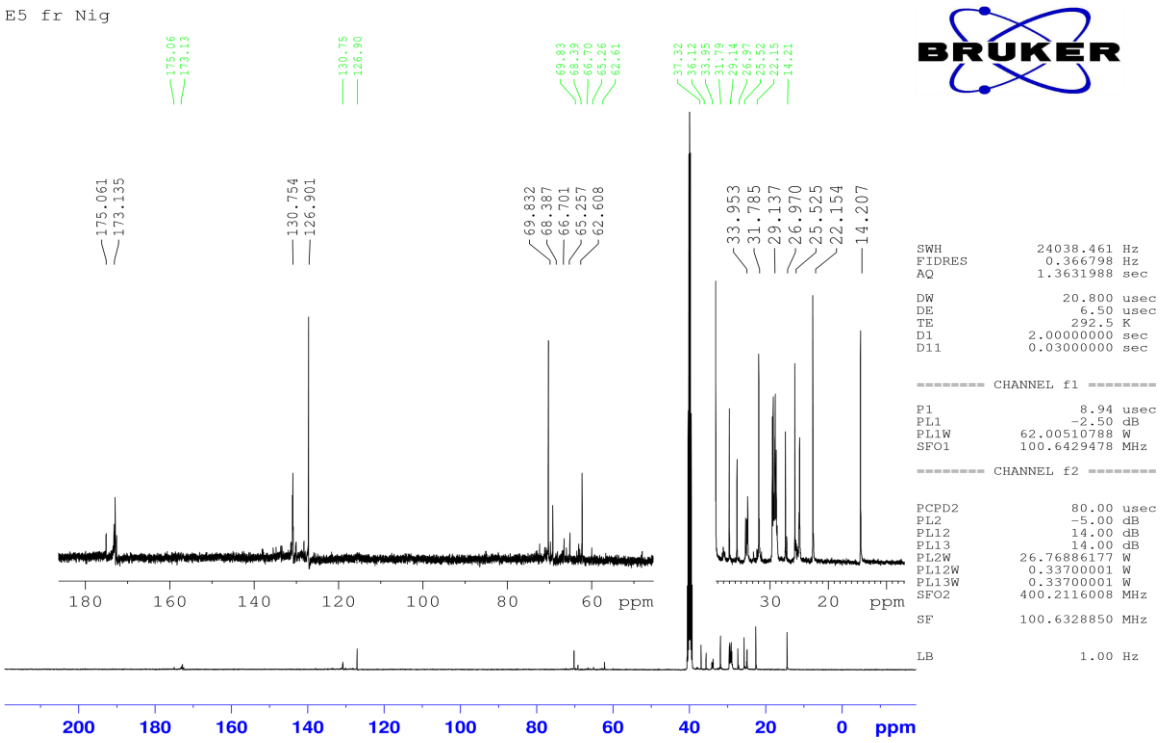

Fig. 2: ^{13}C NMR of Compound E5 in DMSO-d_6.

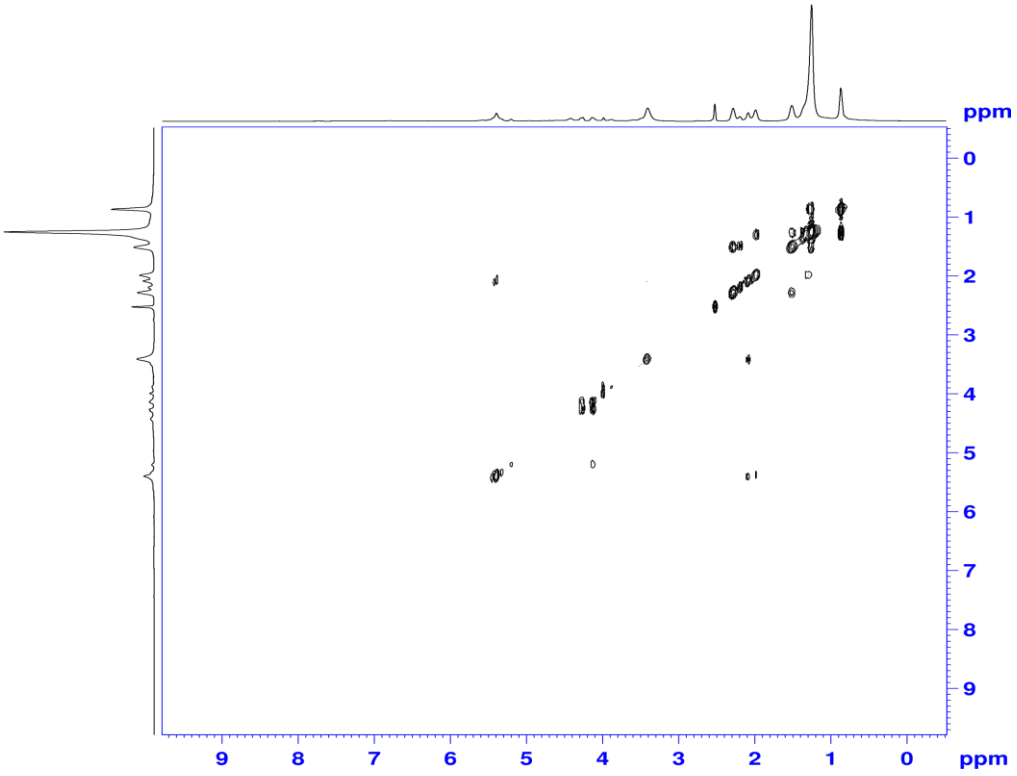

Fig. 3: COSY of compound E5 in DMSO-d_6.

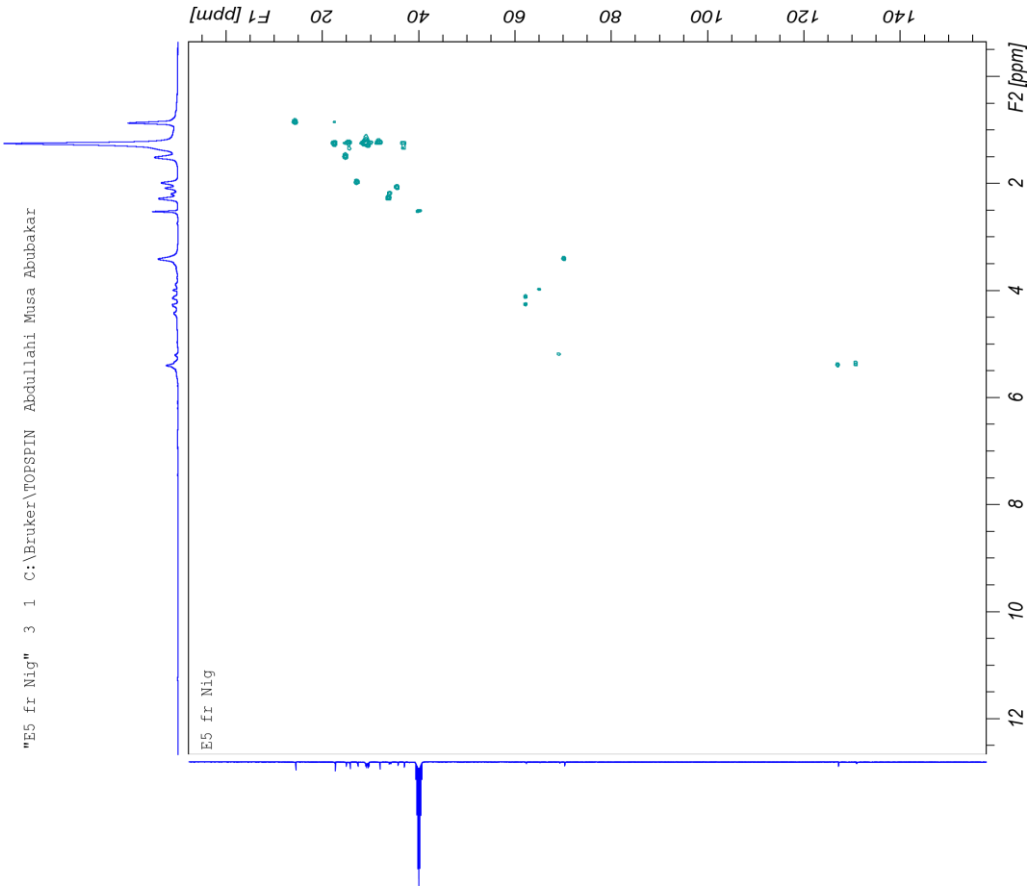

Fig. 4: HSQC of compound E5 in DMSO-d_6

Agilent Technologies

Sample ID: E5 Method Name: DATA COLLECT ONLY

Sample Scans: 32 User: FTIR

Background Scans: 32 Date/Time: 10/25/2018 3:00:01PM

Resolution: 8 cm-1 Range: 4,000.00 - 650.00

System Status: Good Apodization: Happ-Genzel

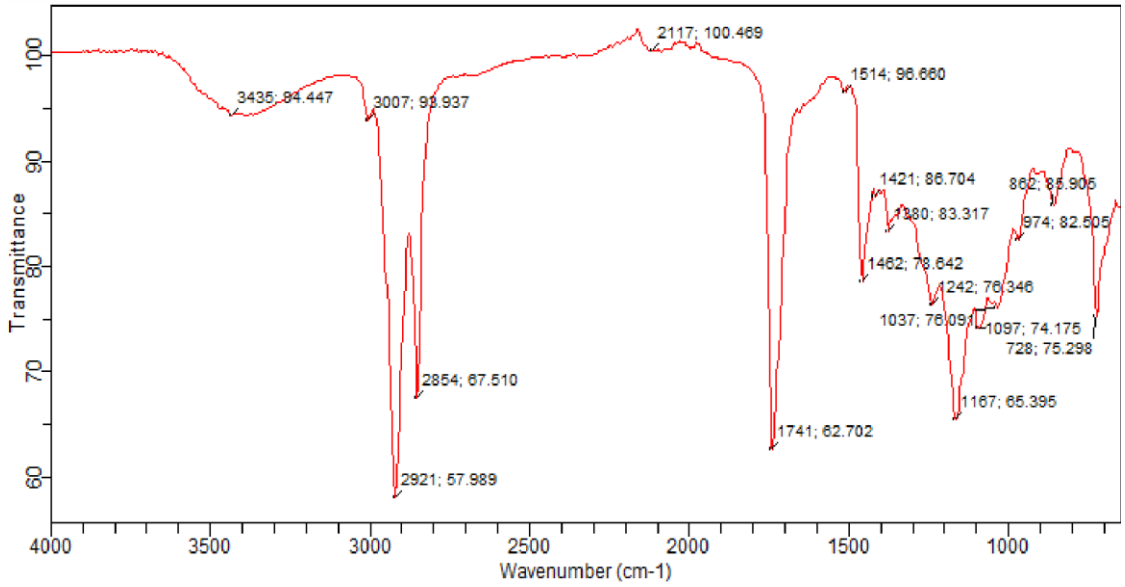

Fig. 5: FT-IR of Compound E5.

Table 3: ^{1}H NMR chemical shift data of compound E5.

Experiment δ_H	Literature δ_{H*}	^{1}H Assignment	^{1}H Position in E5
0.849	0.880	RCH_3	H1
1.264	1.268	RCH_2R	H2, H2’
1.512	1.582	RCH_2R	H5
1.968	1.968	$RCH_2C{=}C$	H3
2.092	2.024	$RCH_2C{=}C$	H3’
2.258	2.285	$RCH_2C{=}O$	H6
2.506		DMSO	
3.397	7.26	H_2O	
3.977	3.923	RCH_2OR	H7, H9
4.143	4.180	RCH_2OR	H8
4.288	4.381	RCH_2OR	H10
4.412	4.402	R_2COH	H11, H11’
5.406	5.337	RC=CH	H4

Key: δ_{H*} Tetraoleoylcardiolipin Spectra in $CDCl_3$.[8]

Table 4: ^{13}C NMR chemical shift data of compound E5.

Experiment δ_C	Literature δ_{C*}	^{13}C Assignment	Carbon Position in E5
14.207	14.137	RCH_3	C1
22.154	22.708	RCH_2R	C2
25.525	24.963	RCH_2R	C9
26.970	27.261	RCH_2R	C6
29.137	29.353	RCH_2R	C4
31.787	29.805	RCH_2R	C5
33.953	31.938	RCH_2R	C11
36.12	34.114	RCH_2R	C3
37.32	34.312	RCH_2R	C10
62.608	62.736	R_3COR	C14
65.257	63.508	R_3COR	C16
66.701	66.799	R_3COR	C17
68.387	69.523	R_3COH	C18
69.832	70.487	R_3COR	C15
126.901	129.657	RC=CR	C7
130.754	129.989	RC=CR	C8
173.135	173.489	RCOOR	C12
175.061	174.112	RCOOR	C13

Key: δ_{C*} Tetraoleoylcardiolipin Spectra in $CDCl_3$.[8]

Table 5: COSY (^1H- ^1H) and HSQC (^1H- ^{13}C) data of compound E5.

^1H Assignment	$^1H - ^1H$ Correlation	$^1H - ^{13}C$ Correlation	^{13}C Assignment
H1	H1 – H2	H1 – C1	C1
H2, H2’	H2’ – H3	H2 – C2	C2
H5	H2’ – H5	H2’ – C4	C9
H3	H5 – H6	H2’ – C5	C6
H3’	H3 – H4	H2’– C10	C4
H6	H3’ – H4	H3 – C6	C5
DMSO	H7 – H8	H3’ – C6	C3
H_2O	H8 – H9	H4 – C7	C11
H7, H9	H10 – H11	H4 – C8	C10
H8		H5 – C9	C14
H10		H6 – C11	C16
H11, H11’		H7 – C14	C17
H4		H8 – C15	C18
		H9 – C16	C15
		H10 – C17	C7
		H11 – C18	C8
			C12
			C13

DISCUSSION

The phytochemical analysis carried out on the bark of *Ficus polita* plant shows the presence of saponin, steroid, triterpenoid, tannin and lipids in all the fractions. Carbohydrate and anthraquinone are present in all the fractions with exception of methanolic fraction. Flavonoid however is present in crude ethanol extract and ethyl acetate fractions. However, alkaloid is present only in crude ethanol extract. The result corresponds to the report in the literature that different solvent extraction and geographical area may result in obtaining different phytochemical constituents.[11][12][13]

The compound E5 spectral data is identical with the literature spectral data obtained. The compound E5 was elucidated to be Tetraoleoylcardiolipin ($C_{81}H_{150}O_{17}P_2$).

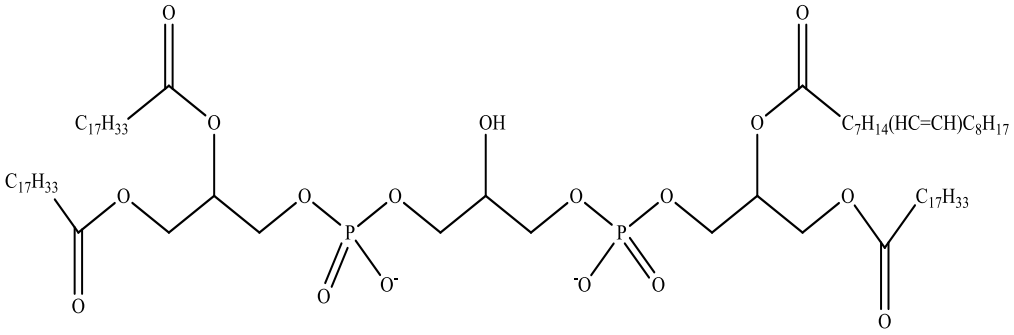

Figure 6: Structure of Tetraoleoylcardiolipin (E5).

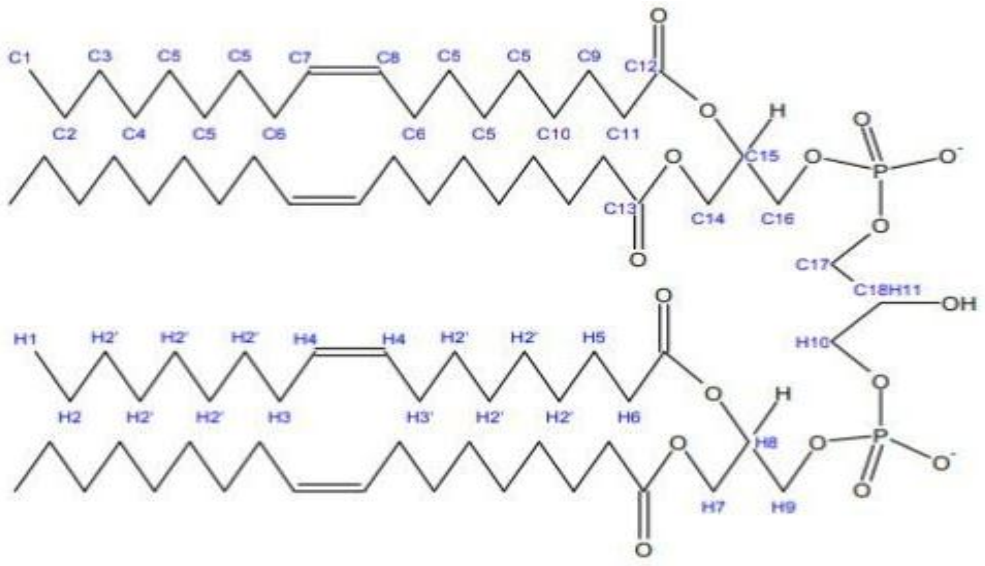

Figure 7: Proposed ^{1}H and ^{13}C assignment of Compound E5.

The characteristic ^{1}H NMR shows overlapping around 1.264 ppm, proton that is bonded carbon connected to oxygen, alcoholic proton, vinylic proton and there is presence of allylic

proton and also proton adjacent to carbonyl carbon as shown in figure 1. The characteristics of ^{13}C peaks in the spectrum of E5 revealed that the carbons are mainly aliphatic, carbon directly bonded to oxygen, alcoholic carbon, vinylic carbons, and esters as shown in figure 2. The COSY and HSQC correlation correspond with the structure of the compound. The FT-IR Spectrum of the compound shows a characteristic absorbance for OH (3435cm^{-1}), C=C (3007cm^{-1}), C-H (2921cm^{-1} and 2854cm^{-1}), and C=O (1741cm^{-1}), CH_2 and CH_3 (1462cm^{-1}), CH_3 (1380cm^{-1}), C-O (1242cm^{-1}), P=O (1167cm^{-1}) and P-OR (1037cm^{-1}) as shown in figure 5.

The compound E5 is cardiolipin derivatives, is a kind of disphosphatidylglycerol lipid in which all four of the phosphatidyl acyl groups are specified as oleoyl. It has two phosphatidic acid moieties connect with a glycerol backbone in the center and potentially carries two negative charges with IUPAC name [(2R)-3-[[3-[[(2R)-2,3-bis[[(Z)-octadec-9-enoyl]oxy]propoxy]-hydroxyphosphoryl]oxy-2-hydroxypropoxy]-hydroxyphosphoryl]oxy-2-[(Z)-octadec-9-enoyl]oxypropyl](Z)-octadec-9-enoate.

CONCLUSION

The *Ficus polita* bark methanol extract shows presence saponins, tannins, steroids, triterpenoids and lipids. Isolation and identification of bioactive compound indicates that the compound isolated is a cardiolipin derivative, tetraoleoylcardiolipin ($C_{81}H_{150}O_{17}P_2$).

AKNOWLEDGEMENT

We acknowledged the support given to me by Alh. Goni Baba Gana's family, Alh. Bulama Lawan's family, my colleagues, Umar Ya'u, Ali Ya'u Yusuf, Abdulsalam AS, M. Kaka YSU, Abdullahi Firat, Dr. Fatih Briyan and the entire staff of Pure and Industrial Chemistry Department, Bayero University Kano.

REFERENCES

1. Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HMP, Ambassa P, Ngadjui BT. (Antimicrobial activities of the methanol extract, fractions and compounds from Ficus polita Vahl. (Moraceae)). BMC Complementary and Alternative Medicine, 2011; 11: 6.
2. Mustapha AA. (Ethnobotanical field survey of medicinal plants used by traditional medicine practitioners to manage HIV/AIDS opportunistic infections and their prophylaxis in Keffi Metropolis, Nigeria). Asian J Plant Science and Research, 2014; 4(1): 7-14.

3. Danjuma MN, Darda'u H. An Ethno-Survey of Medicinal Trees of Kabobi Village, Northern Katsina, Nigeria. Academic Research International, 2013; 4(3): 174-183.
4. Negbenebor HE, Shehu K, Mairami FM, Adeiza ZO, Nura S, Fagwalawa LD. (Ethnobotanical Survey of Medicinal Plants Used by Hausa People in the Management of Diabetes Mellitus in Kano Metropolis, Northern Nigeria). European J Medicinal Plants, 2017; 18(2): 1-10.
5. Goni ZZ, Idriss MM. In-vivo Antidiabetic Activity of Ficus polita Bark Extracts. World J Pharmaceutical Res., 2019; 8(13): 261-270.
6. Kamga J, Sandjo LP, Poumale HM, Ngameni B, Shiono Y, Yemloul M, Rincheval V, Ngadjui BT, Kirsch G. Politamide, a new constituent from the stem bark of FicuspolitaVahl (Moraceae). Arkivoc, 2010; (ii): 323-329.
7. Bajpai VK, Majumder R, Park JG. Isolation and purification of plant secondary metabolites using column-chromatographic technique. Bangladesh Journal of Pharmacology, 2016; 11: 844-848.
8. BMRD; Maria N, Lawrence JC, Christopher S, Mark EA, John LM. Tetraoleoylcardiolipin bmse001105. Biological Magnetic Resonance Data Bank, http;//www.bmrd.wisc.edu/metabolomics/mol_summary/show_data.php/id=bmse001105 &whichTab=1
9. Abubakar AS, Adoum OA, Hassan Y, Ibrahim B. Phytochemical Screening and Anti-plasmodial Activity of *Balsamodendron africanum* (A. Rich) (Burseraceae). American Journal of Bioscience and Bioengineering, 2016; 4(6): 65-69.
10. Marka R, Talari S, Penchala S, Rudroju S, Swamy RN. Preliminary Phytochemical Analysis of Leaf, Stem, Root and Seed Extracts of *Arachis Shypogaea* L. Int. J. Pharm. Sci. Rev Res., 2013; 20(1): 134-139.
11. Akesa TM. Phytotaxonomy and phytochemicals of Eight species of the Family Moraceae in Benue State, Nigeria. International Journal of Scientific & Engineering Research, 2016; 7(2): 588-595.
12. Usman H, Kaigama AU, Ibisagba OO, Fulata AM, Ahmed IA. Phytoconstituents evaluation and antimicrobial efficacy of the crude flavonoids and saponins rootbark extracts of *Terminalia avicennioides* and *Ficus polita.* J Herbmed Pharmacology, 2018; 7(2): 106-111.
13. Sodipo OA, Akanmu AO, Mamza UT, Idriss MM, Wampana B. (Antidiabetic effect of the aqueous stem bark extract of *Ficus polita* Vahl in Alloxan-Induced Diabetic Albino Rats). Nigerian J Pharm and Biomed Res, 2016; 1(1): 75-81.