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Isolation and Characterization of Bioactive Compound from Ethanol Extract of Root Bark of *Grewia mollis* (Dargaza'a) Widely Growing in the Wild in North Eastern Nigeria

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ABSTRACT: Grewia mollis is a plant, has been used as a source of food and in traditional medicinal preparations. Hence, the objective of the present study was to isolate and characterize the bioactive compound from ethanol extract of root bark of Grewia mollis (Dargaza'a) widely growing in the wild in North Eastern Nigeria using standard methods. The isolated compounds were also subjected to antimicrobial, antioxidant and cytotoxic assay. The isolation from the ethanol extract afforded a compound B that showed some degree of bioactivities. Compound B isolated showed good antioxidant activity by bioautography method. For the cytotoxic activity the result revealed that Compound B was moderately active with LC_{50} value of 14.06 µg/ml. The result for the antimicrobial activity of the isolated compound B using bioautography showed moderate inhibition by inhibiting three of the test organisms. The structural elucidation of the isolated compound B were done using UV, IR, ^{1}H and ^{13}C-NMR, DEPT and COSY and also by comparing the melting point and Rf- values of the isolate with the standards reference from literatures and compound B was found to be (2-(3,4- dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran -4- one) (quercetin)

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African plants constitute a rich untapped pool of natural products. Scientific investigations of medicinal plants have been initiated in many countries because of their contribution to health care (Fatope, 1995). In developing countries it was estimated that about 80% of the population relies on plant based preparation used in their traditional medicinal system and as the basic need for human primary health care (WHO, 2000; Hamayun *et al*., 2006).The global demand for herbal medicine is growing (Muregi *et al*., 2003; Zowai ., 2003). Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in health care (Hamayun *et al.,* 2006). Medicinal plants are able to produce a large number of diverse bioactive compounds, particularly secondary metabolites. For this reason, extensive studies using different plant extracts have been reported by several scientists, to investigate the antibacterial, anti-inflammatory, analgesic, antioxidant and many other medicinal values of these extracts Mshelia *et al.,* 2008. The Nigerian flora is rich in medicinal plants that are commonly used in folklore medicine for the treatment of various diseases. Grewia mollis (Tiliceae) commonly known in Northern Nigeria as dragaza'a is a shrub or small tree that grows up to 10.5m tall, and grows in tropical areas (Burkill, 2000; Sharma, 2002 Katende *et al.,* 1995). The fruit is edible and very

sweet (Person, 1982). It is popularly used in folk medicine to treat malaria fever (Fowler, 2006). The mucilaginous bark and leaves are applied to ulcer, cuts, sores and snake bites (Brink, 2007). The bark and root preparation are taken to treat cough. Extracts of stem bark and leaves are drunk to treat fever. The decoction of the stem bark is taken to treat diarrhoea, and maceration is taken to ease child birth. The mucilage is credited with laxative properties while an infusion of the bark is used to treat colic (Lockett *et al.*, 2000). The pounded leave mixed with water are taken against stomach problems and also given by constipated domestic animals (Ruffo *et al.*, 2002). The decoction of the leaves is used in baths and drinks against rickets in children and difficult birth (Kokwaro, 1993). A decoction of the roots is drunk in case of palpitation (Katenda *et al.*, 1995). The sap from root shavings is placed under the eyelid to treat sore eyes, where as a liquid obtained by kneading the root bark in water is drunk to treat stomach ache, colic and poisoning by certain plants (Neuwinger, 2000). The paste of ground root is applied to rheumatic swellings and inflammation while the fruit is used as febrifuge and in treatment of malaria fever (Lockett *et al.*, 2000; Fowler, 2006). Some findings demonstrated that the mucilage obtained from the stem bark can serve as a good binder in paracetamol formulations (Martins *et al.*, 2008; Muazu, *et al*., 2009). A reports suggest that high concentration of stem bark in dietary may cause some adverse effects, especially liver injury (Wilson, 2010). Therefore the objective of the present study was to isolate and characterize the bioactive compound from ethanol extract of root bark of *Grewia mollis* (Dargaza'a) widely growing in the wild in North Eastern Nigeria

MATERIALS AND METHODS

Collection and preparation of plant material: The root bark of *Grewia mollis* was collected in Hawul local Government Area of Borno State.

The collection was done in April and the soil on the root was removed by gently washing it under running tap water and the bark removed.

The root bark was then air dried under a shade in the laboratory at room temperature and pulverized using motorized miller.

Extraction of Plant Material (Soxhlet extraction method): The extraction was carried out using the soxhlet extraction with the following solvents hexane, ethyl acetate, acetone, ethanol and water in order of increasing polarity for about 6 to 8 hours (Vogel, 1979).

Antibacterial activity (Bioautography) according to Masoko; Eloff, 2006; Begue; Kline, (1972).

Brine shrimps: Cytotoxicity analysis was carried out according to Adoum, 2009; Clarkson and Thompson (2000); Meyer *et al.,* (1982); Abdulrani *et al.,* (2010)**.**

Qualitative Analysis of antioxidant (Bioautography) was carried out using 1, 1-diphenyl-2- icrylhydrazyl (DPPH) (Brand-William *et al.,* 1995; Masoko; Eloff, 2006).

Purification: The purification of extracts by solvent-solvent separation according to the one described by Gailliot, (1998); Houghton; Ramon, (1998); Suffness; Douros, 1979).

Isolation: The isolation was done using Column and Preparative TLC chromatography (Mohammed, *et al.*, 2016; Alluri, *et al.,* 2005; Moshi *et al.*, 2010).

Analysis of Extracts/Fractions: The extract/fraction was analyzed by separation on Merck TLC F_{254} analytical plates using three different solvent systems of varying polarities, namely BEA (benzene/ethanol/aluminium hydroxide (90: 10: 1), CEF (Chloroform/ethyl acetate/formic acid) 5: 4:1 and EMW (ethyl acetate /methanol/water (40: 5.4: 4). Separated components were visualized under visible and UV light. Plates were afterward sprayed with P-anisaldehye sulphuric acid or vanillin sulphuric acid spray reagents and heated for about five minutes at 100^{o}C for development of colour (Wagner; Bladt, 1996).

Isolation: The fraction F_4 from column chromatography using silica gel (100-200mesh) and eluated with solvent system of increasing polarity (hexane: ethyl acetate: methanol) of the n- butanol fraction of solvent – solvent separation of the ethanol extract form soxhlet extraction. Fraction FC_4 and FC_5 showed very good antibacterial activity using bioautography. FC_4 was re-subjected for further separation using column chromatography on silica gel (60-120mesh) with solvent systems hexane: ethyl acetate (7:3) and then hexane: ethyl acetate (5:5). Four fractions were obtained after re-combining based on TLC profile, all the fractions showed some degrees of antibacterial activity using bioautography. Fraction FCC_1 was washed with hexane in ethyl acetate (9:1) to obtain compound A. Fraction FCC_2 was re-applied on silica (60-120mesh) and eluted with chloroform: hexane (8:2) to obtain four fractions after recombining and all of them showed some degree of antimicrobial and antioxidant activity. The fractions FCD_1, FCD_2 and FCD_4 are too small in quantity and are not

subjected for further purification. Fraction FCD_3 was washed with hexane: chloroform (9:1) to obtain compound M after crystallization. FC_5 was re-subjected to column chromatography on silica gel (100-200) mesh with solvent system ethyl acetate: methanol (9:1) to obtain four fractions after recombining. Fraction FD_2-FD_4 were remixed and applied on column using sephradex and eluted with chloroform: methanol (7:3) to obtain two fractions FDD_1 and FDD_2. Fraction FDD_1 is very small in quantity and shows relatively very low degree of antibacterial activity therefore it was not subjected for purification. The fraction FDD_2 was washed with chloroform in acetone and allowed to crystallize to obtain compound B which was active on the test microorganism using bioautography.

Melting Point Determination using melting point apparatus and the electrothermal melting point apparatus.

UV–Vis Spectroscopy method: The Genesys 10S UV–Vis spectrophotometer was used for the analysis and scans were performed over a wavelength range of 200 to 600 nm.

Fourier Transform Infrared Spectrophotometer (FTIR): For IR analysis, Perkin Elmer frontier MIR/NIR Fourier Transform Infrared spectrophotometer (FTIR) was used for the analysis. The spectra were recorded at room temperature between 4000 and 400 cm^{-1} with 4 cm^{-1} resolution running 10 scans.

Nuclear Magnetic Resonance Spectroscopy: The *NMR* analyses of compound B were run for proton (^1H), carbon 13 (^{13}C), distortionless enhancement through polarization transfer (DEPT), and correlated spectroscopy (COSY) as reported by (Gumel *et al.,* 2012).

RESULT AND DISCUSSION

The Percentage Recovery, Colour and Texture of Solvent-Solvent Extraction of Ethanol Extract (14.0000gm) is presented in table 1. Table 2 showed the Brine Shrimp Lethality Test of Compounds B from the Root bark of *Grewia mollis* the result indicate that compound B showed moderate cytotoxic activity with LC_{50} value of 14.06 µg/ml.

Table 3 shows the result for the Melting Point, Rf Values, LC_{50}, Antioxidant and Antimicrobial Activity of Compound B. the isolated compound B had a melting point of 316ºC with the RF values of 0.9342 in EMW, 0.6308 in CBM and 0.8382 in CEF mobile systems. The result also showed a moderate cytotoxic effect with LC_{50} value of *14.06* µg/ml and a good antioxidant activity of (+++) by bioautography method. The antimicrobial activity showed moderate activity using bioautography by inhibiting three of the test organism.

Table 1: Percentage Recovery, Colour and Texture of Solvent-Solvent Extraction of Ethanol Extract (14.0000gm)

S/No	Solvent	Mass of fraction (g)	Percentage of fraction	Colour of fraction	texture fraction
1	n-butanol	4.2300	30.2143	Light brown	Powder
2	Water	1.8745	13.3900	Light brown	Powder
3	n-hexane	0.4471	3.1936	Green	Crystalline
4	Carbon tetrachloride	0.4288	3.0629	Yellow	Crystalline
5	Chloroform	1.0507	7.5050	Yellow	Crystalline
6	35% water in methanol	4.5232	32.3086	Brown	Powder

Table 2: Brine Shrimp Lethality Test of Compounds B from the Root bark of *Grewia mollis*

Conc. (ppm)	number of nauplii	no. of dead nauplii				percentage mortality of nauplii				LC_{50} (µg/ml)		
		0 hr	6 hr	12 hr	24 hr	0 hr	6 hr	12 hr	24 hr	6 hr	12 hr	24 hr
10	16	0	4	5	7	0	25.00	31.25	43.75	1441.43	265.0	14.06
100	16	0	5	6	9	0	31.25	37.50	56.25			
200	17	0	7	10	14	0	41.18	58.82	82.35			
500	15	0	5	11	15	0	33.33	73.33	100.00			
1000	18	0	8	12	18	0	44.44	66.67	100.00			

Figure 1 and Table 4 showed the UV spectrum and UV spectrum data of compound B with two major absorption bands in (MeOH) at 363nm and 262nm with another weaker absorption at 298nm. Figure 2 and Table 5 shows the Fourier transform infrared spectroscopy FTIR spectra and data of compound B. The FTIR showed frequencies at 3395.26 cm^{-1} and 1662.92cm^{-1} indicating the presence of hydroxyl group and keto group in conjugation and the absorption peaks at 1520.06 cm^{-1} and 1165.67 cm^{-1}

indicating the presence of ethylenic double bond and aromatic ring respectively. Figure 3 and 4 showed the 1H-NMR spectrum of compound B. The spectrum in fogure 3 showed the complete spectrum showing all peaks from delta 0 to 12.5 ppm, while that of figure 4 showed peaks from delta 6.0 to 8.1ppm presumably the most important peaks.

Table 3: The Melting Point, Rf Values, LC_{50}, Antioxidant and Antimicrobial Activity of Compound B

Isolate	M. P (^{0}c)	RF values of isolates			LC_{50} (µg/ml)	AO	Antimicrobial activity			
		EMW	CBM	CEF			ST	EC	SA	SD
B	316	0.9342	0.6308	0.8382	14.06	+++	++	++	++	-
Ascorbic acid						+++	ND	ND	ND	ND

Key: SA= Staphylococcus aureus, SD =Shigella dysenteriae, ST=Salmonella typhi, EC= Escherichia coli, AO= Antioxidant activity

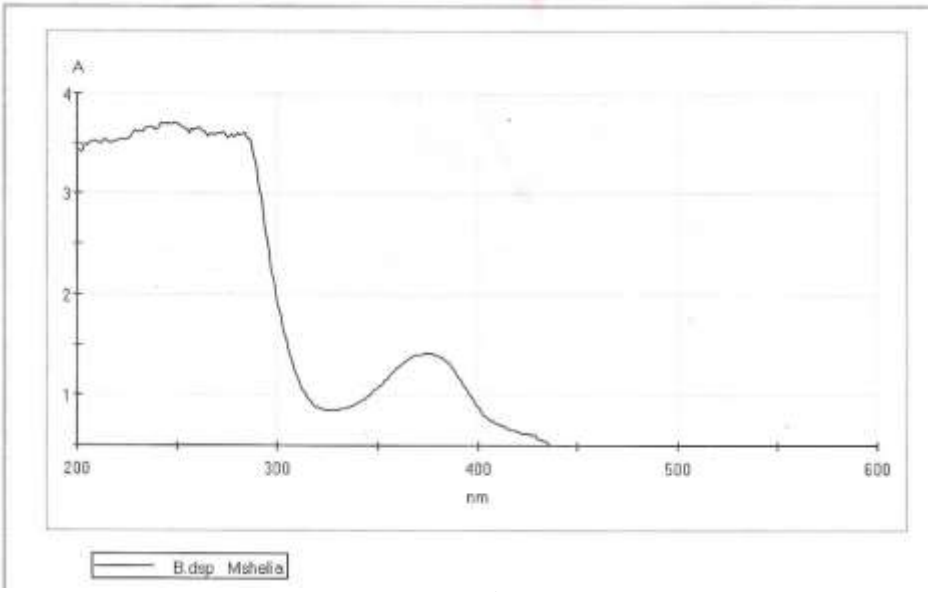

Fig 1: UV Spectra of compound B

Table 4: UV spectroscopy of compound B

Absorption peak (nm)	Functional group	Electron transition
262	C=C (aromatic)	$\pi - \pi^*$
298	Attributed to C-ring only	$\pi - \pi^*$
362	C=O	n - π^*

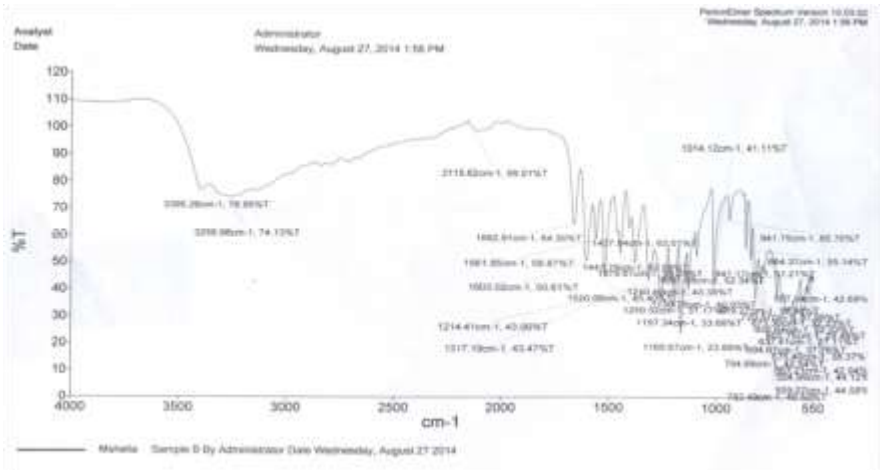

Fig 2: Fourier transform infrared spectroscopy FTIR Spectra of compound B

Table 5: Fourier transform infrared spectroscopy FTIR spectra of compound B

Frequency (cm^{-1})	Group

3395.26	O-H stretching of phenols
3034.42	C-H stretching of aromatics
1662.92	C = O aryl ketonic stretch
1603.02	C = Cethylenic double bond
1561.85, 1520.06	C= C aromatic ring stretch
1440.03	In plane O-H bending of phenols
1317.19	In plane bending of C-H bond in aromatic hydrocarbon
1214.41	C – O stretching of phenols
1165.67	C-CO-C stretch and bending in ketone
841.17, 794.89, 671.31, 655.64	C-H out of plane bending of aromatic hydrocarbon

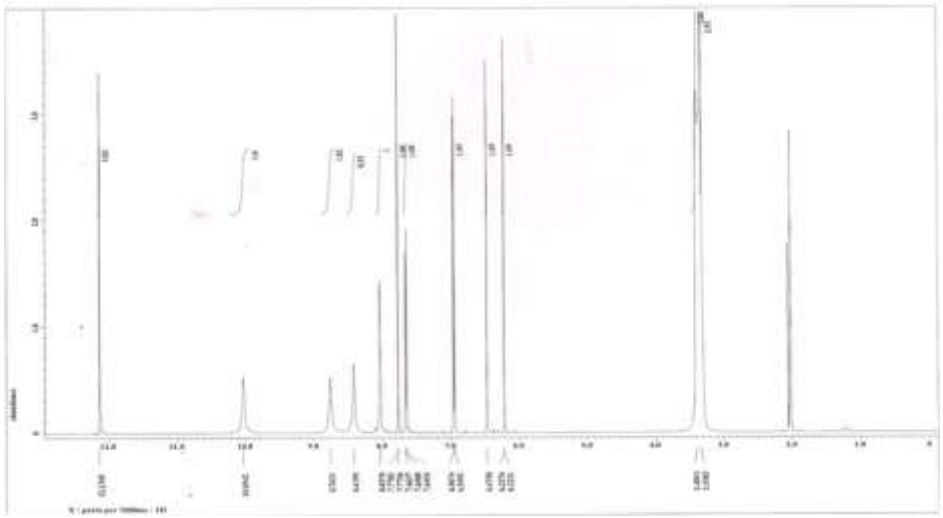

Fig 3: ^{1}H-NMR Spectrum of compound B

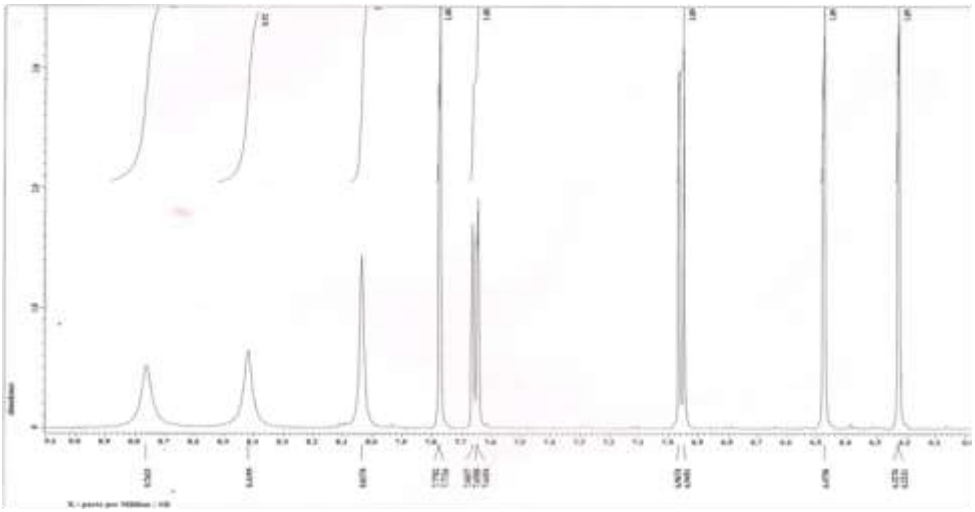

Fig 4: ^{1}H-NMR Spectrum of compound B

Figure 5 and 6 showed the ^{1}H- ^{1}H COSY Spectrum of compound B. Figure 3 showed the whole spectrum for delta 0-12 ppm, while figure 5 showed the spectrum with the peaks between 4.0 to 8.2pmm on the x-axis and 5.5 to 9.5ppm on the y-axisFigure 5: Figure 4:^{1}H-^{1}H COSY Spectrum of compound B. Figure7 and 8 showed the ^{13}C- NMR Spectrum of compound B. Figure 7 showed the ^{13}C- NMR peaks of compound B with the chemical shift between 0-220 ppm, while figure 8 showed the ^{13}C- NMR Spectrum of compound B showing the peaks between delta 90.0 to 210ppm. Figure 9 and 10 showed 13C-DEPT Spectrum of compound B. the DEPT spectrum in figure 8 showed all the peaks that falls between delta 0 to 220ppm including the solvent peak. Figure 9 showed the presumably the most inportant peaks of compound B tha falls between delta 90.0 to 210ppm.

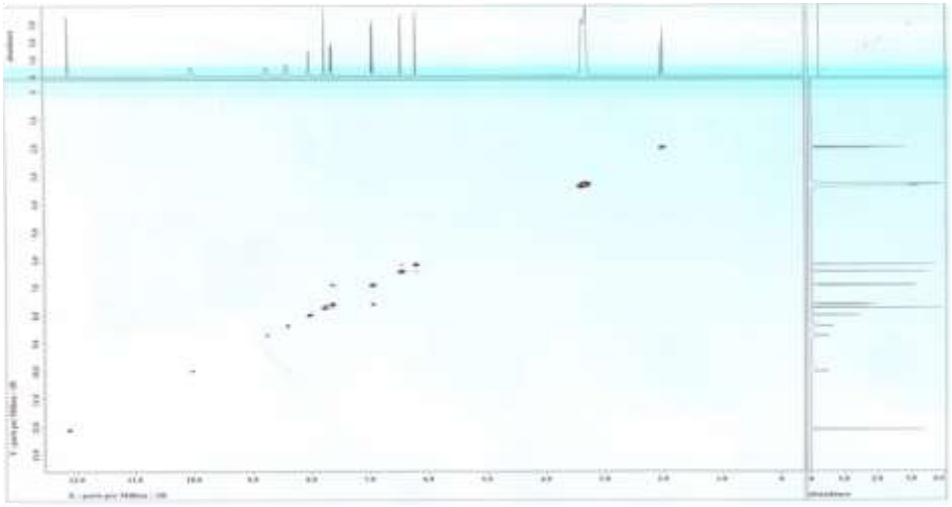

Figure 5: ^{1}H- ^{1}H COSY Spectrum of compound B

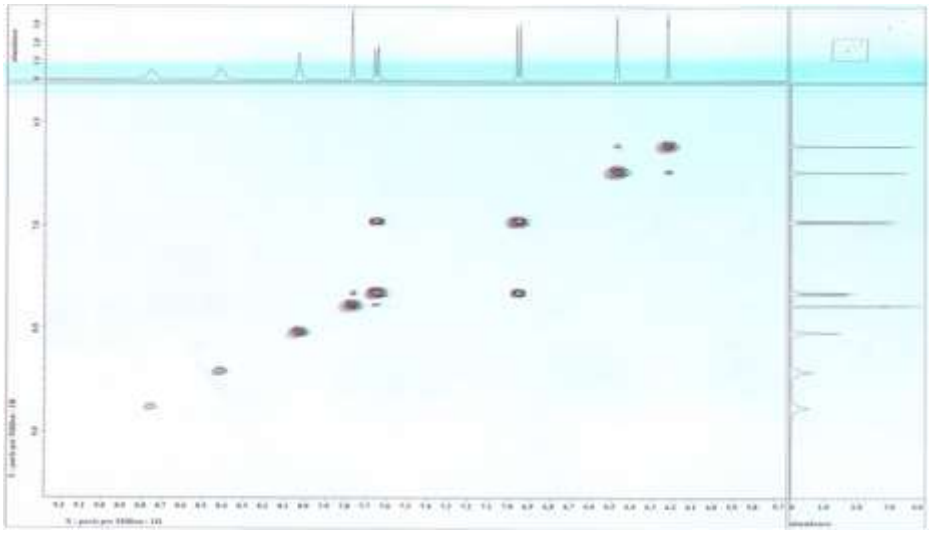

Fig 6: ^{1}H- ^{1}H COSY Spectrum of compound B

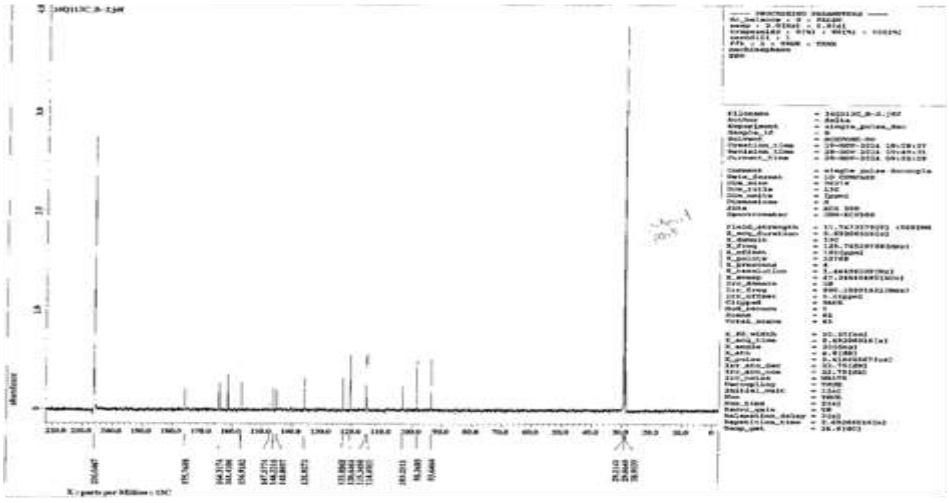

Figure 7: ^{13}C- NMR Spectrum of compound B

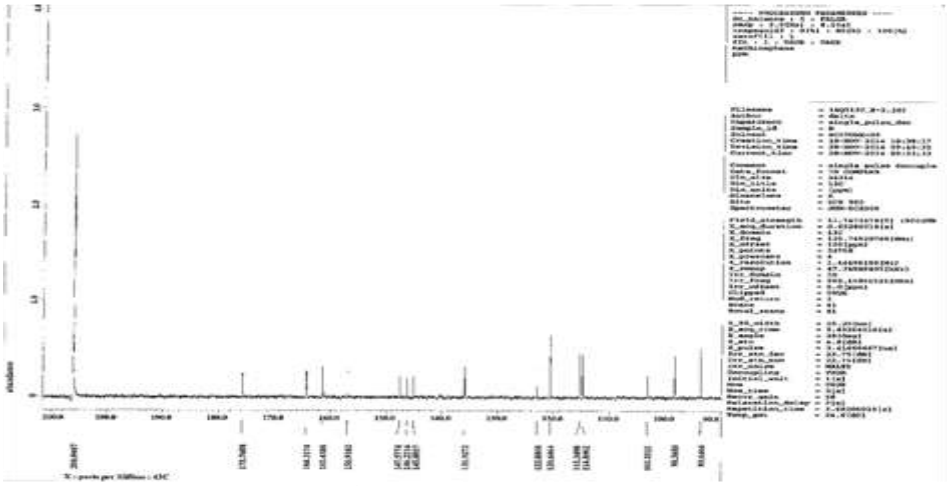

Fig 8: ^{13}C- NMR Spectrum of compound B

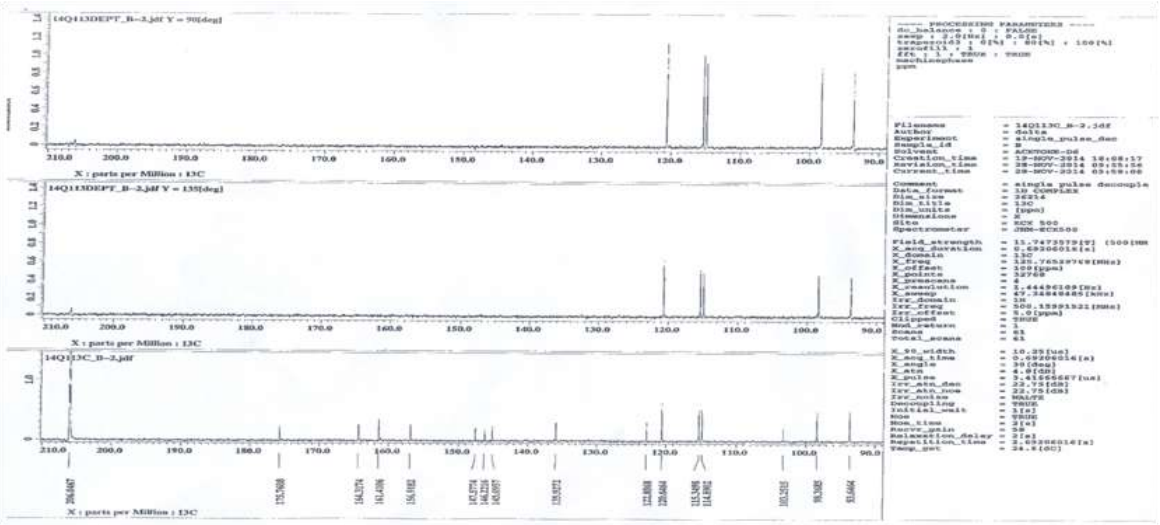

Fig 9: ^{13}C-DEPT Spectrum of compound B

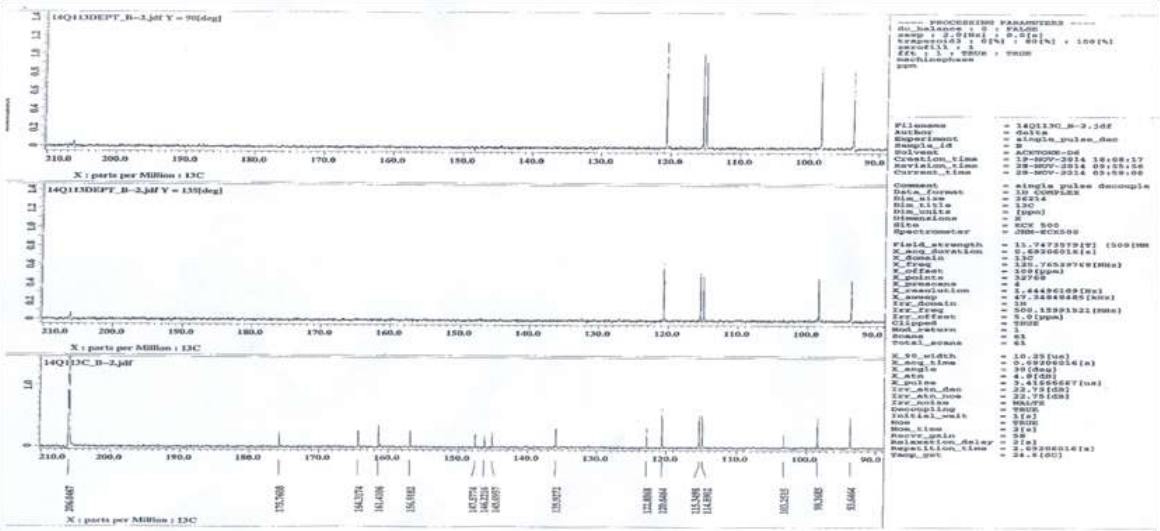

Fig 10: DEPT Spectrum of compound B

Figure 3-10 and Table 6 and showed the spectra NMR data of compound B. The ^{1}H-NMR spectral data of compound B showed that it has five aromatic protons between δ_H of 6.2231 – 7.7781 ppm which consist of three singlet aromatic protons (δ_H 6.2231, 6.4750 and 7.7736ppm) and two doublet aromatic proton at δ_H (6.9491 and 7.6454 ppm) (Megawati and Fajriah, 2013). The δ_H 6.2231 (IH, s) and 6.4750 (IH, S) are due to meta coupled protons of A – ring of H – 6 and H – 8 of a flavonoid nucleus. The signals at δ_H 6.9491, (1H, d,J = 7.28 Hz, 7.7736 (1H, S) and 7.6637 (1H, d, J=7.32Hz) are assigned to H -5'', H – 2'' and H – 6'' respectively of the ring B. The H-NMR spectrum showed some signals representing the hydroxyl protons as shown by the following literatures (Fatemeh *et al*., 2006; Leena and Aleykutty, 2016). The hydroxyl protons at δ_H 12.1345 (1H,S, C 5-OH), δ_H 10.0342(1H, S C7-OH), δ_H 8.7631(1H, S C3-OH), δ_H 8.4198(1H, S C3'-OH) and δ_H 8.0370(1H, S C4'-OH)

Table 6: ^{1}H-NMR, ^{13}NMR, DEPT and ^{1}H-^{1}H COSY of compound B

position of carbon	δ_C (ppm)	DEPT (Types of carbon)	δ_H (ppm)	COSY
2	156.918	C		
3	135.9272	C		
4	175.7608	C		
5	161.4106	C		
6	98.3685	CH	6.2231 (IHd J=1.8H2)	8
7	164.3174	C		
8	93.6464	CH	6.4750 (IH)	6
9	156.577	C		
10	103.2515	C		
1'	120.6464	C		
2'	114.8902	CH	7.7736 (IH:J=1.84H2)	
3'	145.0957	C		
4'	146.2216	C		
5'	115.3496	CH	6.9491 (IH J= 7.24H2)	6'
6'	122.8868	CH	7.6454 (IH:J = 7.32H2)	5'

^{13}C – NMR and DEPT spectrum showed that compound B has 15 carbon atoms, five methine carbon at δ_C (98.3685, 93.6464, 114.8902,115.3490 and 122.8868ppm) and ten quaternary carbon at δ_C 156.9180, 135.9272, 175.7608, 161.4106, 164.3174, 156.5770, 103.2515, 120.6464, 145.0757 and 146.2216ppm. Based on the correlation data for (^{1}H - ^{1}H COSY), it shows that there are two coupling between δ_H 6.2231 ppm (H – 6) and δ_H 6.4750 ppm (H – 8) and δ_H 6.9491 (H – 5') and δ_H 7.6454 (H – 6'). On comparing the data obtained for compound B it can be confirmed to be 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one (quercetin)**.**

They had similar UV, IR, H^{1}NMR, H^{1}-H^{1} COSY, and DEPT spectra with those of an authenticate sample of 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one (quercetin) from literatures (Jan *et al*., 2004; Ahmadu *et al.*, 2007; Wang *et al*., 2012; Bakkialakshmi and Barani, 2013; Megawati and Fajriah, 2013; Selvaraj *et al*., 2013; Rajan and Muthukrishnazna, 2013; Osuji *et al*., 2013; Hao-Bin *et al*., 2013; Abdel-Aziz *et al*., 2014; Miyazawa and Hisama, 2014; Sathyadevi and Subramanian, 2014; Svetiana *et al*., 2015; Fadeyi *et al*., 2015; Naturajan and Anton, 2015; Wenjun *et al.*, 2015; Svetiana *et al.*, 2015; Naturajan and Anton, 2015; Fadeyi *et al.*, 2015b; Wianowska *et al.*, 2017; Genene &Hazare 2017; Elufioye, 2017; Patel and Jat, 2017; Mi *et al.*, 2017; Alessandro *et al.*, 2017). The structure of compound B is elucidated to be 2-(3', 4'-Dihydroxyphenyl)-3, 5, 7-trihydroxy-1-benzopyran-4-one (quercetin)

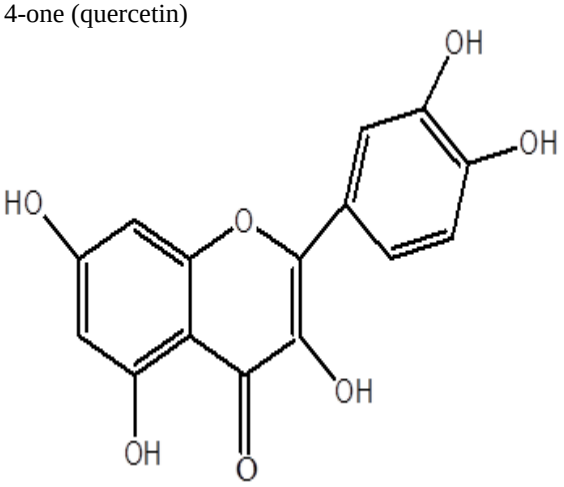

Fig 11: Compound B: 2-(3', 4'-Dihydroxyphenyl)-3, 5, 7-trihydroxy-1-benzopyran-4-one (quercetin)

Conclusion: The plant Grewia mollis contain a bioactive compound that have been previously isolated from the plant that had some degree of antimicrobial, cytotoxicity and antioxidant activities. The isolated bioactive compound was elucidated to be *2-(3', 4'-Dihydroxyphenyl)-3, 5, 7-trihydroxy-1-*

benzopyran-4-one (quercetin) which have justified the use of the plant by the people of Gombe and Borno state in traditional medicine.

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