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Research Article

THE GC-MS ANALYSIS OF ACETONE EXTRACT OF THE ROOT BARK OF GREWIA MOLLIS WITH THE TLC ANALYSIS, ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF ITS SOLVENT - SOLVENT FRACTIONS

[1]Mshelia E. H., [1,] *Kadam Tadzabia, [2]Watirahyel Pindar and [3]Mohammed A.H.

[1]Department of Chemistry, Faculty of Natural and Applied Science, Nigerian Army University Biu, Borno State, Nigeria.
[2]Department of Integrated Science, Federal College of Education (Tech.) Gombe, Nigeria.
[3]Department of Chemistry, School Science, Federal College of Education (Tech.) Gombe, Nigeria.

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ABSTRACT

Medicinal plants are a source of biologically important compounds, which play an important part in the observed therapeutic uses of different plants. Grewia mollis is a well-known medicinal plant among the Bura people of Borno state, and have been used by folk lore in the treatment of various diseases. These work look at the GC-MS analysis of the acetone extract of the root bark of Grewia mollis obtained from soxhlet extraction with the TLC analysis, antimicrobial, antioxidant and cytotoxic activities of its solvent – solvent fractions. The GC-Ms was analyzed on Shimadazu GC-MS QP 2010 equipped with flame ionization detector while the TLC was virtualized using Merck silica gel F254 plates. The antimicrobial activity was determined using disc agar diffusion, the qualitative antioxidant using DPPH while the cytotoxicity was determined using brine shrimp method.The GC-MS result showed there were sixteen compounds in the acetone extract while the chloroform fraction had the highest percentage recovery from the solvent-solvent extraction and carbon tetrachloride was the least recovered. EMW was the best eluting solvent while hexane was the fraction with the highest components. The hexane and carbon tetrachloride fraction inhibited the growth of two organisms each while the remaining fractions inhibited the growth of three organisms each. The brine shrimp cytotoxicity assays shows that three of the fractions chloroform fraction with LD50 of 9.05µgm/ml, carbon tetrachloride with LD50 of 31.36 µgm/ml, and n-butanol with LD50 of 65.97µgm/ml were highly active and the remaining moderately active, while carbon tetrachloride and chloroform fractions showed moderate antioxidant activity.

Keywords: Grewia mollis, Antimicrobial, Antioxidant and Cytotoxicity.

INTRODUCTION

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. Plants have been used as herbal drug since time immemorial (Srivastava *et al.,* 1990). These herbal drugs are being used as herbal remedies to treat various diseases across the world. The curative properties of several herbs find the place in the 'Atharvaveda' from which Ayurveda originated. The traditional medicinal herbs which are responsible for strengthening the body immune system showed the presence of many essential and nutritional elements (Sofowora, 1996).

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 (Sermakkani, and Thangapandian, 2012; Hamayun*et al.,* 2006). The global demand for herbal medicine is growing (Muregi*et al.,* 2003) andinterest in the 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). When the herbs are used in the treatment of certain illnesses, it should be known that, besides the pharmacological effect they have, the medicinal plants could be toxic if the content of minerals in them is elevated. Although the effectiveness of medicinal plants is mainly associated with their constituents such as novel chemical constituents the secondary metabolites, (Gordon and David, 2001). The primary benefits of using plants derived medicine are that they are relatively safer than synthetic alternative offering profound therapeutic benefits and more affordable treatment. Besides the rapid evolution of numerous defense mechanisms, against antimicrobial agents and resistance to old and newly produced drugs underlies the urgency with which alternative chemotherapeutic agent with possible normal mechanism of action to the microbes needs to be developed.

The antimicrobial compounds from plant may inhibit microbial growth by different mechanisms than those presently used antimicrobial and may have a significant clinical value in treatment of resistant microbial strains (Zaria*et al.*, 1995). The investigation of plant for bioactive secondary metabolites is an area which most plant scientist have recently focused on with the aim of discovering new clinically useful and commercially important plant product (Zhou *et al.,* 2019). Therefore several medicinal plants have been evaluated for possible biological activity and potential cure for a variety of ailments especially of microbial origin. (Mshelia *et al.,* 2008). Screening of plant extracts for anti-microbial, antioxidant and cytotoxic activities have provided interesting results, in that most plant – derived bioactive compounds showed positive results on animals.

Gas chromatography – Mass spectrometry is an important technique that has been adapted to evaluate different phytoconstituents present in various plant extracts with their structures (Thamer and Thamer, 2023; Velmurugan and Anand, 2017). This technique has superior separation potency that tends to produce a high accuracy and precision of chemical fingerprint (Vinodh*et al.,* 2013). Moreover, quantitative data along with the coupled mass spectral database can be given by GC-MS that is of tremendous value for achieving the correlation between bioactive compounds and their applications in pharmacology (Kumar *et al.*, 2019; Gani 2003; Fernie, 2004;

Corresponding Author: Kadam Tadzabia,
1Department of Chemistry, Faculty of Natural and Applied Science, Nigerian Army University Biu, Borno State, Nigeria.

Kell*et al.,* 2005). 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), the mucilaginous bark and leaves are applied to ulcer, cuts, sores and snake bites and also used in soup preparation (Brink, 2007), while 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 diarrhea, 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 to 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) 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). This work determine the compounds present in the acetone extracts obtained by soxhlet extraction of the root bark of Grewia mollis. The solvent extraction of the acetone extract was carried out to separate the compounds based on their polarity. The number of compounds in different fractions was determined by TLC chromatography to ascertain the number of compounds in each fractions. Finally the antimicrobial, antioxidant and cytotoxic activities of the different fractions were evaluated

MATERIAL AND METHODS

Collection of Plant Material: The root bark of *Grewia mollis* was collected in Hawul local Government Area of Borno State. The collection was done in September when the leaves were green. The infected parts were removed and the healthy fresh bark was air dried under a shade and pulverized using motorized miller.

Extraction of plant material: The powdered root bark of Grewia mollis were serially extracted with hexane, ethyl acetate, acetone, ethanol and distilled water using soxhlet extractor apparatus for 8 hours each (Vogel, 1979). The extracts were evaporated to dryness on rotary evaporator and the percentage yield of each extracts were determined.

GC-MS Instrumentation For The acetone extract

The Samples chemical compositions were analyzed on Shimadzu GC-MS-QP 2010 plus instrument (Shimadzu, Japan) equipped with GC-MS flame ionization detector (FID) carrying Agilent DB-5 ms column (30 m long x 0.25 mm internal diameter x 0.25 µm film thickness), was used. Samples were prepared by dissolving aliquot amount in 2 ml solvent chloroform. About 1.5 ml of this solution was with-drawn using a borosilicate glass syringe and filtered into an amber GC-MS vial (VZS-0209A-100; Cronus, UK) using 0.02 µm disposable PTFE filter cartridge (Sartorius Stedim, Germany).

The GC-MS method was observed according to Gumel *et al.,* (2012) with slight modification. A sample (1 µl) was automatically injected into the GC-MS at a split ratio of 1:50. The injection temperature was 280°C. The GC oven temperature ramping was set as follows: 40°C for 1 min then increased to 120°C at 15°C per- minutes; held at 120°C for 2 min then increased to 250°Cat 10°C per-minutes; then held at 250°C for 15 minutes. Helium (0.41 bar) was used as the carrier gas at a flow rate of 48.3 ml per-minute. Mass spectra were acquired at 1250 scan speed, 50-600 m/z scan mass using electron impact energy of 70 eV at 200°C ion-source temperature and 280°C interface temperature.

Solvent- Solvent Separation

The separation technique used for the solvent-solvent separation was the one used by USA National Cancer institute described by Stuffiness and Douro's (1979). The acetone extract obtain from soxhlet extraction was subjected to solvent-solvent separation with different solvents.

The extract was dissolved in equal volume of chloroform and water. The water fraction was mixed with an equal volume of n- butanol in a seperatory funnel to yield the water and butanol fractions. The chloroform fraction was dried in a stream of air and extracted with equal volume of hexane and 10% water in methanol mixture. This yielded the hexane fraction and the 10% water in methanol mixture. The 10% water in methanol mixture was then diluted to 20% water in methanol by adding water. This was then mixed with carbon tetrachloride in a seperatory funnel giving the carbon tetrachloride fraction. The 20% water in methanol fraction was further diluted to 35% water in methanol and mixed with chloroform to yield the chloroform and 35% water in methanol fractions. The fractions were allowed to dry under stream of air over night or water bath.

Thin Layer Chromatography (TLC)

The numbers of compounds present in each fraction namely n-butanol, water, n-hexane, carbon tetra chloride, chloroform and 35% water in methanol fractions were determined by TLC. 10µl of a 10mg/ml concentration were spotted on Merck silica gel F_{254} plates. Three eluting system selected to separate high, intermediate and low polarities (ethyl acetate/methanol/water (40:5.4:4) EMW polar, chloroform/ethyl acetate/formic acid 5:4:1 CEF intermediate polarity/acidic and Benzene/ethanol/ammonium 90:10:1 BEA non-polar/basic) were used. Separated compounds were examined under visible light marked and sprays with anseldehye/sulphuric acid spray reagent (Wagner and Bladt, 1996; (Rarh, 2012)

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Brine Shrimp Lethality Assay

Brine shrimp eggs were commercially available. For this experiment, brine shrimp egg without shells "Artemia Revolution" 120g were obtained from NT labs (Fry care) laboratories LTD UK, Serial No. 7//3380900038///3.Made in England. Eggs were stored in a refrigerator at 5^0C (NT laboratory LTD UK 2015).

Preparation of Artificial Sea water

Artificial sea water was prepared by dissolving 35g of sea salt in1litre of distilled water for hatching the brine shrimp eggs (NT laboratory LTD UK 2015).

Hacthing of Brine Shrimp

An artificial seawater was Prepared at full strength. To obtain an optimum result a solution of specific gravity of 1.022 at 24^0C was prepared by dissolving 35g sea salt sodium chloride NaCl pre- liter of water. The seawater was added to the brine shrimp Hatcher in a heated aquarium aerate from bottom of the unit so that all eggs are kept in suspension and moving. The brine shrimp bottle was shaken before dispensing into the aquarium (each drop gives from 1500 to 2000 nauplii, three drops (5000 nauplii) and are hatched in approximately 250ml sea water.

The hatcher is illuminated very well for a minimum of three hours preferably for 12hours. The hatching time depend on temperature at 24^{0}C (which is average tropical aquarium temperature) hatching take place between 24-48 hours (maximum hatch 44-48hours). The Nauplii is then used directly for the cytotoxicity test (NT laboratory LTD UK, 2015);

Preparation of Test Sample: Samples were prepared by dissolving 20mg of the fractions in 10ml of suitable solvent (stock solution # 1). Solution of varying concentrations (1000, 500, 250,125,100 µg /ml) were obtained by the serial dilution technique of the stock solution.

Cytotoxicity Test (Bioassay): Brine shrimp lethality bioassay was carried out using brine shrimp larvae (Artemiasalinarevolution) to determine the cytotoxicity of the fractions. To each sample vial corresponding to 1000,500,250,125, and 100µg/ml, 4ml of artificial seawater was added and 10 brine shrimps were introduced into the tubes using pipette, and the final volume in each vial was adjusted with artificial seawater to make a total volume of 5ml. The test tubes were left uncovered in the light, the nauplii were counted against a lighted background using magnifying hand lens and the number of the surviving shrimps were counted and recorded after 24hours. Control test was also carried out using artificial seawater only. Nauplii were considered dead if they were lying immobile at the bottom of the vial (Ghosh and Catterjee, 2013; Adoum *et al.,* 1997)

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Statistical Analysis The percentage of deaths and (LC_{50}) were determined using statistical analysis as described by Adoum, (2009).

Percentage of mortality (%M)

$$= \frac{Total\ number\ of\ nauplii - number of\ nauplii\ alive}{total\ number\ of\ nauplii} \text{X100}$$

Lethal Concentration (LC_{50}) Determination: The lethal concentrations of fractions resulting in 50% mortality of the brine shrimp (LC_{50}) was determined from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis, the LC_{50} was derived from the best-fit line obtained. LC_{50} values were obtained from the best-fit line, plotted of concentration against Percentage mortality.

Qualitative antioxidant determination

The fractions were re-dissolved in appropriate solvent and spotted on the silica-gel $60F_{254}$ plates and then develop the chromatogram in appropriate solvent systems. The plates were sprayed with a methanolic solution of DPPH (2mg/ml). Thus, antioxidants activity appears as yellow bands on a light purple background and the intensity of the yellow colour shows the degree of antioxidant activity. After spotting the fractions on the TLC plates, even uneluted plates can also be used to determine the qualitative antioxidant analysis. The uneluted plates were sprayed with 0.2% DPPH methanol solution and sample spots will be evaluated for radical scavenging activity (Marwah*et al.*, 2007).

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Antimicrobial activity determination

Source and Maintenance of Organism Gram negative and Gram positive microorganism were obtained and confirmed microbiology and parasitology laboratory, Federal Teaching Hospital, Gombe. The organisms were maintained on Muller-Hinton agar (MHA) (Oxoid, UK) to obtain the isolated colonies.

Disc-Agar Diffusion Method Fractions were tested for antibacterial activity by the disc diffusion method. A single colony was aseptically transferred with an inoculating loop to about 20 ml of the prepared nutrient agar. Filter papers cut out with a diameter of 1cm was transferred to the oven and sterilized for one hour. With the aid of sterilized forceps, the filter papers were then transferred to the various fractions at a concentration of 100µg/ml. The filter paper was left in the extracts for about 20 minutes so as to soak the fractions very well. The filter papers were then transferred to a cultured agar plates, the plates are then incubated at 37ºC for 24 hours in the incubator. Negative controls were performed using filter paper discs loaded with acetone and standard loaded with gentamicin and ampiclox. The zone of inhibition was determined after 24hours (Youmans*et al.,* 1967)

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RESULTS AND DISCUSSION

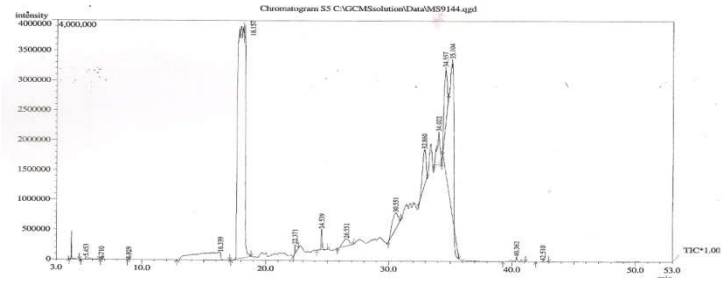

Figure 1: GC-MS spectrum of Acetone extract

Table 1: Compounds identify by the GC-MS spectroscopy present in the Acetone extracts of the root bark of Grewia mollis

Peak	Retention time (min)	%composition of compounds	Name of compounds	Formula
1	4.257	0.22	Methyl benzene	C_7H_8
2	5.453	0.22	Dimethyl sulfoxide	C_2H_6OS
3	6.710	0.00	1,6-Dianhydro-2-deoxy-β-d-ribohexopyranose	$C_6H_8O_3$
4	8.929	0.00	2,2-Dimethyl butane	C_6H_{14}
5	16.339	6.05	Dimethylphthalate	$C_{10}H_{10}O_4$
6	18.157	48.50	Diethyl phthalate	$C_{12}H_{14}O_4$
7	22.371	0.12	1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$
8	24.539	0.53	Dibutyl phthalate	$C_{16}H_{22}O_4$
9	26.551	1.32	Lup-20(29)-en-3-ol	$C_{30}H_{50}O$
10	30.551	2.95	Lup-20(29)-en-3-ol acetate	$C_{32}H_{52}O_2$
11	32.860	3.60	Lup-20(29)-en-3,28 - diol	$C_{30}H_{50}O_2$
12	34.022	2.84	C(14α)-Homo-27-norgammacer-13-en-21-01-3-methyl (3α,β)	$C_{31}H_{52}O_2$
13	34.557	5.11	Fagarasterol	$C_{30}H_{50}O$
14	35.104	28.13	Acetic acid,17-(1,5-dimethyl hex-4-enyl)-4,4,8,10,14-pentamethyl-2,3,4,5,6,7,8,9,10,11,12,13,14,15,16-tetradecahydro-1H-cyclopentaphenanthanone	$C_{32}H_{52}O_2$
15	40.362	0.36	Triphenyl phosphine oxide	$C_{18}H_{15}OP$
16	42.510	0.04	Pentadecianoic acid 2,6,10,14-tetramethyl, methyl ester	$C_{20}H_{40}O_2$

Table 2: Percentage of recovery, color and texture of solvent-solvent extraction of acetone extract of *Grewia mollis*

S.No	Solvent	Percentage of fraction	Colour of fraction	Texture of fraction
1	n-butanol	13.6000	Light green	Gummy
2	Water	16.8660	Brown	Powder
3	n-hexane	4.5673	Greenish yellow	Gummy
4	Carbon tetrachloride	10.6500	Light brown	Crystalline
5	Chloroform	21.6050	Greenish yellow	Crystalline
6	35% water in methanol	14.1905	Light brown	Powder

Table 3: Number of compounds in fractions of acetone extract of Grewia mollis root bark

S.No.	Fraction	Visible light			Sprayed with anisaldehyde		
		BEA	EMW	CEF	BEA	EMW	CEF
1	n-butanol	0	2	0	0	5	1
2	Water	0	0	0	0	3	0
3	Hexane	3	2	2	8	3	4
4	Carbon tetrachloride	0	2	0	5	5	2
5	Chloroform	0	0	1	1	2	1
6	35% water in methanol	0	0	0	0	2	0

Table 4: Brine Shrimp Lethality Test for Solvent-solvent fraction of the acetone Extracts of root bark of Grewia mollis

Fraction	Concentration (ppm)	% Mortality of naupiles	LC_{50}-(µg/ml)
Butanol	10	20	65.97
	100	60	
	200	70	
	500	100	
	1000	100	
Water	10	30	440.72
	100	30	
	200	30	
	500	60	
	1000	80	
Hexane	10	20	468.64
	100	10	
	200	60	
	500	50	
	1000	80	
Carbon tetrachloride	10	20	31.36
	100	50	
	200	90	
	500	100	
	1000	100	
Chloroform	10	60	9.05
	100	80	
	200	100	
	500	100	
	1000	100	
35% water in methanol	10	50	369.14
	100	30	
	200	50	
	500	40	
	1000	80	

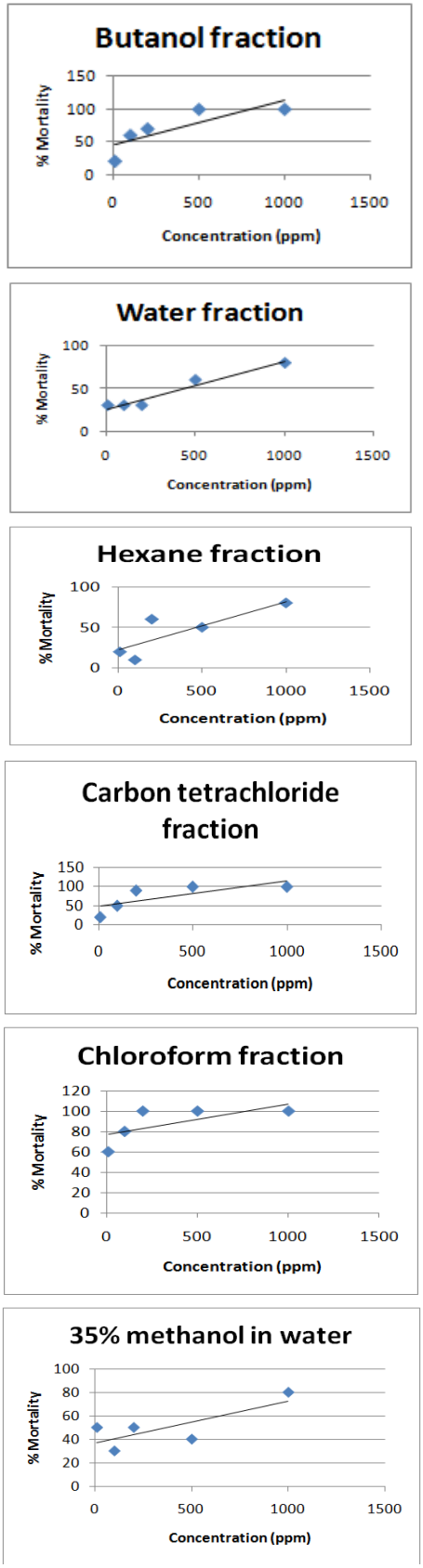

Figure 2: Cytotoxic effects of the fractions of solvent extraction of acetone extract of root bark of Grewia mollis on brine shrimp

TABLE 5: Antimicrobial, cytotoxicity (LC_{50}) and antioxidant activities of fractions of solvent/solvent extraction of acetone extract of root bark of Grewia mollis

Fraction	Salmonella typhii	Staphylococcus aureus	Escherichia coli	Shigella dysentariae	LC_{50}	Antioxidant activity
n-butanol	9	0	8	6	65.97	+
Water	10	12	16	0	440.72	-
n-hexane	8	0	10	0	4 68.64	-
Carbon Tetrachloride	14	24	0	0	31.36	++
Chloroform	16	22	0	11	9.05	++
35% water in Methanol	12	20	0	4	369.14	+
Gentimicin	26	18	18	19	ND	ND
Ampicillin	30	20	22	18	ND	ND
Water	0	0	0	0	-	ND

RESULTS AND DISCUSSION

Figure 1 showed the spectrum for the GC-MS spectroscopy of the acetone extract of the root bark of Grewia mollis while table 1 showed the compounds present in the extract. The result showed that there are sixteen different compounds in the extracts which includes terpenes, alcohols, esters carboxylic acids carbonyl compounds and hydrocarbons. This result showed that the acetone solvent was able to extract different classes of compounds because it is a semi polar solvent.

Table 2 showed the percentage recovery, colour and texture of the fractions of solvent- solvent extraction of the acetone extract from serial extraction using soxhlet extractor of the root bark of Grewia mollis. The highest percentage recovery was obtained with chloroform fraction which is greenish yellow in colour and crystalline. The least percentage recovery was obtain in n–hexane fraction which was also greenish yellow but gummy in nature. The carbon tetra chloride and chloroform are both crystalline in nature but different in colour. The high percentage of the chloroform fraction indicates that most of the compounds in the acetone extract are soluble in the chloroform and less soluble in the non-polar solvent such as hexane with least percentage recovery.

Tables 3 showed the number of compounds present in each fraction obtained from the solvent-solvent extraction of the acetone extract of the root bark of Grewia mollis. The result showed that under the visible light hexane fraction revealed highest number of compounds while water extract did not show any spot. The EMW was the best mobile phase when sprayed with anisaldehyde/sulphuric acid mixture compared with the other mobile phase. The high number of compound seen in hexane fraction when eluted with BEA was because it is an on-polar solvent mixture.

Table 4 showed the brine shrimp lethality test for the solvent- solvent fractions of the root bark of Grewia mollis, while figure 2 showed the graph for the best trend line fit linear regression analysis for the determination of LC_{50} value of the different fractions in table 4. The chloroform extract had the highest cytotoxicity activity with LC_{50} value of 9.05µg/ml, followed by the carbontetrachloride fraction with LC_{50} value of 31.36µg/ml, and while the hexane extract had the least cytotoxic activity with LC_{50} value of 468.64 µg/ml followed by water fraction with LC_{50} value of 440.72µg/ml.

Table 5 shows the antimicrobial and antioxidant activities with the LC_{50}values of the fractions of solvent-solvent extraction of acetone extract of the root bark of Grewia mollis. The antimicrobial activity result showed that all the fractions showed activity on at least one test organisms. The hexane and carbon tetrachloride fractions inhibited the growth of two organisms while the remaining fractions showed activity on three organisms at varying degrees. The fractions showed larger zones of inhibition on staphylococcus aurous compared to the other organisms. The shigella dysentatiae were more resistant to the fractions compared to the other test organisms. The antimicrobial activity of the extracts may be due to the dimethyl phthalate and Diethyl phthalate (Shobi*et al.*, 2018; Arun*et al.*, 2013) as shown by the GC-MS spectroscopy.

The phthalates compounds have been blamed for environmental pollution but this notion, has progressively changed in light of the mounting evidences that phthalate compounds are found in secondary metabolites of organisms, such as plants, animals and microorganisms (Mohamed *et al.*, 2019). The phthalates compounds from plant source has low toxicity, medical effectiveness, and many natural anti-tumor agents derived from various medicinal plants (Ortiz, and Sansinenea, 2018; Zhang, *et al.*, 2018). Phthalates compounds have been proven to have antibacterial and antifouling characteristics (Ingole, 2016), and a tyrosinase inhibitor that can suppress melanogenesis (Zhang, *et al.*, 2018). The antimicrobial activity can also be attributed to the presence of alkane derivatives (Adeleye, Daniels and Omadime, 2010; Rahbar, Shafagha and Salimi, 2012) or derivatives of Methyl-benzene (Preeti*et al.,* 2022).

The toxicity of herbal extracts is usually expressed as $LC_{50}$and was based on comparison to Meyer's or Clarkson's toxicity indices. According to Meyer's *et al.*, (1982), toxicity index, extracts with LC_{50}< 1000 µg/ml are considered as toxic, while extracts with LC_{50} > 1000 µg/ml are considered as non-toxic (Meyer *et al.*, 1982). Clarkson's toxicity criterion for the toxicity assessment of plant extracts classifies extracts in the following order: extracts with LC_{50} above 1000 µg/ml are non-toxic, LC_{50} of 500 - 1000 µg/ml are low toxic, extracts with LC_{50} of 100 - 500 µg/ml are medium toxic, while extracts with LC_{50} of 0 - 100 µg/ml are highly toxic (Clarkson *et al.*, 2004). The cytotoxic effect of the fraction determined from the LC_{50} value showed three of the fractions were highly active based on Clarkson et al (2004) toxicity index, with their LC_{50} values less than 100µg/ml. The highly active fractions were chloroform with the LC_{50} value of 9.05, carbon tetrachloride with LC_{50} value of 31.36µg/ml and butanol fraction with the LC_{50} value of 65.97µg/ml. The remaining three fractions were moderately active since their LC_{50} values falls between 100-500 µg/ml ranges according to Clarkson et al (2004). The fraction are 35% water in methanol LC_{50} value of 369.14 µg/ml, water fractions with LC_{50} value of 440.72 µg/ml and the hexane fraction with the LC_{50}

value of 468.64 µg/ml. The cytotoxicity effect may be attributed to the presence of compounds such as Lup-20(29)-en-3-ol, Lup-20(29)-en-3-ol acetate, Lup-20(29)-en-3,28 - diol (Basri *et al*., 2014), phthalate compounds (Ortiz, and Sansinenea, 2018; Zhang, *et al*., 2018) which were detected in the acetone extract by the GC-MS method.

The result for the antioxidant activity of fractions showed that the carbontetrachloride and the chloroform fractions of the acetone extract had moderate antioxidant activity whereas the n-butanol and 35% methanol in water showed weak antioxidant activity, while the remaining fraction did not show any antioxidant activity. The antioxidant activity may be due to the presence of compounds that possess hydroxyl group in their molecules that will scavenge for free oxygen to remove them. The presence of compounds such as Lup-20(29)-en-3,28 –diol, Lup-20(29)-en-3 – ol, Fagarasterol and Pentadecianoic acid-2,6,10,14-tetramethyl, methyl ester (Hanan,2012; Aparna *et al.,* 2012) may be responsible for the antioxidant activity due to the presence of the hydroxyl group in their molecules.

CONCLUSION

From the present research it can be concluded that the acetone extract of the root bark of *Grewia mollis* contain different classes of organic compounds. The best way to separate these components is by the use of solvent extraction followed by column and preparative TLC chromatography using BEA and EMW as the solvent system for the elution. The chloroform fractions were the most active fraction from the solvent extraction because it had the best antimicrobial, cytotoxic and antioxidant activities, followed by the carbon tetrachloride fraction. The recommendation is that the chloroform and the carbontetrachloride fractions should be subjected to further separation method such as column and preparative chromatography so as to isolate the active compounds.

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