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CHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF *Prosopisfarcta (Fabacea) FRUIT* FROM IRAQ-KURDISTAN REGION

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ABSTRACT

The chemical constituents of di chloro methane and hexane extracts of *Prosopis Farcta* (Fabacea family)were studied by gas chromatography, mass spectrometry. Their relative concentrations were determined and both extracts contained 8 expected compounds. There were 7 compounds found in hexane extract which are: 1- methyl pyrrole (8.08%), alpha-ethyl aspartate (9.96%), spiropentane propyl (13.75%), ethyl oleate (43.43%), 1-iodo-n-Nonyliodide (7.6%), Borane-diethyl (decyloxy) (9.34%), Sulfurous acid, 2-ethyl hexyl ester (7.84%). While one compound was found in dichloromethane extract namely: 3-O-methyl-d-glucose (100%) was recorded as the principle constituents. The antibacterial activities of dichloromethane and hexane extracts were measured against three pathogenic bacteria, which are *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus Pneumonia* using diffusion method. Significant dissimilarities in the antibacterial activity of plant extracts were noticed depending on the type of bacterial strain and the concentration of plant extracts.

KEYWORDS: Prosopisfarcta, , extracts, Chemical constituents, antibacterial activities

INTRODUCTION

Production of chemical medication in recent decades has been greatly influenced by utilization of herbal medicines due to the presence of active chemical substances in plants possessing astrong safety profile.Nawadays,it has been reported that synthetic additives serve as antibacterial agents in food industries has anoticeable contribution in causing potential problems leads to various disease conditions,Therefore,to decrease the risk of free radical production from chemical substancesentering into the human body,exploting the plant extracts has attracted agreat attention as sources of anti bacterial and oxidant agents (Skrovankova et al.2012).In folk medicine,the fruit is used as adiuretic,and against constipation, hemorrhoids, tooth pain, diabetes, kidney stones, skin conditions, and more(LPWG,2017,14October2015.UsDA**).**

Prosopis farcta fruit is a valuable commodity of considerably commercial importance particularly for Kurdistan. This plant is multi-branched up to 2m high. The stems are old and grayish to whitish in color. Leaves contain 2-pinnate with 3-6 pinnae pairs. Leaflets areoblong-elliptical in 8-14 pairs. Flowers are small, cream-colored in spikes up to 10 cm long. Pods ovoid or irregularly swollen up to 5 cm long dark brown when ripe (Alsherif, 2007).*Prosopis fracta* fruit is shown in (Figure 1).

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Fig. (1). *Prosopisfracta (Fabacea)*

Prosopis farcta(kharnek) or vernacular name "Yanbut" is a genus of flowering plants in the pea family Fabacea. It contains many species of spring trees and shrubs found in subtropical and tropical regions of Americas, Africa, Western Asia, and south Asia(Herbarium of Harvard University1919).

They often thrive in dry soil and are unaffected to drought, on occasion developing extremely deep root systems. Their wood is usually rigid, and durable. Their fruits are pods and may contain large amount of sugar. The common name means "burdock" in Latin and it is originated in Greek language (Herbarium of Harvard University1919 ,UsDA).

Prosopis species have been found to contain 5-hydroxy tryptamine, apigenin, isorhoamnetin-3-diglucoside, 1-arabinose, quercetin, tannin and tryptamine. *Prosopis* species are known to contain alkaloids, as shown in (**Table 1**) (www.hort.purdue.edu.Retrieved 2008)

Table(1). The Alkaloids constituents of *prosopis* species (Graziano ,*etal*.1971,Tapia,*etal*.2000,Luis Astudillo,*etal*.2000,www.hort.purdue.edu.Retrieved(2008),Dukes(2010),Constantino Manuel Torres and David B.Repke.2006).

***Prosopis*species type**	**The Alkaloids constituents**
Prosopisalbo	Beta-phenethylamine and tryptamine
Prsopisalpataco	"Aerial parts" contain tryptamine-phenethylamine derivatives
Prosopisargentina	"Aerial parts" contain tryptamine-phenethylamine derivatives
Prosopischilensis (vertfication needed)	"Aerialparts" contain Beta-phenylethylamine and derivatives plus tryptamine
Prosopisargentina	Exudate contains tryptamine-phenethylamine derivative
Prosopisglandulose	Alkaloids in bark and roots, tyrosine and N-methyl tyramine(astimulate) in leaves.
prosopisjulifloro	5-HTP(plant and trypamine (plant)
Prosopisnigra	Harman, eleagnine and N-acetyl tryptamine
Prosopispugionate	"Aerial parts" contain tryptamine-phenethylamine derivatives

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The tannins present in *Prosopis* species are of phytogallotannis and pyrocatecollic types. Tannis of tropical woods tend to be of acathetic nature rather than of the gallictype present in temperate woods. The tannins areprimarily found in the bark and wood while their concentration in the pods is low (Pasiecznik,2001).The aim of this study is to investigate the chemical constituents of *Prosopis farcta* using solvent extraction in several polar and non-polar solvents. The GC-Mass was used to identify the extracted chemical constituents. The biological activity of the two extracts was investigated against three pathogenic bacteria.

MATERIAL AND METHODS

Plant material

The fruit of *Prosopis farcta* were collected in spring 2012 from Duhok city in Kurdistan region area, Iraq and identified by Dr. SalimShahbaz from faculty of Agriculture.

Chemicals

All chemicals used were of analytical grade; dichloromethane (DCM), n-Hexane, ethyl acetate and ethanol were purchased from (UNI-chem. China).

Experimental Section

The extraction was performed as described elsewhere (Hong-Xiali,*et al*. 2002). The fruits were cleaned, dried and ground to powder by a food processer. 1.115Kg of powder was weighed and transferred to Stoppard flask, and treated with ethanol until the powder is fully immersed. The flask was shaken from time to time for one week. The plant was filtred; the filtrate was evaporated by a vacuum rotary evaporator. Then 11g of the extracts was dissolved in 10% ethanol and partitioned individually with hexane. dichloromethane and finally with ethyl acetate to get three extracts; (4.4g) hexane extract, (6.5g) DCM extract and the residue of ethanol extract was (0.5g), while ethyl acetate gave no extract.

Hexane extract and dichloromethane extract were injected to (GC-Ms) as shown in (Scheme 1) . No compounds were detected in ethyl acetate and ethanol while hexane and DCM gave a considerable amount of compounds that were detected by GC-MS.

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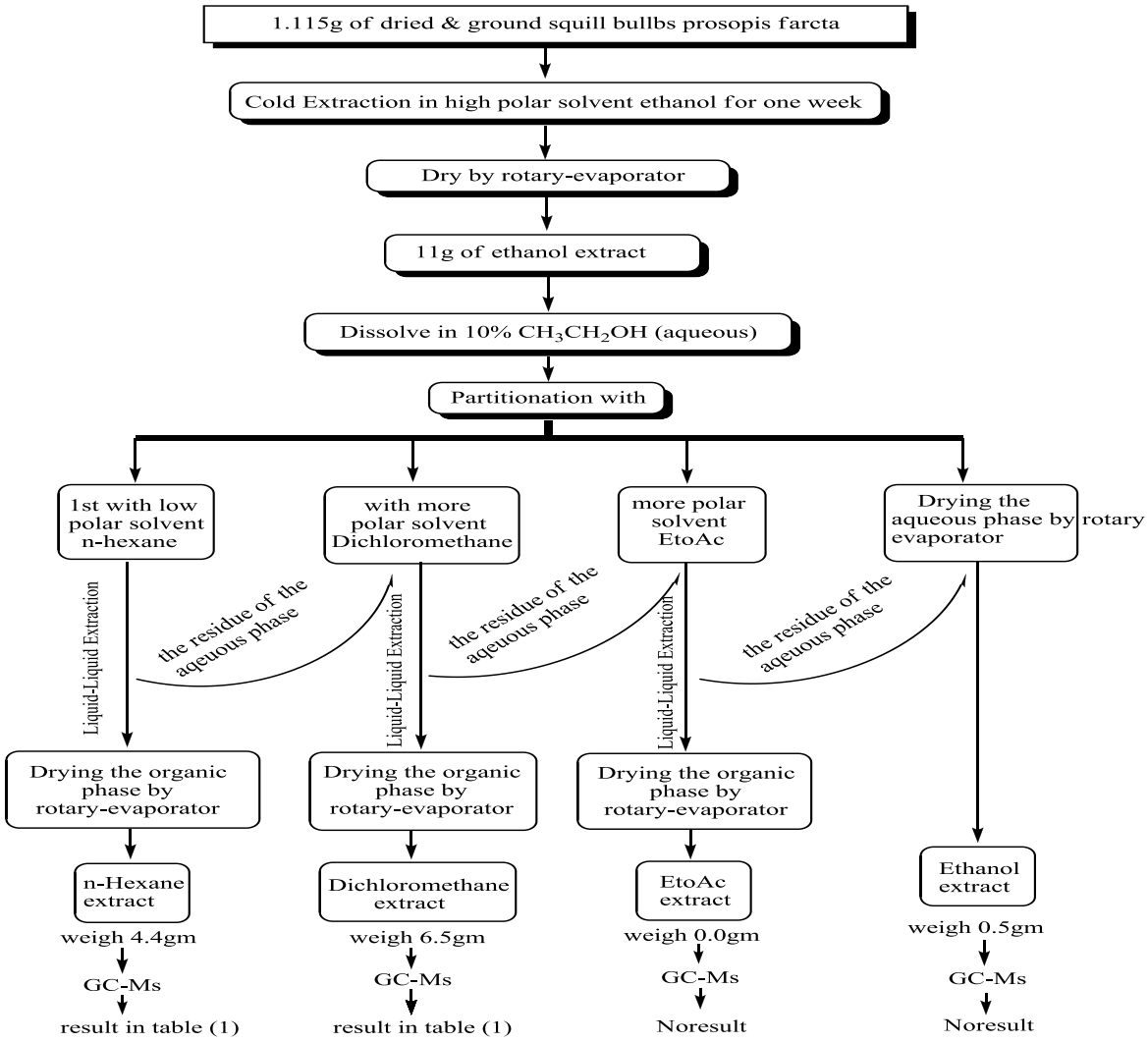

Scheme (1) : General extraction methodes of fruit of *Prosopis Farcta*

Instrumentation

GC-Ms technique was used, in chemistry department, Faculty of Science, University of Malaya UM, Kuala Lumpur, Malaysia, to identify the expected components of the plant extracts, using the thermo scientific GC-Ms [SHIMADZUQP2010] gas chromatography with software: GCMs solution ver.2.53.

The gas chromatography was interfaced to a Mass spectrometer equipped with Elite-1 fused silica capillary column of length: 30.0 m, diameter: 0.25 mm, film thickness 0.25 µm and composed of 100% dimethyl polysiloxane. The column oven temperature was maintained at 40^{o}C and injector temperature at 240^{o}C. The oven temperature was programmed as follows: 40^{o}C for 2 minutes raised to 280^{o}C for 7 minutes at the rate of 5^{o}C/min. Helium of the 99.9995% purity was used as the carrier gas at 1.115 ml/min. the sample (1µL) was injected in the split mode of 10:1. The total GC running time was 32 min with ion source and interface temperatures maintained at 200^{o}C and 240^{o}C respectively. The mass spectra were taken with scan range of 40-1000 m/z of 0.5 seconds intervals at 70 eV ionization. The relative percentage area of each component was calculated by comparing it with the total area.

Antibacterial activity

Antibacterial activity of all of the prepared (hexane and di chioro methane extract) were tested against three clinical isolates of Gram positive and Gram negative pathogenic bacteria which are *Staphylococcus aureus, Escherichia coli* and*Streptococcus Pneumonia*according to

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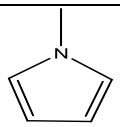
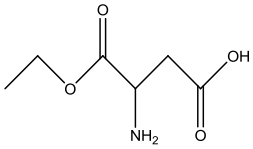
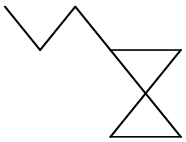
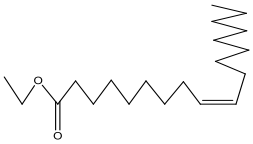
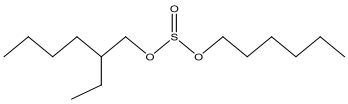
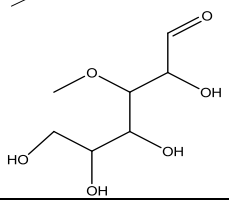
NCCLS(Swenson,*etal*.2003)with slight modifications.

Mullaer-Hinton agar was prepared and poured into each sterilized petri-dish to a depth of (5mm). The elute were inoculated with 0.1 ml of bacterial suspension (with optical density 0.1 at wave length 450 mm using UV Spectrophotometer HehIOS) by sterile glass spreader. Equidistant holes were made in the agar using sterile cork borers ($\emptyset = 7mm$). Four different concentrations were prepared for ethanol and hexane plant extract (10, 15, 20, and 25) mg/ml. 100 micro liters of each plant extract concentration were applied to the holes using micro pipette. Distilled water was used as control. After 24-48 hour of incubation period, inhibition zones were measured in (mm) as diameters of clear zones around the wells. All tests were duplicated.

RESULTS AND DISCUSSION

Eight compounds were identified in the hexane and dichloromethane extracts of *Prosopis farcta*. The reported peaks of the total ion current chromatography obtained with details of compound number, retention time, peak area, structures of expected compounds, Names of the identified components, their molecular formula and molecular weight are shown in (Table 2).

Table (2). Expected identified compounds in the hexane and dichloromethane extract of *prosopis farcta*

Comp. No.	Rt.	Molecular Formula	M.wt	Peak Area %	Structures of expected compound	Name
1	12.182	C_5H_7N	81	8.08		1-methyl pyrrole
2	33.567	$C_6H_{11}NO_4$	161	9.96		Alpha-ethyl aspartate
3	36.656	C_8H_{14}	110	13.75		Spiropentane-propyl
4	36.745	$C_{20}H_{38}O_2$	310	43.43		ethyloleate
5	45.088	$C_9H_{19}I$	254	7.60		1-Iodo-n-nonyl iodide
6	47.841	$C_{14}H_{31}BO$	266	9.34		Borane-diethyl (decyloxy)
7	51.498	$C_{14}H_{30}O_2S$	278	7.84		Sulfurous acid, 2-ethyl hexyl hexyl ether
8	27.752	$C_7H_{14}O_6$	194	100.0		3-O-methyl-d-glucose

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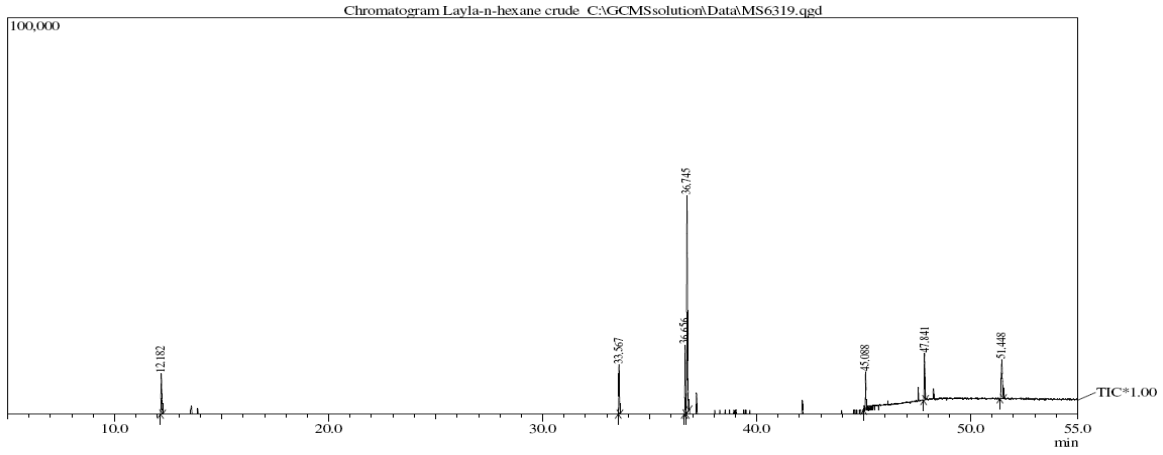
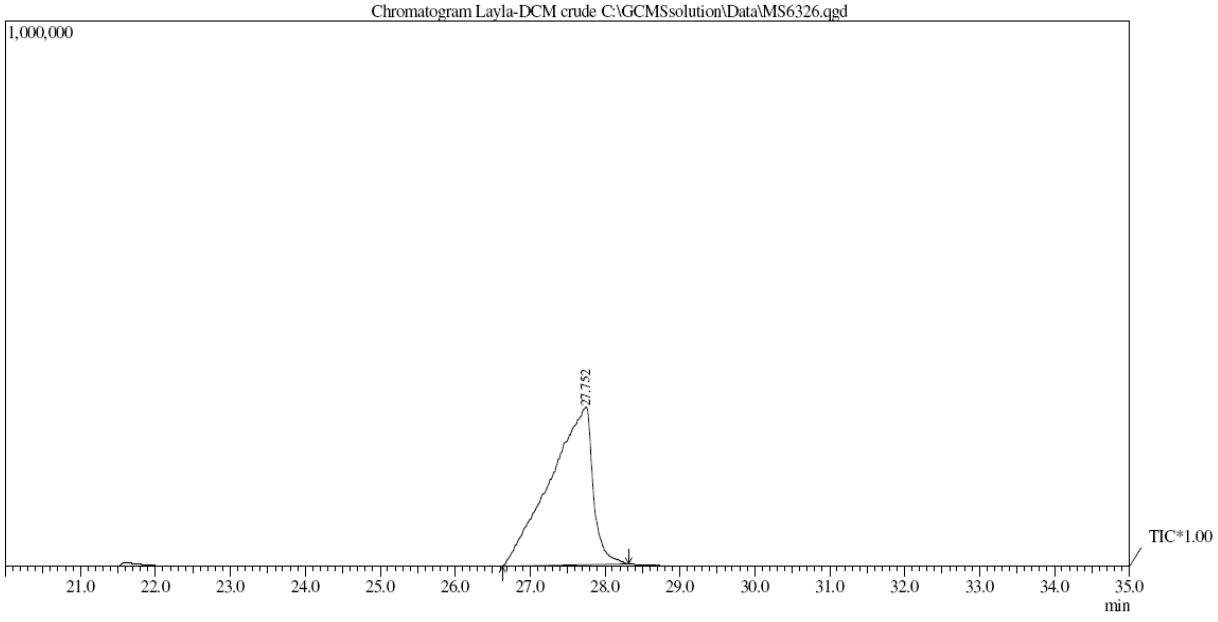
The GC-Ms chromatograms of hexane and dichloromethane extracts are shown in **(Figure 2**) and **(Figure 3**). Seven expected compounds were identified in the hexane extract as shown in **(Table 2)**: 1- methyl pyrrole (8.08%), alpha-ethyl aspartate (9.96%), spiropentane propyl (13.75%), Ethyl oleate (43.43%)(Hong-Xiali,*etal*.2002), 1-iodo-n-Nonyliodide (7.69%) (Tony,*etal*.2007), 2-ethyl hexyl ester (7.84%). The major chemical constituents in the hexane extract at Rt 36.745 fragment peaks at m/z 310(M), 264, 222, 180, 152, 137, 123, 55.



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	12.182	12.142	12.242	26034	8.12	10194	8.08	2.55		
2	33.567	33.533	33.608	24703	7.70	12564	9.96	1.97		
3	36.656	36.617	36.692	33302	10.38	17344	13.75	1.92		
4	36.745	36.700	36.833	142143	44.31	54794	43.43	2.59		
5	45.088	45.000	45.150	24937	7.77	9586	7.60	2.60		
6	47.841	47.800	47.892	28805	8.98	11784	9.34	2.44		
7	51.448	51.358	51.533	40846	12.73	9888	7.84	4.13		
				320770	100.00	126154	100.00			

Figure 2: GC-Ms chromatogram of the hexane extract of prosopis farcta



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	27.752	26.633	28.317	11495347	100.00	289295	100.00	39.74		
				11495347	100.00	289295	100.00			

Figure 3: GC-Ms chromatogram of the Dichloromethane Extract of prosopis farcta

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Fig. (4): shows the mass spectrum of ethyl oleate which harmonized with the pattern of mass spectrum(Dukes2010) . The main fragment ions were formed at m/z 264(M-C_2H_5OH), 222 (M-$C_4H_8O_2$), 180(M-$C_7H_{14}O_2$) and the base peak is 55(M-C_4H_7). At Rt 45.088 the second major compound fragment peaks at m/z 282(M), 183, 127, 85, 57.

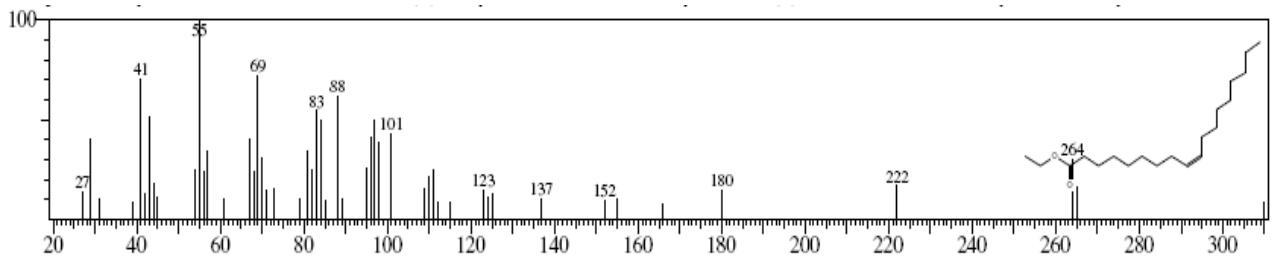

Figure 4: Mass spectrum of ethyl oleate

In the mass spectrum of 1-iodon-Nonyl iodide **(Figure 5)** there is a main fragment ions which formed as m/z 183(M-C_7H_{15}), 127(M-$C_{11}H_{23}$), 85(M-$C_{14}H_{29}$), and the base peak is 57(M-$C_{16}H_{33}$) which concur with the pattern of mass spectrum indicated by(Tony,*etal*.2007).

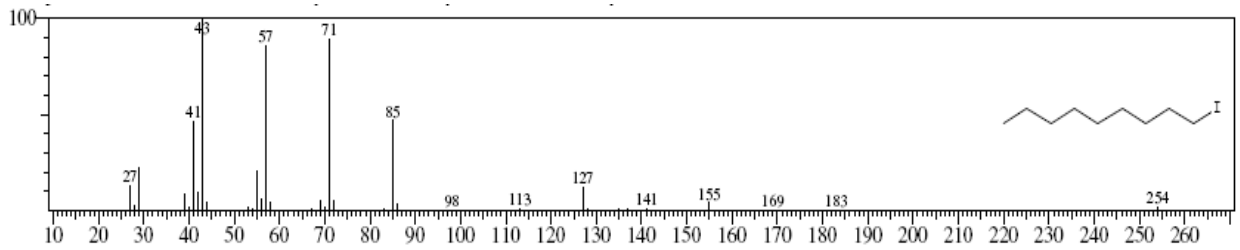

Figure 5: Mass spectrum of 1-iodo-n-Nonyl iodide

The only compound found in dichloromethane is 3-O-methyl-d-glucose (Pilli,*etal*.2010)**(Table 2)**. The major chemical constituent at Rt (27.752) in the dichloromethane extract gave fragment peaks which shown in **(Figure 6)** the molecular ion appeared as m/z 99(M-C_4H_9), 86(M-C_5H_{10}), and the base peak is 57(M-C_7H_{14}). The fragment peaks coincides with the pattern of mass spectrum indicated by(Pasiecznik,2001).

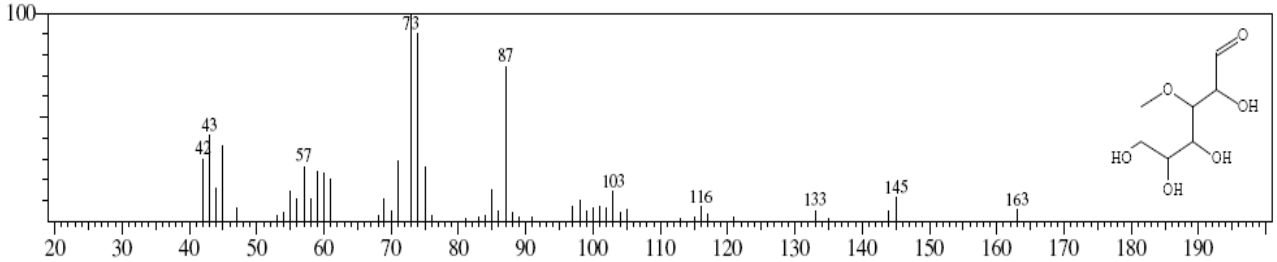

Figure 6: Mass spectrum of 3-O-methyl-d-glucose

The results of the antibacterial activity of hexane and dichloromethane extracts are shown in **(Table 3)**. The obtained results from this study show considerable differences in the antibacterial activities among extracts depending on the bacterial strain and the concentration of extracts used. The obtained result revealed that all plant extracts had a pronounced effect against the growth of Gram positive and Gram negative pathogenic ones. This could be due the fact that

pathogenic bacteria undergo many mutations and consequently become more resistance(Turkoglu,*etal*.2007). The plant extract shown more potent activity against Gram positive than negative bacteria. This may due to the presence of the phospholipid membrane carrying the structural lipo-polysaccharide in Gram negative bacteria. The Gram positive bacteria should be more susceptible having only one outer peptidoglycan layer which is not an effective permeability barrier (Nostro,*et a*l.2000).

Table (3): Antibacterial activity (inhibition zone/mm) of n-hexane and Dichloromethane extracts against Gram positive and Gram negative pathogenic bacteria.

Extract	mg/ml	Test organism		
		S. aureus	***E. Coli***	***S. Peneumonia***
Hexane	10	13mm	10mm	12mm
	20	12mm	9mm	10mm
	30	10mm	8mm	9mm
	40	8mm	7mm	8mm
Dichloromethane	10	14mm	9mm	15mm
	20	15mm	13mm	14mm
	30	13mm	8mm	13mm
	40	12mm	8mm	10mm

CONCLUSION

The examination of the phytochemicals in the Hexane and dichloromethane extracts of *Prosopisfarcta* revealed the presence of **8** compounds namely: 1- methyl pyrrole (8.08%), alpha-ethyl aspartate (9.96%), spiropentane propyl (13.75%), ethyl oleate (43.43%), 1-iodo-n-Nonyliodide (7.69%), Borane-diethyl (decyloxy) (9.34%), Sulfurous acid, 2-ethyl hexyl ester (7.84%) and 3-O-methyl-d-glucose (100 %). The plant extracts have shown a potent activity against three isolates of Gram positive and Gram negativebacteria.

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