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Isolation and Identification of Alkaloidic Extract of *Capparis spinosa*L Buds and Study of Its Cytoxicity and Antibacterial Activity.

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Abstract
This study was carried out for isolation and identification of alkaloids from *Capparis spinosa* L. buds, by using several solvents, qualitative reagents and gas chromatography-mass spectrum (GC – mass) technique. The isolated alkaloidic extract was found to contain many active chemical compounds. Antibacterial activity of alkaloidic extract was investigated against some pathogenic bacteria which represented by *Escherichia coli* and *Staphylococcus aureus*.
The alkaloidic extract concentrations (125 , 250 and 300 mg/ml) recorded inhibition zone diameters equal to (16 , 19 and 19 mm) against *E. coli* bacteria and (16 , 20 and 20 mm) against *S. aureus* bacteria. The concentration of (250 mg/ml) was found to be the maximal inhibitory concentration against both pathogenic bacteria. It is recommended to use alkaloids of *Capparis spinosa* to treat different diseases caused by these bacteria instead of antibiotics but this demands more clinical and pharmaceutical studies.
Keywords: *Capparis spinosa* buds, Alkaloids Extract, Pathogenic Bacteria, GC-mass technique, Maximal inhibitory concentration.

1.Introduction

Herbal drugs play a major role in the treatment of many common diseases. *Capparis spinosa* L. (Cappariaceae) is a common perennate shrub and a favored plant for restoring vegetation on dry regions in west or central Asia. It is particularly widely grown in the Mediterranean basin (Kusmenoglu *et al*,1997; Baytop,1984). *Capparis spinosa* is a much branched, thorny, woody climber growing to a height of (3-4) m. It is distributed in dry parts of India. In Ayurveda, *C.spinosa*is used a stomachic, tonic, appetizer, removes Kapho and vata, cures fever, tumors, used in inflammation, wound healing and diseases of muscles, also acts as blood purifier. The ground root is used as a cure for snake bite; the plant is also useful in skin diseases(Chopra *et al*, 1986; Chiej,1984; Huseini *et al*, 2005).
Capparis spinosa is highly variable in nature in its native habitats and is found growing near the closely related species such as *Capparis sicula*, *Capparis orientalis* and *Capparis aegyptia*.
Scientists can use the known distributions of each species to identify the origin of commercially prepared capers (Fici, 2001; Fragiska, 2005). The buds and fruits of this medicinal plant are used as folk medicine.
The plant was reported to have diuretic, tonic, expectorant, anti-tumor, anti- P. H. inflammatory, hypotensive and spasmolytic effects and was used for rheumatism, gout, paralysis, tuberculosis complaints. The decoction of *C.spinosa* fruits, is used in the treatment of cough and diabetes, and the ground fruits are used against rheumatism (Senay *et al*, 1997; Davis, 1965). In China, *C.spinosa* L. is mainly distributed in the Xingiang Autonomy Region. Its fruits have been used to treat different diseases. The bioactivity prompted us to continue investigation its active fractions and chemical components(Xiaolu *et al*, 2010). Medicinal plants have many active chemical compounds resulting from secondary metabolism. These chemical compounds are abundant as natural families which are represented by essential oils, phenols, glycosides, alkaloids, steroids, saponins and terpenes (Mohammed, and Al-Maliki, 2014; Nizar, 2010).
Alkaloids are heterocyclic nitrogenous compounds are widely present in plant kingdom and they have high dramatically effect to treat various diseases, and also these compounds have antibacterial, antifungal, anti-parasitic anti- cancer and anti-tumor actions. Typical alkaloids are derived from plant source, they are basic compounds contain one or more nitrogen atoms (usually in heterocyclic ring). Also alkaloid molecule must contain nitrogen connected to at least two carbon atoms and have at least on ring (Shenta, and Al-maliki, 2013; Al-maliki, 2011).
The current study was focused on investigation the antibacterial activity and cytoxicity of alkaloids fraction extracted from *Capparis spinosa* buds against two pathogenic bacteria.

2. Material and Methods

2.1 **Plant Collection:** *Capparis spinosa* L. buds were collected from Abu Al-khaseeb region in Basrah governorate, Southern of Iraq, cleaned with cold distilled water, dried in the shadow at room temperature, ground, powdered and kept in dark plastic containers until of use. The plant was taxonomies in Biology department, at college of education for pure sciences in Basrah University.

2.2 **Chemicals:** The chemical compounds used in this research were of analytical grade they are ethanol, acetic acid, α -napthol, sulphuric acid, ferric chloride, bismuth sub-nitrate, potassium hydroxide, ninhydrine, ammonium hydroxide, chloroform, mercuric chloride, potassium iodide, sodium citrate, sodium carbonate, copper sulphate and benzene.

2.3 **Pathogenic Bacteria:** Pathogenic standard bacteria strains were isolated and identified which are represented by *Escherichia coli* (negative towards Gram stain) and *Staphylococcus aurous* (Positive towards Gram's stain).

2.4 **Culture Medium:** Mackoakey agar manitol salt sugar medium was prepared according to information determining by manufacturing company and it was supplied from biology dept., Education College for pure sciences at university of Basrah.

2.5 **Isolation of Alkaloids from *Capparis spinosa* L. buds**

Twenty five grams of *Capparis spinosa* L. buds powder, were dissolved with 500 ml of (10% V/V) ethonolic acetic acid, the mixture was stirred on magnetic stirrer for 24 hr. After that, the mixture was filtered by Buchner funnel, the precipitate was removed and the filterate was concentrated to quarter of its volume by using rotary evaporator and acidified with 5 ml of concentrated sulphuric acid. The acidic fraction was basified by using ammonium hydroxide to pH equal to 9. Then the extraction process was carried out by using separation funnel by adding (3 * 20 ml) of chloroform. After that, alkaloids were extracted and dried(Harborne, 1984).with yield equal to 6.23gm.

2.6 **Preliminary Qualitative Analysis**

The extracted alkaloids were undergone several tests as the following :

1- Dragendroff Test :-

One ml of alkaloids extract was treated with 2 ml of Dragendroff reagent and the mixture was mixed and shaken gently(Harborne, and Bxter, 1993).

2- Marquis Test: -

One ml of alkaloids extract was mixed with (1.5 ml) of Marquis reagent, then the solution was shaken gently(Harborne, 1984).

2.7 **Functional Groups Detections** (Bruneton, 1995).

To detect on structural and functional groups, the isolated alkaloids were undergone the following tests:

1- Double bond test: was achieved by using potassium permanganate reagent.

2- Alkaloidic groups test: was carried out by using Dragendroff reagent.

3- Aldehyde and ketone groups test: was applied for by using 2, 4 di-nitrophenyl hydrazine (DNPH) reagent.

2.8 **Gas Chromatography – Mass Spectroscopy**

The alkaloids isolated from *Capparis spinosa* L. buds were separated and identified by using gas chromatography-mass spectrum (GC – Mass) technique by using GC-Mass instrument type (Agilent Technoloies GC 7890A GC system)in university of Tarbiat Modares at Tehran in Islamic Iran Republic. The alkaloidic sample was injected by concentration of in gas chromatography instrument with standard optimizations. Different peaks were separated and recorded at different retention times. Then separated chemical compounds were identified by using mass spectrum.

2.9 **Estimation of Antibacterial activity and minimal inhibotony concentration**

Several concentrations of alkaloids isolated from *Capparis spinosa* L. buds, are (125, 250 and 300 mg/ml), mg/ml were treated against growth of pathogenic bacteria stains which are represented by *Escherichia coli* and *Staphylococcus aureus* for assay of inhibition zone diameters and maximal inhibitory concentration by using MacCkonkey agar and Manitol Salt agar culture medium depending on diffusion method in petri dishes. After that the concentrations used against the bacteria, were put in the incubator for 24hr, then antibacterial activity was calculated(Cowan,1997; Collee, Fraser, and Bimon, 1996).

2.10 **Estimation of Cytoxicity of Alkaloidic Extract**

Cytoxicity of alkaloids extract isolated from *Capparis spinosa* L. buds, was estimated. Series concentrations of alkaloidic extract, were prepared, where 2000 mg was dissolved in 10 ml of Ringer's solution, then it was diluted with the ratios (1:1,1:10, 1:100 and 1:1000 V/V). Negative control factor was used, contains Ringer's solution as normal saline and positive control factor was used represented by tap water. After that 0.8 ml of each concentration, was put in sterilized test tube is Eppendrof tube contains an anti-clotting substance, then to each tube, 0.2 ml of blood was added and the total volume in each tube became 1 ml. the tubes were incubated in the incubator at 37 ℃ for 30 min., and centrifuged for 5 min. with speed equal 3000 rpm. Finally all tubes were tested to observe the hemolysis (Xian-guo, and Ursula, 2007).

3. Results and Discussion

The importance of various medicinal plants, results from their biochemical activities in treat different diseases since these plants contain many active chemical compounds and which produce biochemically from secondary metabolism. It is known that these chemical compounds are called naturally secondary metabolites which are biosynthesized by different metabolic pathways in presence of several enzymes in medicinal plants. The biochemical synthesis resulting from these enzymes, lead to produce the active chemical compounds which are represented by alkaloids, phenols, glycosides, saponins, terpenes, steroids and essential oils (Chauhan *et al,* 2010; Mohammed, and Al-maliki,2014). Alkaloids are present naturally in medicinal plants as natural products. From these plants, *Capparis spinosa* L. which is considered as an important plant which has many active metabolites produced naturally from secondary metabolism pathway. Alkaloidic compounds have many dramatic physiological effect therefore they are capable of having biochemical activities to kill pathogenic micro-organisms such as bacteria and fungi.

In this study alkaloids fraction was extracted and isolated with extraction percentage equal to 24.92%. This percentage is considered somewhat very good and this means presence of alkaloids in a high quantity and qualitatively in different types.

Table (1) shows qualitative preliminary analysis of alkaloids isolated from *Capparis spinosa* L. buds which ensure presence of alkaloids as basic nitrogenous compounds. It was noticed by using Dragendroff and Marquis reagents, this led to form very good orange precipitate and pink maroon color respectively therefore these positive qualitative results indicate presence of alkaloidic compounds in *Capparis spinosa* L. buds. Different previous studies showed abundance of alkaloids as active chemical compounds, in leaves, seeds, stem and fruits of *Capparis spinosa* L. Plant(Nizar *et al*, 2010; Satyanarayana *et al*, 2009; Bouriche, 2011). Also, the roots of *Capparis spinosa* (caper) was used as ethanolic extract to investigate the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in CCl4-intoxicated mice(Aghdel, 2007).

The biochemical advantage of abundance of alkaloids in medicinal plants including *Capparis spinosa* L. is elimination of toxic materials from plant, storage of some essential elements such as nitrogen, regulators of growth and protection of plant from attack of fungi and insects (Borner, and Varner, 1965).

The detection of functional structural groups of alkaloidic extract isolated from *Capparis spinosa* L. buds, showed existence of double bond system (C=C), because of disappearance of potassium permanganate color. Also presence of alkaloidic nitrogenous groups represented by (C=N) bond since orange precipitate was formed. Formation of yellow – orange precipitate assures existence of aldehyde and ketone groups as in table (2).

Table (1):Preliminary qualitative analysis of alkaloids isolated from *Capparis spinosa* L. buds.

Reagent	Test result	Notes	Conclusion
Dragendroff	+	Formation of orange precipitate	Presence of alkaloids
Marquis	+	Appearance of pink-maroon color	Presence of alkaloids
$FeCl_3$ (1%)	-	No bluish-green- color	No phenols
Benedict	-	No red precipitate	No glycosides
$HgCl_2$ (5%)	-	No white precipitate	No saponins
Ninhydrin(1%)	-	No violet color	No amino acids

The abundance of structural and functional groups in alkaloids, gives these active chemical compoundsbiochemical activity in treat various diseases caused by pathogenic fungi, parasites and bacteria(Khan,2010; Shenta, and Al-maliki, 2013).

Table (2): Functional and structural groups detection results of alkaloids isolated from *Capparis spinosa* L. buds.

Reagent	Detection result	Indications	Conclusions
$KMno_4$	+	Disappearof potassium permangane color	Presence of double bond system
Dragendroff	+	Formation of orange precipitate	Presence of alkaloidic nitrogenous groups
DNPH	+	Formation of yellow orange precipitate	Presence of aldehyde and ketone groups

The result of gas chromatography (GC) – mass spectrum technique, was used successfully in current study to isolate the alkaloidic compounds. GC analysis showed presence of twelve peaks with their retention times, which indicate separation of twelve alkaloids, so these active compounds were gotten in a high accuracy because of abundance of optimizations for gas chromatography technique, which have led to the excellent separation to all alkaloidic extract compounds, abundant in *Capparis spinosa* L. buds. Then mass spectra depending gas chromatography results, were recorded for all alkaloidic compounds.

The alkaloids separated and recorded are twelve chemical compounds 1-methyl-2-butyl-pyrrolidine, 2-pyrrolidineethanamine, 2,3 dihydroxy-6-methyl-4H-pyrane-one, 5-oxo-pyrorrolidine, 2-(2-hydroxyethyl) piperidine, aziridine, piperidine-4-ol, 3-piperidinol, 2-methyl aziridine , 1-(2-butenyl) pyrrolidine, 2H-1-benzopyran-2-one, 2,4-dimethoxy-5-pyrimidine carboxaldehyde. It is seems that these active alkaloidic compounds act together to give the biochemical activity according to synergistic interaction principle (Bouriche *et al*, 2011). Therefore these results are shown in figures (1 & 2).

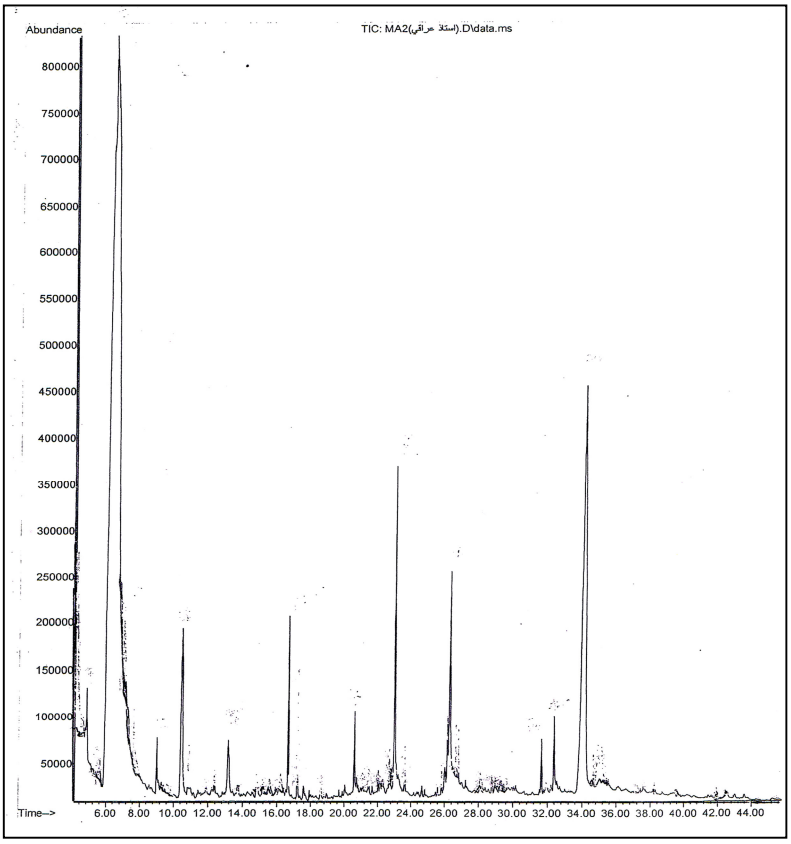
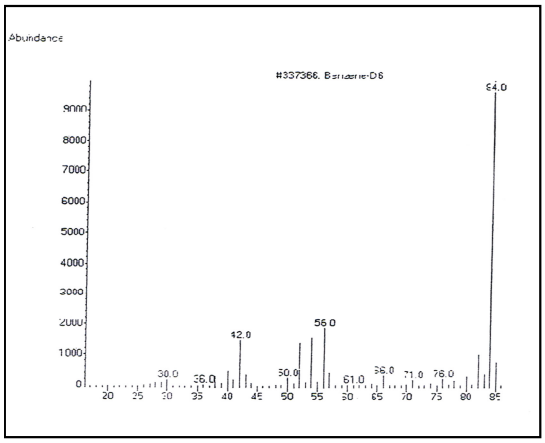
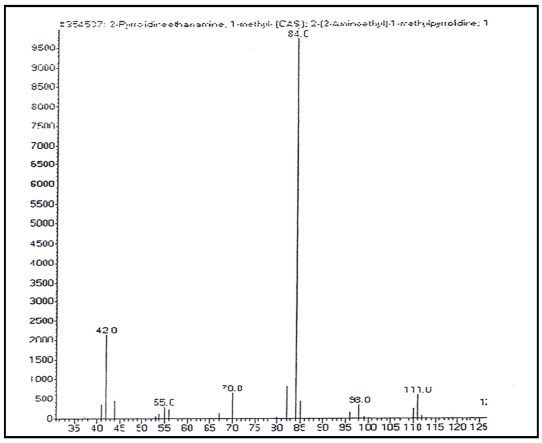


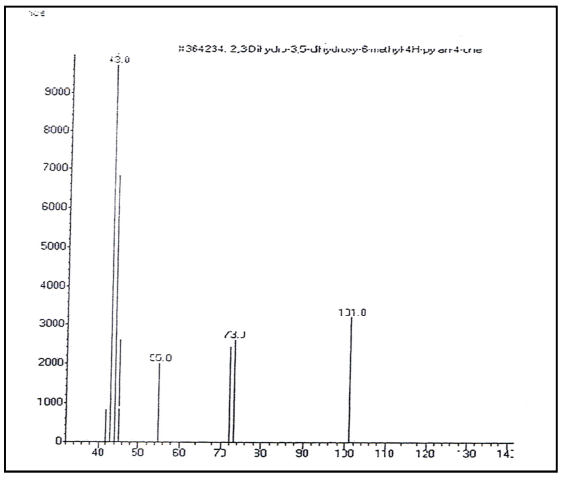
Fig (1): Chromatogram of alkaloidic compounds Separated by gas chromatography GC technique.



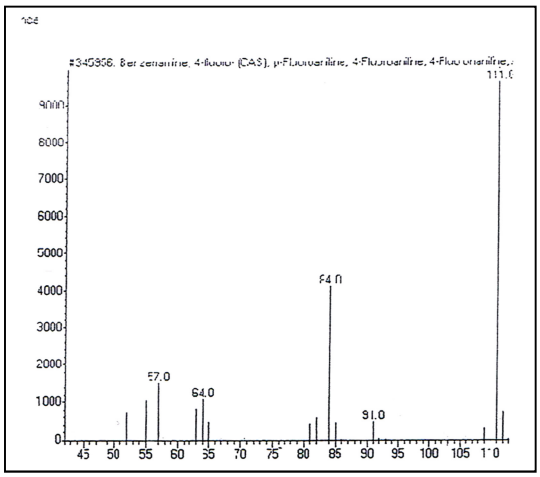
1- Methyl -2- butly – pyrrolidine



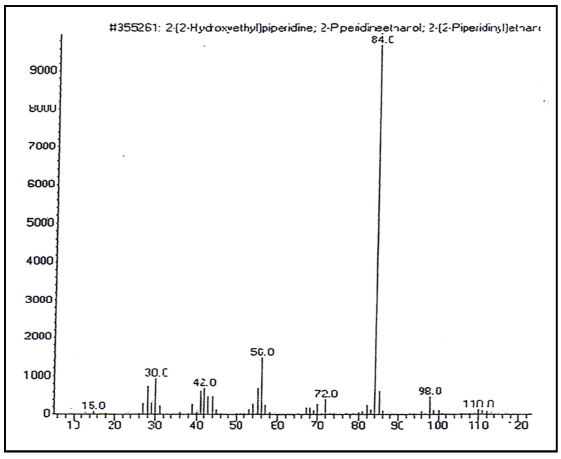
2- Pyrrolidineethanamin



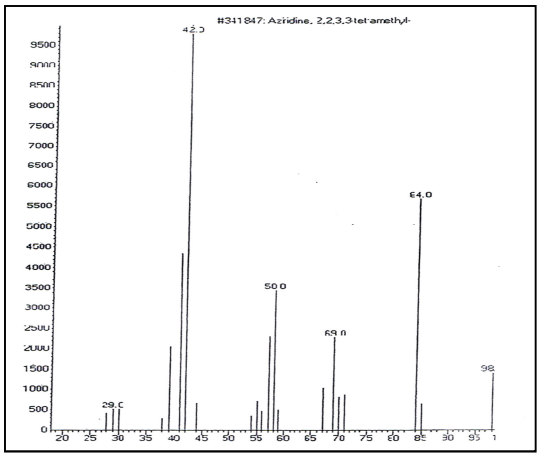
2, 3 – Dihydroxy -6- Methyl – 4H – pyran – one



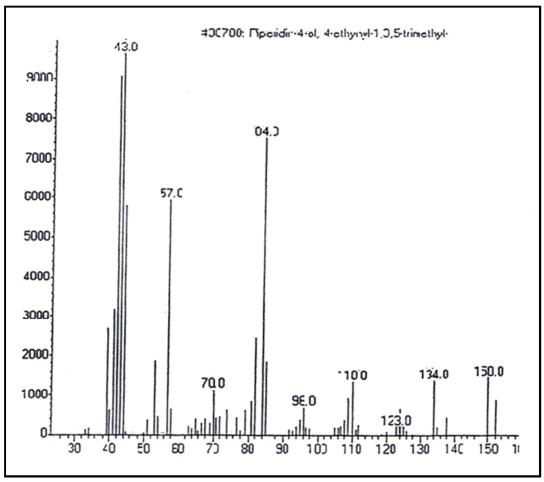
5 – Oxo – pyrrolidine



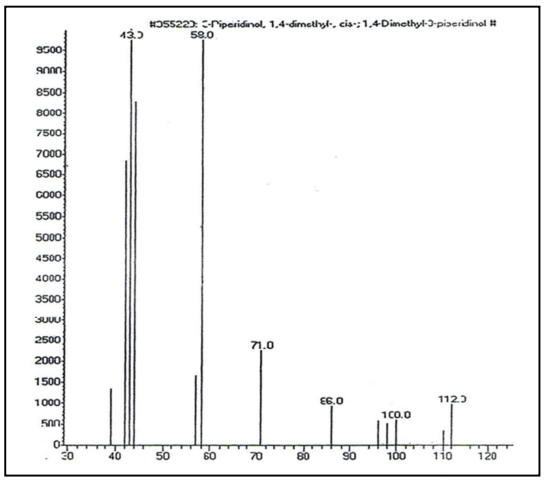
2- (2- hydroxyethyl) piperidine



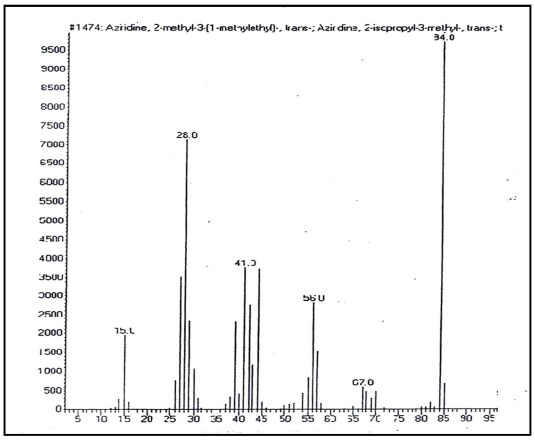
Aziridi



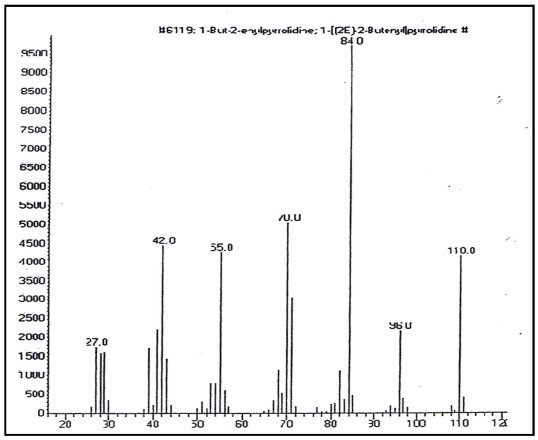
Piperidin -4- 01.



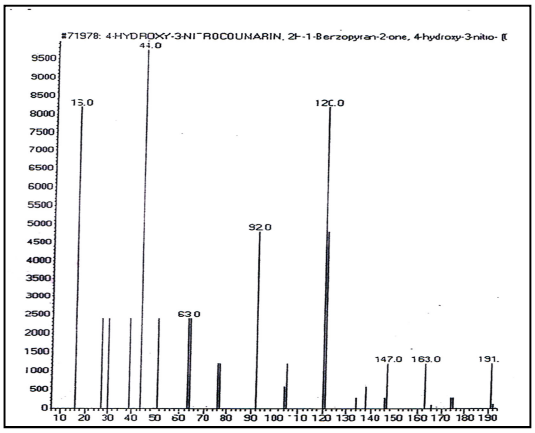
3- Piperidino



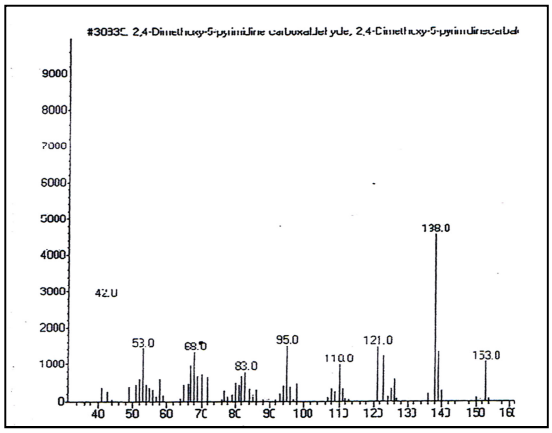
2- Methylaziridine



1- (2-butenyl) pyroli



2H -1- benzopyran -2- one



2, 4-Dimethoxy -5- pyrimidine carboxaldehyde

Fig (2) Mass spectra of alkaloidic compounds isolated from *capparis spinosa* L. buts and Separ

The GC-mass spectrum technique is very fast, characteristic, qualitative, quantitative, separation, purification and identification method, so the knowledge of chemical structures of alkaloids, give a fantastic step to synthesis of these active compounds in laboratory or isolation these alkaloids in high quantities, then use of them as herbal drugs(El-Hawary, *et al*, 2011).

3.1 **Results of antibacterial activity of alkaloids extract**

Three concentrations of alkaloidic extract were carried out to investigate the biochemical activity of these concentrations on pathogenic bacteria. The concentration of 125 mg/ml recorded inhibition zone diameters are 16 mm and 16 mm against *E. coli* and *S. aureus* bacteria respectively, also the concentration of 250mg/ml showed inhibition zone diameters equal to 19 and 20 mm against *E. coli* and *S. aureus* bacteria respectively. But the concentration 300 mg/ml recorded the same antibacterial activity of concentration 250 mg/ml because the inhibitor zone diameters are similar for both concentrations, this ensures that the concentration 250 mg/ml is the maximal inhibitory concentration (MaxIC) as shown in table (3). Some studies indicated that alkaloids activity isolated from medicinal plants because these chemical compounds have physiological effects and dramatic medicinal features for killing the pathogenic microorganisms including bacteria(Saikat *et al*. 2010; Singh *et al* 2008).

Table (3):Inhibition zone diameters resulting from effect of alkaloids extract against pathogenic bacteria.

Conc. of alkaloid extract (mg/ml)	Inhibition zone diameters (mm)	
	E. coli	*S. aureus*
125	16	16
250	19	20
300	19	20

It was noticed that the increase of concentration led to increase of inhibition zone diameter. The biochemical mechanism of effect of alkaloids isolated from medicinal plants including *Capparis spinosa* L., is explained by chemical bonding between these active compounds with nucleic acids (DNA and RNA), then inhibition of metabolism of these acids. Also the functional chemical group in alkaloids, is amine which is represented by (-N=C), has ability to decompose and destruct the cell of pathogenic bacteria(Shah *et al*, 2006). In the cell of pathogenic bacteria, the alkaloidic compounds denaturant the cell proteins and they do an interaction with enzymes of proteins biosynthesis, containing thiol group (-SH). Also alkaloids are capable of linking with enzymes of protein metabolism, such as DNA – polymerase, DNA – ligase and RNA – polymerase(Goodwin, and Mercer,1983).

3.2 **Results of alkaloids extract cytoxicity**

The cytoxicity results of alkaloids isolated from *Capparis spinosa* L. buds toward red blood cells are shown in table (4) (Chiej, 1984). It was noticed that the concentration of (1:1 mg/ml) gave a positive toxicity against red blood cells then it showed a hemolysis toward these cells, but the concentrations of (1:10, 1:100 and 1:1000 mg/ml) didn't give any hemolysis against red blood cells, this means that alkaloids extract can be used safely to treat the various diseases caused by pathogenic bacteria which are represented by *E. coli* and *S. aureus*.

The importance of determination of maximal and minimal inhibitory concentrations of any active chemical compounds including alkaloids, lead to investigate of the toxicity of any medicinal plant, then this observation lead to follow the healthy damage which cause to disorder in the human body(WHO, 2013) .

Table (4):Cytoxicity results alkaloids isolated from *Capparis spinosa* L. buds.

Alkaloids extract conc. (mg/ml)	Hemolysis
1:1	T +
1:10	NT –
1:100	NT –
1:1000	NT –
Control negative (blood + Ringer solution)	NT –
Control positive (tap water + blood)	T ++++

T = Toxic, NT = Non Toxic

4. Conclusions

The alkaloidic compounds isolated from *Capparis spinosa* L. buds, showed a very good activity for inhibition of pathogenic bacteria growth which represented by *E. coli* and S. aureus, where these compounds recorded a very good inhibition zone diameters, this ensures the great ability of alkaloids to kill these pathogenic microorganisms. Therefore these active chemical compounds can be used as herbal substituent's for treating the infections caused by these bacteria,

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