



Antibacterial effect of Cappariloside (C₂₂H₂₈N₂O₁₁) and Vitexlactam (C₂₂H₃₅N₄O₄) alkaloids compounds isolation from *Capparis spinosa* roots & *Vitex agnus-castus* fruits.

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Abstract:

The isolation of alkaloids compounds from *Capparis spinosa* roots and *Vitex agnus-castus* fruits as crystal yellowish white powder. The chemical and physical properties of isolated alkaloids compounds were studied by using thin layer chromatography (TLC), melting point (MP), ultraviolet spectrum (UV) and FTIR spectrum. The isolated alkaloids compounds are shown high antibacterial activity against pathogenic bacteria (*S. aureus*, *P. aeruginosa*, *P. stutzeri*, *E. aeruginosa*, *S. viridans*, and *S. pneumoniae*). The results explained broad spectrum antibacterial property of alkaloids compounds against all pathogenic bacteria.

Introduction:

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative sources. Due to the widespread and often indiscriminate use of antimicrobial drugs, many bacteria have the genetic ability to transmit and acquire resistance to drugs and these strains are particularly evident in the hospital environment (Shah, 2005; Evans, 1999).

Plants are rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (Fatima, 2006). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials (Khalaf, 2007). In this study two medicinal plants *Capparis spinosa* L. and *Vitex agnus-castus* L. which are used by traditional medicine in Iraq. *C. spinosa* is commonly known as Iraqis caper, the plant is used traditionally as antibacterial, anti-inflammatory, anti-diabetic, anthelmintic (Ibrahim, 2012) and *V. agnus-castus* is one of medicinal plants, known as Kaff maryam in Iraq and researches have revealed its antimicrobial, diuretic, digestive, antifungal, against anxiety, and also stomachache (Ekundayo *et al.*, 1990).

Alkaloids are a large family of nitrogen containing secondary metabolites found in approximately 20% of the species of vascular plants. As a group, they are best known for their striking pharmacological effects. Alkaloids are usually synthesized from one of a few common amino acids- in particular, Aspartic acid, Lysine, Tyrosine, and Tryptophan and classified according to the amino acids that provide their nitrogen atom and part of their skeleton (Gershenzon, 1998). They are heterogeneous group, ranging from simple compounds like coniine, the major alkaloid of hemlock, *Conium maculatum*, to the pentacyclic structure of strychnine, *Strychnos bark*.

These compounds generally exert pharmacological activity particularly in mammals such as humans. Even today many of our most commonly used drugs are alkaloids from natural sources and new alkaloidal drugs are still being developed for clinical use (Robert and Wink, 1998).

Many alkaloids serve as models for the chemical synthesis of analogues with better properties. Important examples are Vittatine alkaloid has antibacterial activity against the *S. aureus* and the *E. coli* (Dubouzet *et al.*, 2005), Study by (Pick *et al.*, 2006) confirmed that bromotyrosine alkaloids have effective antimicrobial activity against Gram positive bacteria including mycobacteria.



Materials and Methods:

1-Sources of studied bacterial strains

Pure cultures of *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus viridans*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*. The culture of pathogenic bacteria was obtained from bacteriology laboratory of Department of Biology, College of Science, Universities of Kufa and Myssan. The bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slants at 4 °C.

2-Plants Collection and authentication:

The plants *C. spinosa* and *V. agnus-castus* were collected from the College of Agriculture /University of Basrah, and center of Myssan city in September 2012 and was identified by Dr. Ahmad Abies Motar, Biology Department, College of Science/ Kufa University. The plant (aerial and roots parts) cleaned by tap water, dried in air, then milled several times while getting affine powder for each plants parts.

3-Isolation and purification of alkaloids compounds

The method is used by (Eltayeb *et al.*, 1997):

- 1- Five g of powdered dry extract of *C. spinosa* roots and *V. agnus-castus* fruits were in 500 ml glass beaker and add 250 ml of 2% Oxalic acid.
- 2- The mixture left in a vibrator for 30 minute.
- 3- The solution filtered by paper (Whatman-No.1).
- 4- Taking the leaky and added 5 ml of (NaOH 60%) solution
- 5- In water bath for 10 minutes at temperature 75 C°.
- 6- Left at room temperature for 24 hours, then central exclusion process solution speed 3000 rpm for 15 minute and took the deposit.
- 7- Added 25 ml Hcl concentration 0.5 M, left to cool.
- 8- Five ml of NaOH 60 % added to extract solution.
- 9- In a water bath to 100 C° for 10 minutes and leave to dry at room temperature.
- 10- Crystallization process by using methanol 80% and leave to dry at room temperature, and then the test was conducted TLC to the value of the relative effect of compound and some chemical tests and physical tests to ensure its purity.

4-Identification of alkaloids compounds

To determine the purity and relative to front (R_f) of isolated compounds. A thin layer chromatography was carried out for (30±5 min). On glass plates (2 x 10 cm) TLC plates are used in glass tanks, using ascending chromatography. The sample was spotted or lined by capillary tube, the spot was allowed to dry and then the plate was placed in the glass chromatography tank with the solvent previously placed in the bottom of the tank to a depth of 10 mm. (Harborne, 1984; Kaufman *et al.*, 1999). Relative flue (R_f) calculated from below equation:

$R_f = \text{distance a compound moves} / \text{distance solvent front}$ (Harborne, 1984)

Melting point electrothermal is used for the determination of melting point of the isolated compounds. Melting points of single compounds Cappariloside and Vitexlactam were determined in open capillary tubes Electrothermal 9100 apparatus and were unconnected (Harborne, 1984). Ultra violate (UV) spectra of purified single compounds were obtained on UV-1601PC spectrophotometer (Shimadzu) using distilled water as solvent. Ultraviolet spectrum of the isolated compounds was carried out in the Department of Chemistry /college of Science/University of Kufa. (Donald *et al.*, 2009).

Spectrum of the isolated compounds was recorded with (FT-IR) to determine functional groups of purified single compounds FTIR spectra were used a recorded in KBr using a FTIR-8400S Fourier transform infrared spectrophotometer (Shimadzu). In the Chemistry Department/college of Science/University of Kufa. (Donald *et al.*, 2009).



5-Concentration of plants extracts

Stock solution was prepared for each extract by dissolving 500 mg of dried extract with 1 ml of distilled water for aqueous extract and ethanol for alcohol extract, so the final concentration of extract would be 500 mg/ml, from this stock solution other concentration were prepared 62.5, 125, 250 mg /ml which was used against bacteria (Nwachukwu and Uzoeto, 2010).

6-Determination of antibacterial activity

Agar well diffusion method was used to determine the antibacterial activity of alkaloids compounds according to (Egharevba *et al.*, 2010).

1. With a sterile wire loop, the tips of 4-5 isolated colonies of the organism to be tested were picked from the original culture and introduced into a test tube containing 10 ml Mueller Hinton, then incubated at 37 °C for about 2 to 5 hours to produce a bacterial suspension of moderate turbidity. Its turbidity was compared to McFarland tube No. 0.5.
2. Within 15 minutes of adjusting the density of the inoculums, a sterile cotton swab was dipped into the standardized bacterial suspension. The excess fluid was removed by rotating the swab firmly against the inside of the tube above fluid level. The swab was then streaked onto the dried surface of a Mueller- Hinton plate in 2 different planes to obtain an even distribution of the inoculums.
3. The plate lids were replaced and the inoculated plates were allowed to remain on a flat and level surface undistributed for 3-5 minutes to allow absorption of excess moisture
4. Four holes with 8 mm diameter which done by corky bore and filled carefully with 100 µl from each concentration of the extracts by using micropipette. Distilled water and ethanol were added to one hole in the cultured media to be as control, then the Petri dishes were incubated in 37 ° C for 24 hour. The inhibition zone was measured by ruler; this was repeated three times.

Results and Discussion

1-Identification of isolated alkaloids compounds

A-Physical Properties

Table (1) illustrated physical properties of isolated compounds, and shown that cappariloside (1*H*-indole-3-acetnitrile 4-*O*-β-(6'-*O*-β-glucopyranosyl)-glucopyranoside) and Vitexlactam (6β-acetoxy-9*x*-hydroxy-13(14)-labdan-16,15 amide) are fairly stable crystalline solids and higher melting points.

At room temperature, we found that the isolated compounds dissolve easily in water and or moderate solubility in methanol but of slightly soluble in ethanol, acetone and chloroform.

The high solubility of our alkaloids compounds in water is ascribed to the presence of polarized groups in the structures of these compounds. This character encourages us to study biological activity of these alkaloids. The structures of the isolated compounds are assigned on the basic of their T.L.C., UV and IR spectra.

B-Thin Layer Chromatography

Table (2) shown the TLC results which noted, that the behavior of our isolated compounds was similar to the behavior of alkaloids (orang, after spraying with Dragendroff reagents) or (brown, after treatment with the Iodine steam reagent) and compounds which isolated from the roots of *C. spinosa* (Cappariloside) and fruits of *V. agnus-castus* (Vitexlactam) were given $R_f = 0.78$ and $R_f = 0.83$ respectively. The difference in the value of R_f of studied alkaloids is ascribed to the degree of polarity and function groups of the each compounds and may be to the mobile phase. Small R_f value indicated low dissolvability of compound in mobile phase therefore the compound slowly move to up. Big R_f value indicated high dissolvability of compound in mobile phase therefore the compound readily move to up (Keroynz and Anthrykin, 1986).



Table (1): Properties of isolated alkaloids compound

No.	Biological name	Chemical name	Molecular formula	M.P.	Weight before isolation (gm)	Weight after isolation (gm)	Shape and Status of isolated compound
1.	Cappariloside	1 <i>H</i> -indole-3-acetnitrile 4- <i>O</i> - β -(6'- <i>O</i> - β -glucopyranosyl)-glucopyranoside.	(C ₂₂ H ₂₈ N ₂ O ₁₁)	220-223 (deco.)	50	1.3	white crystals solid
2.	Vitexlactam	6 β -acetoxy-9 α -hydroxy-13(14)-labdan-16,15 amide.	(C ₂₂ H ₃₅ NO ₄)	225-227 (deco.)	50	0.47	yellowish white crystals solid

M.P. = Melting point

deco. = decomposing



Table (2): T.L.C. results of isolated alkaloids compounds

Properties	Cappariloside	Vitexlactam
R _f	0.78	0.83
Color in visible light	yellow	yellow
Color in Iodine steam	brown	brown
Color in dragendroff reagent	orange	orange
Mobile phase	1-MeOH : NH ₄ OH: CHCl ₃ (8.5: 0.5: 0.5) 2-MeOH : CHCl ₃ (0.5: 19.5) 3-MeOH : NH ₄ OH (9.5: 0.5)	1-MeOH : NH ₄ OH: CHCl ₃ (8.5: 0.5: 0.5) 2-MeOH : CHCl ₃ (0.5: 19.5) 3-MeOH : NH ₄ OH (9.5: 0.5)
Time of posting	30 minute.	32 minute.

C-Ultra violet spectra

Ultra violet spectra by distilled water of alkaloids compounds were determined. The qualitative UV spectra profile of Cappariloside and Vitexlactam that isolated from root of *C. spinosa* and fruit of *V. agnus-castus* were selected at wavelength from 200-400 nm due to sharpness of the peaks and proper baseline. The electronic absorption data of the investigated compounds are gathered in Table (3) and the spectra of these compounds are shown in Figs. (1 & 2), the Cappariloside and Vitexlactam are characterized by two bands, the short band at 241 nm of Cappariloside and 264 nm of Vitexlactam respectively. The appearance of the short bands in the electronic absorption spectra of isolated alkaloids compounds is ascribed to the locally excited by $\pi \longrightarrow \pi^*$ transition with the double band (C=C) of the compounds. The spectra of long band at 277 nm and 288 nm of Cappariloside and Vitexlactam respectively, is ascribed to the $n \longrightarrow \pi^*$ transitions.

Table: (3) UV spectral data of isolated compounds distilled water solvent, λ max(nm).

No.	Compounds	0.05 mg/ml			
		Band I		Band II	
		λ max	Abs.	λ max	Abs.
1.	Cappariloside	277	2.389	241	1.160
2.	Vitexlactam	288	0.784	264	0.713

Abs.= Absorption.



D-FTIR Spectra

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The IR spectra of the studied alkaloids as KBr discs and of their representative spectra are shown in Tables (4) and Fig.(3 & 4). The spectrum of Cappariloside is characterized by seven bands corresponding to the stretching vibrations of the NH, OH, C-H aromatic, C-H aliphatic C≡N, C=C and C-O groups, which occur in (3600), (3454), (3051), (2800), (2470), (1687, 1647) and (1454) cm⁻¹ respectively. The absorption data of IR of Vitexlactam showed seven stretching vibration bands, which confirmed the correctness of the proposed structure. These bands are NH, OH, CH aromatic, CH aliphatic, C=O, C=C and C-O which occur in (3734), (3454), (3028), (2899, 2831), (1691), (1647, 1554), and (1458) cm⁻¹ respectively. The results of functional groups of FTIR of isolated alkaloid compounds agreement with Cappariloside structure (Calis *et al.*, 1999) and Vitexlactam structure (Li *et al.*, 2013).

Table (4): IR spectral data of isolated alkaloids compounds recorded as KBr discs (cm⁻¹)

No	Compounds	NH Str.	OH Str.	Aromat ic CH Str.	Aliphat ic CH Str.	C≡N Str.	C=C Str.	C=O Str.	C-O Str.
1.	Cappariloside	3600 (w)	3454 (m)	3051(w)	2800 (w)	2470 (w)	1687 and 1647 (w)	-	1454 (S)
2.	Vitexlactam	3734 (w)	3456 (m)	3028 (w)	2899 and 2831 (w)	-	1647 and 1554 (w)	1691 (w)	1458 (S)

Str. = stretching

S= strong

M= medium

W= weak

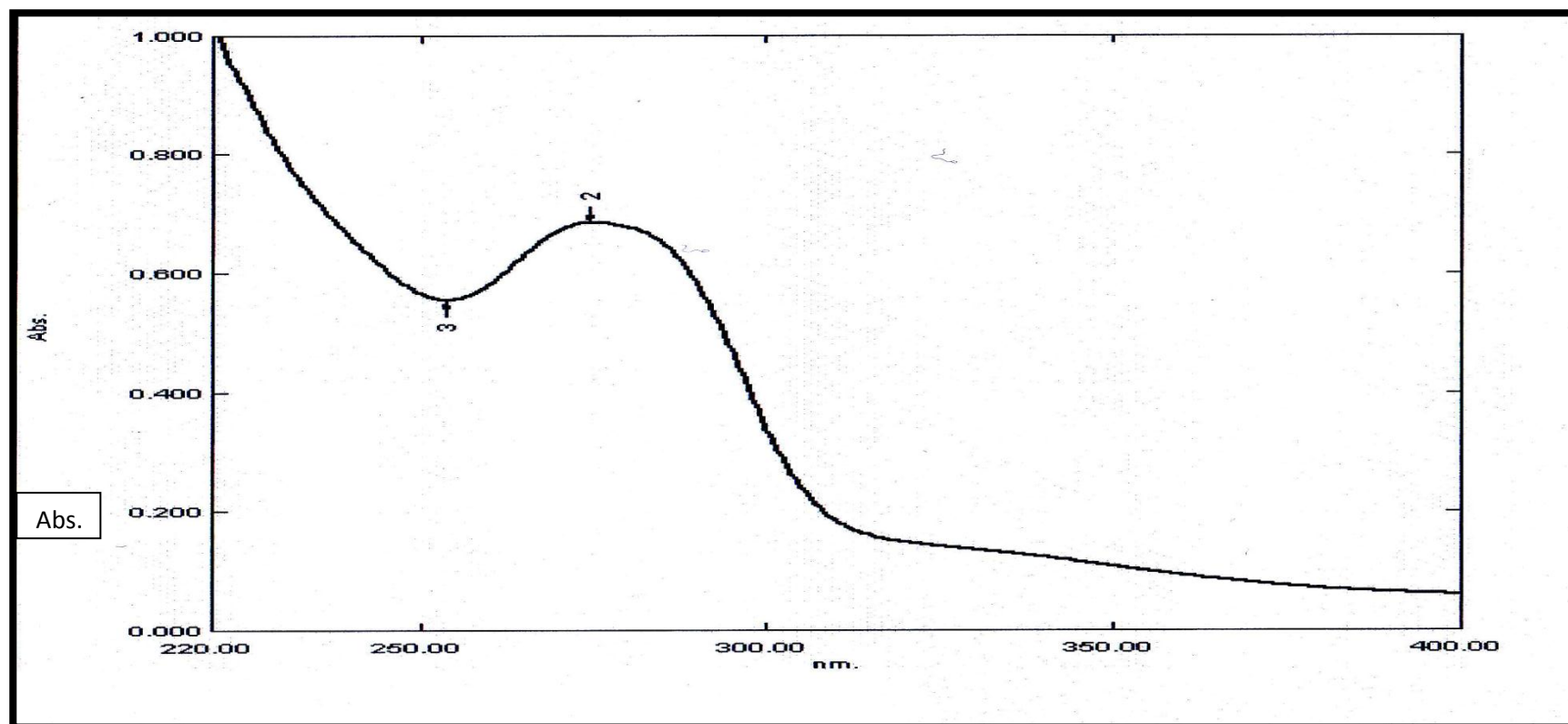


Fig.(1) UV spectrum of Cappariloside



Abs.

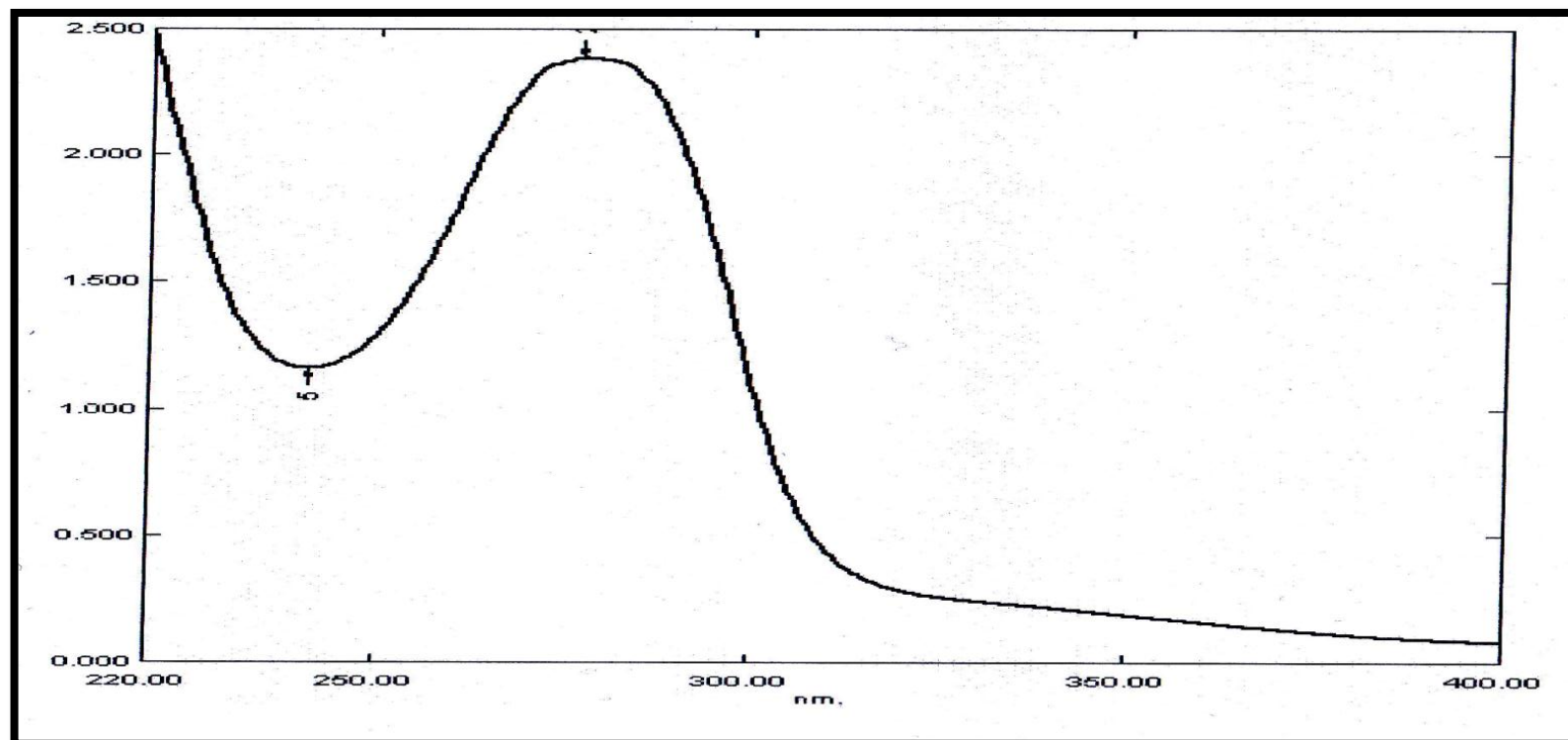


Fig.(2) UV spectrum of Vitexlactam

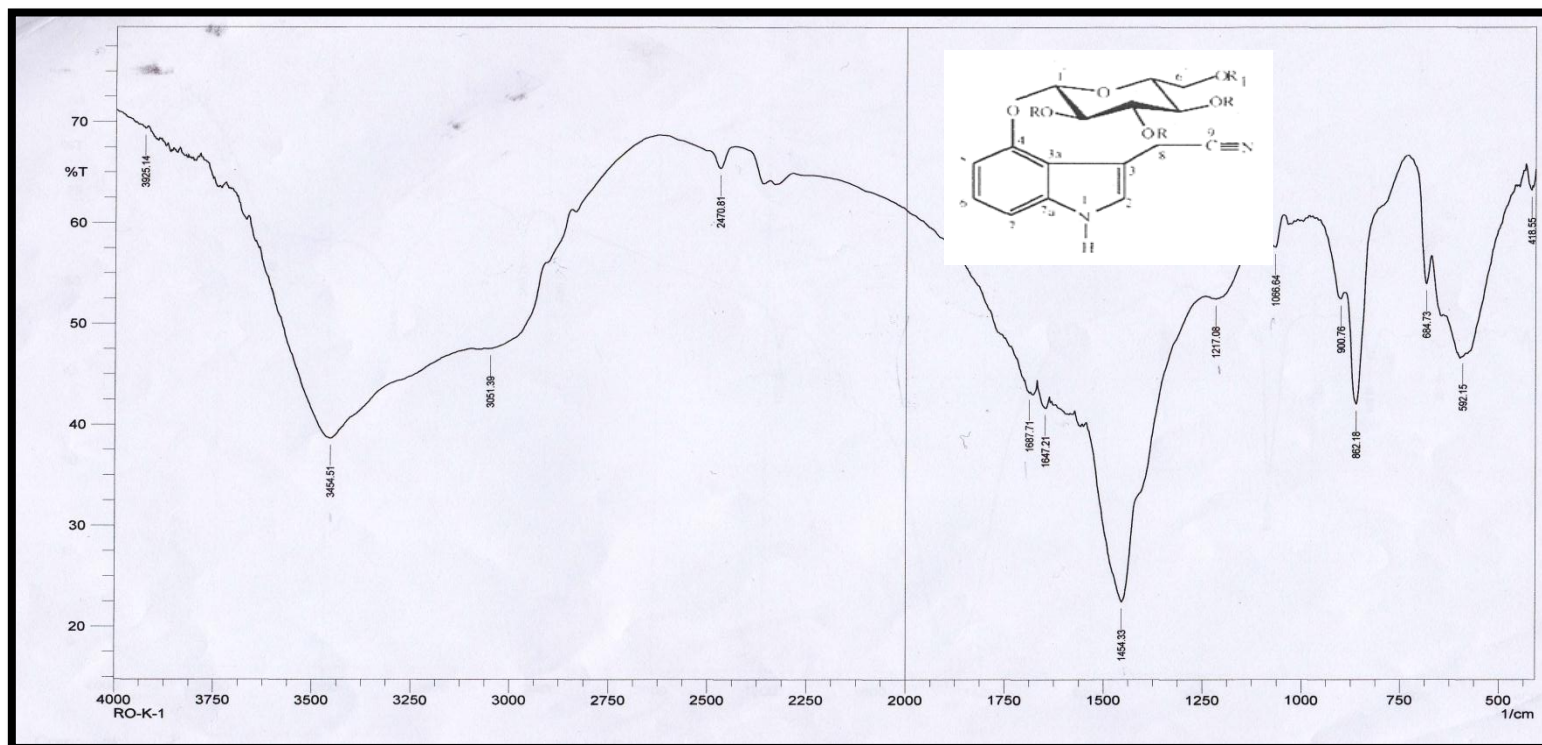


Fig.(3) FT-IR Spectrum of Capparilosides

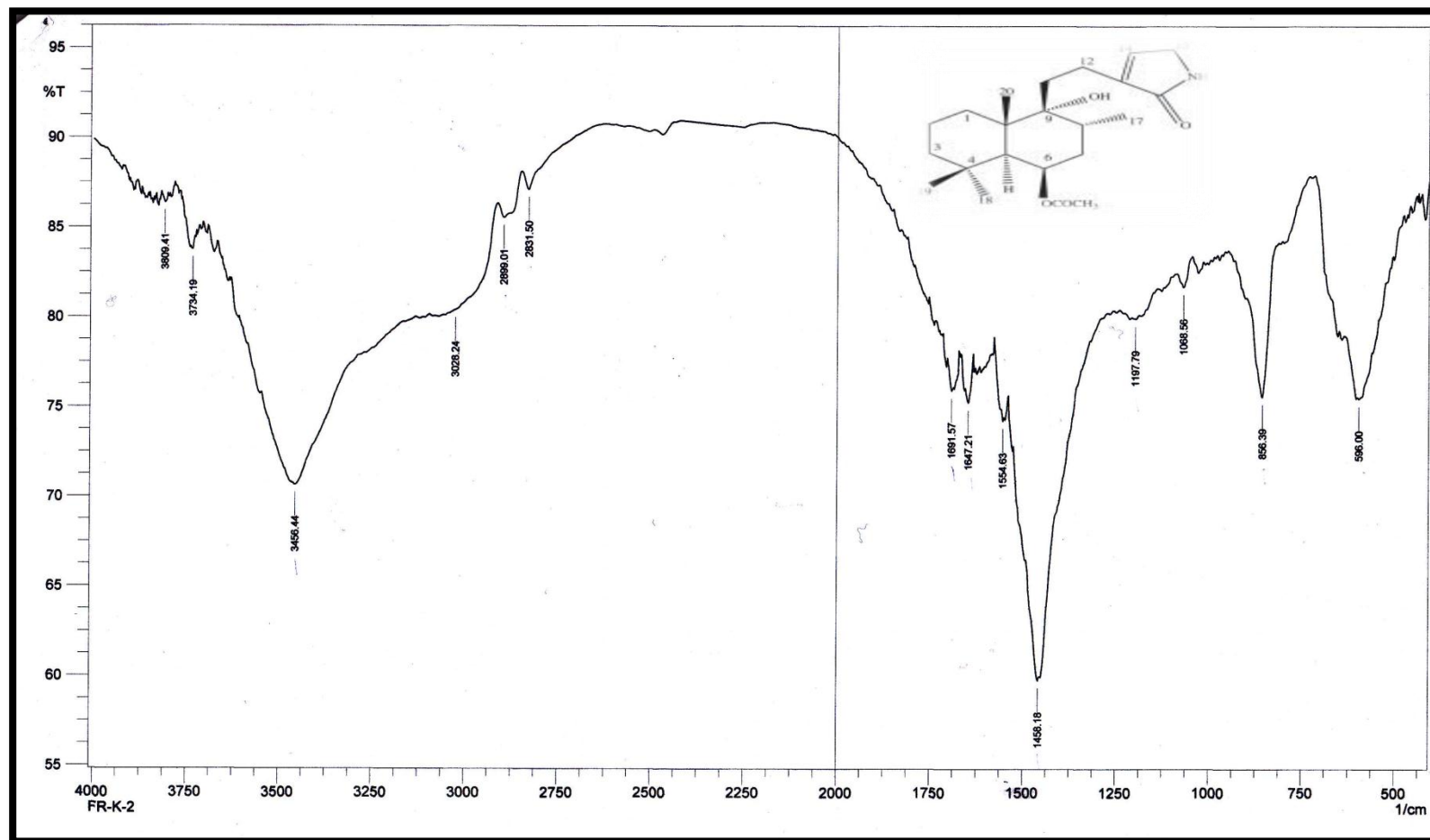


Fig. (4) FT-IR spectrum of Vitexlactam



2-Antibacterial activities of Cappariloside and vitexlactam compounds on some pathogenic bacteria (*S. aureus*, *P. aeruginosa*, *P. stutzeri*, *E. aeruginosa*, *S. viridans*, and *S. pneumonia*)

Bacterial resistance to antibiotics remain a serious public health concern because widespread nosocomial pathogens with immune systems weakness by disease or genetic disposition, *S. aureus* is a problematic nosocomial pathogen and this is complicated further by the fact that humans are a natural reservoir for this organism (Lowy, 1998) and infections caused by multi-drug resistance bacterial species are among the most difficult to treat with conventional antibiotics, in our study the growth of *S. aureus*, *P. aeruginosa*, *P. stutzeri*, *E. aeruginosa*, *S. viridans*, and *S. pneumonia* that were found to be resistant towards many standard antibiotics, was remarkably inhibited by the alkaloids compounds, this shows the potential of these compounds to control the growth of drug resistance microbes. For the reasons outlined above, the alkaloids compounds were extracted and isolated because it is generally recognized that alkaloids have strong antibacterial and biological properties (Kluza *et al.*, 2005).

The antibacterial activity of alkaloids compounds on G⁺ve bacteria revealed the inhibition zone diameter reach to 39.33 mm against *S. pneumonia* at high concentration of Cappariloside and was less susceptible to the Vitexlactam reached to 30.66 mm. while against *S. aureus* the Vitexlactam shown inhibition zone 34.33, and Cappariloside 26.66 mm respectively. *S. viridans* the zone of inhibition of Capparilosides and Vitexlactam have the same effect and there are no significant differences ($P > 0.05$) were recorded between them, the result shown in Table (5). The inhibition zone on Gram negative bacteria of the Vitexlactam has a pronounced effect on the growth of *E. aerogenosa* and the diameter of inhibition zones reached to 30.66 mm at a concentration of 500 mg/ml and 28.33 mm of Cappariloside. On the other hand the Cappariloside and Vitexlactam also shown that no significant differences between the inhibition zone 27.66 mm at concentrations 500 mg/ml on *P. stutzeri*. Finely we found the Vitexlactam more effective 30.66 mm against *P. aerogenosa* than Cappariloside 27.33 mm. The Cappariloside and Vitexlactam showed inhibitory activity against all tested bacterial strains at various concentrations which revealed broad spectrum antibacterial property of compounds this result supports the findings of (Elisabetsky and Costa, 2006) which found that alkaloid used by the plants in defense mechanism against pathogens and predators. In the present study all alkaloids compounds were showed significant antimicrobial potential against test strains.

(Caron *et al.*, 1988) reported similar result which conducted the significant role of the 31 alkaloids isolated from different plants showed antibacterial activity on bacteria tested which were *B. Subtilis*, *S. aures*, *E. coli* and *P. aeruginosa*, *Mycobacterium*, also observed that antimicrobial activity of alkaloids is connected with the stereochemistry of the carbon ring, its aromatic substitution and oxidation.

The results approached with (Ismaeil, 2011) who found that two alkaloid isolated from seed black has antibacterial activity against pathogenic bacteria. It could also be that concluded that the antibacterial alkaloids compounds may inhibit bacteria by a different mechanism than that of currently used antibiotics.



Table (5): Antibacterial activity of Cappariloside and Vitexlactam on some pathogenic bacteria(mm)

Alkaloids compounds	Con Mg/ml	<i>S. aureus</i>	<i>S. pneumonia</i>	<i>S. Viridans</i>	<i>E. aerogenesa</i>	<i>P. aeruginosa</i>	<i>P. stutzeri</i>
Cappariloside	62.5	15.33	25.66	16.33	18.66	16.66	16.33
	125	18.66	29.66	20.66	21.66	19.66	21.33
	250	22.33	34.66	24.66	25.66	23.33	24.66
	500	26.66	39.33	27.66	28.33	27.33	27.66
Vitexlactam	62.5	20.66	17.66	16.66	20.66	20.66	17.66
	125	25.33	21.66	20.33	23.66	24.33	21.66
	250	29.66	25.66	24.66	26.66	27.66	24.33
	500	34.33	30.66	27.66	30.66	30.66	27.66
Control	0.00	-	-	-	-	-	-
Tetracycline	30	13.33	12.66	13.66	-	-	15.33

LSD_(0.05) for interaction= 0.998 0.987 0.9633 0.9452 0.9327 0.9521
Not inhibition= -



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عزل وتشخيص المركبات القلوانية Vitex lactam و Capparilosides من *Vitex* و *Capparis spinosa* roots و دراسة فعالية التضاد المايكروبي لهما *agnus-castus* fruits

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الخلاصة

عزل مركبات قلوانية من جذور نبات *Caparis spinosa* وثمار نبات *Vitex agnus-castus* بشكل مسحوق ابيض مصفر اللون , درست بعض الصفات الكيميائية والفيزيائية للمركبات القلوانية المعزولة باستعمال تقنية كروماتوغرافيا الطبقة الرقيقة thin layer chromatography (TLC) و درجة الانصهار (MP) melting poin وطيف الاشعة فوق البنفسجية ultraviolet (UV) فضلاً عن قياس طيف الاشعة تحت الحمراء واجراء الكشف النوعية لهما , كما حددت فعالية المركبات القلوانية اتجاه نمو العزلات المرضية (*S. aureus*, *P. aeruginosa*, *P. stutzeri*, *E. aeruginosa*, *S. viridans*, and *S. pneumonia*). ووضحت النتائج امتلاك المركبات القلوانية المعزولة خصائص مضادة للبكتريا واسعة الطيف اتجاه كل البكتريا المرضية.