Isolation and Identification of Phenols and an Alkaloidic Compound from *Matricaria chamomilla* Plant Flowers and Study of Their Medicinal Activity Against the Pathogenic Bacteria of Skin Infections

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Abstract

Phenols and an alkaloidic compound were isolated from *Matricaria camomilla* plant flowers . The chemical and physical properties of isolated compounds were studied using qualitative tests , functional groups test , thin layer chromatography (TLC) and infra-red (IR) spectroscopy . The results showed presence of two phenolic compounds in phenols extract and one alkaloidic compound in alkaloids extract . The medicinal activity was measured against growth of standard bacterial strains of skin infections were *Staphylocoous aureus* (positive) (NCTC 6571) and *Escherichia coli* (negative) (NCTC 5933) . It was found that the concentration of (100 mg / ml) of phenols was the highest activity where it recorded inhibition diameters equal to 22 mm and 20 mm for positive and negative bacteria respectively . Also the concentration of (100 mg / ml) of the alkaloidic compound , showed the highest inhibition activity where it recorded inhibition diameters equal to 16 mm and 10 mm for positive and negative bacteria respectively . The minimal inhibitory concentrations (MIC) were estimated for phenols with value 25 mg / ml for both types of pathogenic bacteria and also for alkaloidic compound with values equal to 50 mg / ml and 75 mg / ml for pathogenic positive and negative bacteria respectively . As a result the alkalloidic compound and phenols are recommended to use them as medicinal substituents for treatment of skin infections instead of used antibiotics but this work demands further clinical studies .

Key words : *Matricaria chamomilla* , phenols , alkaloidic compound , pathogenic bacteria , skin infections , medicinal activity .

Introduction

Chamomile (*Matricaria chamomila*) is a herbal medicinal plant with (15 -50 cm) high and it flowers after a few weeks from planting stage .This plant spreads in south and east Europe , mediterranean white sea beaches and united states of America (1) *Matricaria chamomilla* flowers contain active compounds such as volatile oil is extracted by steam distillation and consists of alphabisabolol , bisabolol oxide – A , bisabolol oxide – B (2) , trans – farnesene and spathaulenol (3) . Also the flowers of chamomile contain phenolic compounds as flovonoides such as flavon glycoside , a glycogen apigenin and lutoline . the flowers contain glycosides such as anthamic acid , anthamedine and matricarin (4) . The chamomile has characteristic medicinal properties such as having a resistant effect for bad dreams and physiological disorders (5) .

Alcoholic and aqueous extracts of flowers powder are used for therapeutics of skin infections caused by some pathogenic bacteria , therapy drug of injuries in mouth , therapeutics of respiratory system infection and treatment of digestive disorders (6) . Also *Matricaria chamomilla* extracts are used as anti – contractive development of immunity system and increasing the white blood cells (1) .

Phenols are aromatic compounds contain one or more hydroxyl group and most of them antimicrobial properties (7) and are biosynthesized by shikimic acid in secondary metabolism (8) , whereas flavonoids are a class of phenolic compounds consist of many benzene rings contain hydroxyl groups are conjugated to heterocyclic ring that have one or more oxygen atom (9) . Also flavonoids have antimicrobial characteristics because of their ability to destruct the cellular membrane then dissolve cell proteins (10) .

Tannins are also natural polymeric phenols containing many phenol rings and have ability of proteins precipitation from solutions . They can inhibit growth of pathogenic bacteria and fungi (11) . Alkaloids are basic nitrogenous compounds containing hetrocyclic ring and they have therapeutics importance because of their antimicrobial properties and its ability to bind with nucleic acids (12) .

Staphylococcus aureus (positive towards Gram's dye) and *Escherichia coli* (negative towards Gram's dye) bacteria are from pathogenic germs and they are classified depending on composition of their cellular walls . Also these two types of bacteria cause skin infections (10) , therefore the current study aimed to investigate the medicinal effect of phenols and alkaloids of chamomile against growth of these pathogenic bacteria .

Materials and Methods

First : Materials

1- *Matricaria chamomilla* flowers were bought from local market in Abu Al – Khaseeb region in Basrah Governorate in Iraq , dried well as powder and kept in plastic bottles in laboratory .

2- Chemicals : were supplied as the following :
Ethanol , acetic acid , sodium hydroxide , ammonium hydroxide , chloroform , hydrochloric acid , diethylether , formic acid , sec – butanol , iodine , α - naphthol , potassium hydroxide , lead acetate , mercuric chloride , sulphuric acid , ferric chloride , potassium iodide and sub – nitrate bismuth .

3- Culture medium
Nutrient Agar culture midia was prepared according to information determining by manufacturing company .

4- Standard bacterial strains
Pathagenic bacteria of skin infections were isolated from some patients in Al-Faiha Hospital in Basrah, they are *Staphylococcus aureus* (positive towards Gram's dye) and *Escherichia coli* (negative towards Gram's dye) .

Second : Methods

1- Isolation of phenols from *Matricaria chamomilla* flowers

Forty grams of flowers powder was dissolved in 250 ml of (2%) hydrochloric acid and the mixture was put in water bath for 80 min. at 85 °C . After that the mixture was filtered by Buchner funnel and the precipitate was removed then diethyl ether was added to filtrate by the same volume of filtrate . Mixture was put other time in water bath for 50 min. , then it was evaporated using rotary evaporator . The weight of phenolic product was 6.22 gm (7) .

2- Isolation of alkaloids from *Matricaria chamomilla* flowers

Forty grams of flowers powder was mixed with 250 ml of (10 %) ethanolic acetic acid and put on magnetic stirrer for 24 hr. then the mixture was filtered and precipitate was removed . The filtrate was concentrated to quarter of its volume by using rotary evaporator and acidified with (5) ml of concentrated of sulphuric acid . The acidic fraction was basified with ammonium hydroxide to pH 9 . After that the mixture was put in separation funnel and extracted with chloroform (3 × 20 mL) , finally the alkaloids were isolated in aqueous layer and dried . The product weight was (1.03gm) (9) .

3- Preliminary qualitative tests .

Isolated phenols and alkaloids were underwent to several tests such as :

A- Phenols test : by using (1 %) ferric chloride (13) .
B- Carbohydrates test : by using Molish's reagent (14) .
C- Flavonoids test : by using (5 N) alcoholic potassium hydroxide (15) .
D- Tannins test : by using (1 %) lead acetate (11) .
E- Alkaloids test : by using Dragendroff reagent (9) .
F- Glycosides test : by using Benedict's reagent (7) .
G- Amino acids test : by using (1 %) ninhydrin (7) .
H- Saponin test : by using (5 %) mercuric chloride (13) .

4- Thin layer chromatography (TLC) .

TLC technique was used for separation of phenolic compounds and determination of their purity . Fifty microlitres of phenols were toulrenced on glass plate (2 × 10 cm) covered by silicagel and using (iso – butanol : acetic acid : water) as eluent with ratio (70 : 5 : 25) . The separation time was 32 min. , then the glass plate was dried and the components were developed by UV – lamp at 233 nm , (1%) ferric chloride and iodine vapour . Rate of flow (R_f) values were calculated for all separated phenolic components (7) . Also , TLC was used for isolated alkaloids by the same method but using (sec – butanol : formic acid : water) as eluent with ratio (38.5 : 5 : 6.5) . The separated alkaloid was developed by UV – lamp at 233 nm , iodine vapour and Dragendroff reagent (9) .

5- Infra – red spectroscopy .

Infra – red (IR) spectrum of isolated alkaloid was recorded using (DG- Instrument company in England) spectrophotometer . The alkaloid sample was fixed on NaCl disk with spectral range of (600 – 4000 cm^{-1}) . Also IR – spectrum was recorded for isolated phenols by the same spectrophotometer . The phenols sample was mixed with KBr as a disk with the same spectral range .

6- Functional groups tests (16) .
A- Double bond test : was carried out by using bromine and potassium permanganate solutions .
B- Aldehyde and keton groups test : was done by using 2,4 – dinitrophenyl hydrazine reagent .
C- Phenols test : was examined by using (1%) ferric chloride .
D- Alkaloids test : was carried out by using Dragendroff reagent .

7- Antibacterial activity and determination of minimal inhibitory concentration .

Several concentrations of isolated phenols (25 , 50 , 75 and 100 mg/ml) were used against standard bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) for determination of minimal inhibitory concentration by using Nutrient Agar as a culture medium depending

on diffusion method . Petri dishes were used for each concentration , then they placed in incubator for 24 hr. after that the inhibition zone diameters were measured . The same method was carried out for isolated alkaloid by using the concentrations (50 , 75 and 100 mg/ml) (17,18) .

Results and Discussion

In this work , phenols and an alkaloidic compound were isolated with extraction percentage were (15.55 % and 2.57 %) respectively . Table (1) indicates the chemical qualitative tests results of isolated phenols , which represent presence of phenols , tannins and flavonoids, whereas other chemical families were not found . Some studies ensured presence of flavonoids and tannins in *Matricaria chamomilla* flowers (8 , 19 , 20) .

Table (1) Qualitative tests of isolated phenols from *Matricaria chamomilla* flowers .

Test	Test result	Notes	Conclusion
$FeCl_3$ (1%)	+	Formation of bluish – green colour	presence of phenols
$Pb(Ac)_2$	+	Formation of brown – white precipitate	Presence of tannins
Alcoholic KOH (5N)	+	Formation of yellow precipitate	Presence of flavonoids
Molish	–	No violet ring	No carbohydrates
Benedict	–	No red precipitate	No glycosides
Dragendroff	–	No orange precipitate	No alkaloids
$HgCl_2$ (5%)	–	No white precipitate	No saponin
Ninhydrin (1%)	–	No violet colour	No amino acids

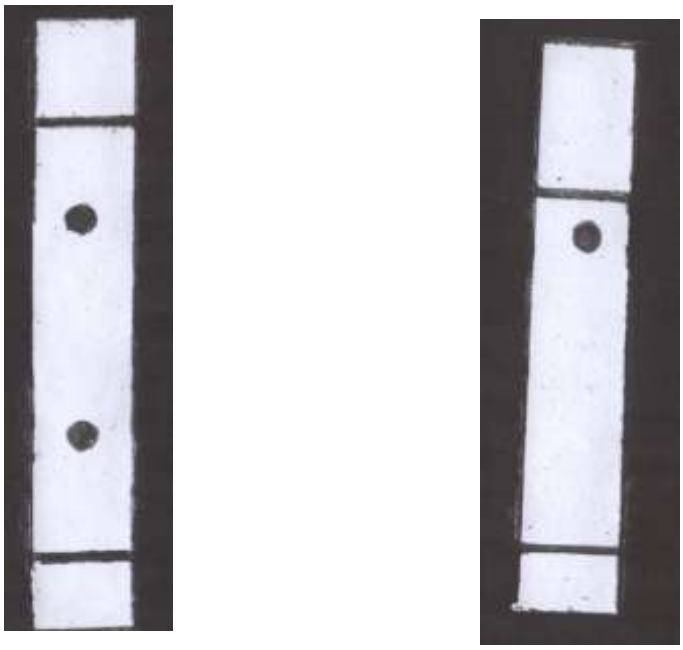
Table (2) Shows the qualitative tests of isolated alkaloid which gave positive response of alkaloids whereas the results indicates that other

chemical compounds were not present . Alkaloids were found to be in several medicinal plants including *Matricaria chamomilla* (19) .

Table (2) Qualitative tests of isolated alkaloids from *Matricaria chamomilla* flowers .

Test	Test result	Notes	Conclusion
Dragendroff	+	Formation of orange precipitate	presence of alkaloids
Molish	–	No violet ring	No carbohydrates
Benedict	–	No red precipitate	No glycosides
$FeCl_3$ (1%)	–	No bluish – green colour	No phenols
$HgCl_2$ (5%)	–	No white precipitate	No saponin
Ninhydrin (1%)	–	No violet colour	No amino acids

Thin layer chromatography results of isolated phenols are shown in table (3) which indicate presence of two spots have rates of flow (R_f) equal to (0.86 and 0.46) , this means separation of two phenolic compounds which were organic compounds were tested by iodine vapour and they have phenolic groups because of appearance of two green spots . Also presence of double bond conjugation system where the two light violet spot were formed



A **B**

Fig. (1) TLC of A) phenolic compounds and B) alkaloidic compound

Table (3) Thin layer chromatography results of isolated phenols from *Matricaria chamomilla* flowers .

Eluent system	Tests	Spots no.	Result	Rate of flow (R_f) values	Conclusion
iso – butanol – acetic acid – water (70 : 5 : 25)	Eyes	2	light green	0.46,0.86	pure compounds
	I_2 – vapour	2	brown	0.46,0.86	presence of organic compounds
	UV – lamp	2	light violet	0.46,0.86	presence of double bond conjugation system
	$FeCl_3$ (1 %)	2	bluish green	0.4,0,86	presence of phenolic compounds

A study ensured presence of phenols as flavonoids compounds in *Matricaria chamomilla* (1) .

Table (4) indicates the thin layer chromatography results of isolated alkaloid from *Matricaria chamomailla* flowers where one spot was separated has rate of flow (R_f) value equal to 0.93 and this ensures presence of one alkaloidic compound . By using iodine vapour test , this alkaloid was found to be a nitrogenous organic compound , also by developing , one orange spot was appeared and change of litmus paper colour from red to blue , ensures presence of alkaloids as basic compounds .

Table (4) Thin layer chromatography results of isolated alkaloidic from *Matricaria chamomilla* flowers .

Eluent system	Tests	Spots no.	Result	Rate of flow (R_f) values	Conclusion
sec – butanol – formic acid – water (38.5 : 5 : 6.5)	Eyes	1	light orange	0.93	pure compound
	I_2 – vapour	1	brown	0.93	presence of nitrogenous compound
	Dragend - roff	1	orange	0.93	presence of alkaloid

FT – IR – spectrum results of isolated phenols are shown in figure (1) and table (5) where several functional and / or structural groups absorption bands were recorded . Appearance of broad peak at wavenumber 3400 cm^{-1} indicates presence of phenolic hydroxyl group (-OH) which represent the stretching vibration because of presence of hydrogen bonding belonging to flavonoids and tannins . The two medium bands at (2870 cm^{-1} and 2930 cm^{-1}) represent stretching vibration of aromatic (C – H) bond . Also , appearance of a weak band at (1715 cm^{-1}) belongs to stretching vibration of (C = O) group of keton . The intensity medium band at (1610 cm^{-1}) indicates the aromatic (C = C) bond also the presence of a bond have medium intensity at (1020 cm^{-1}) represent stretching vibration of (C – O) bond of aryl ether . The appearance of overtone band at (2000 cm^{-1}) which is supported by the (C = C) band at (1610 cm^{-1}) indicates the aromatic ring . And the band at (732 cm^{-1}) represents presence of substitution on phenyl group (21) .

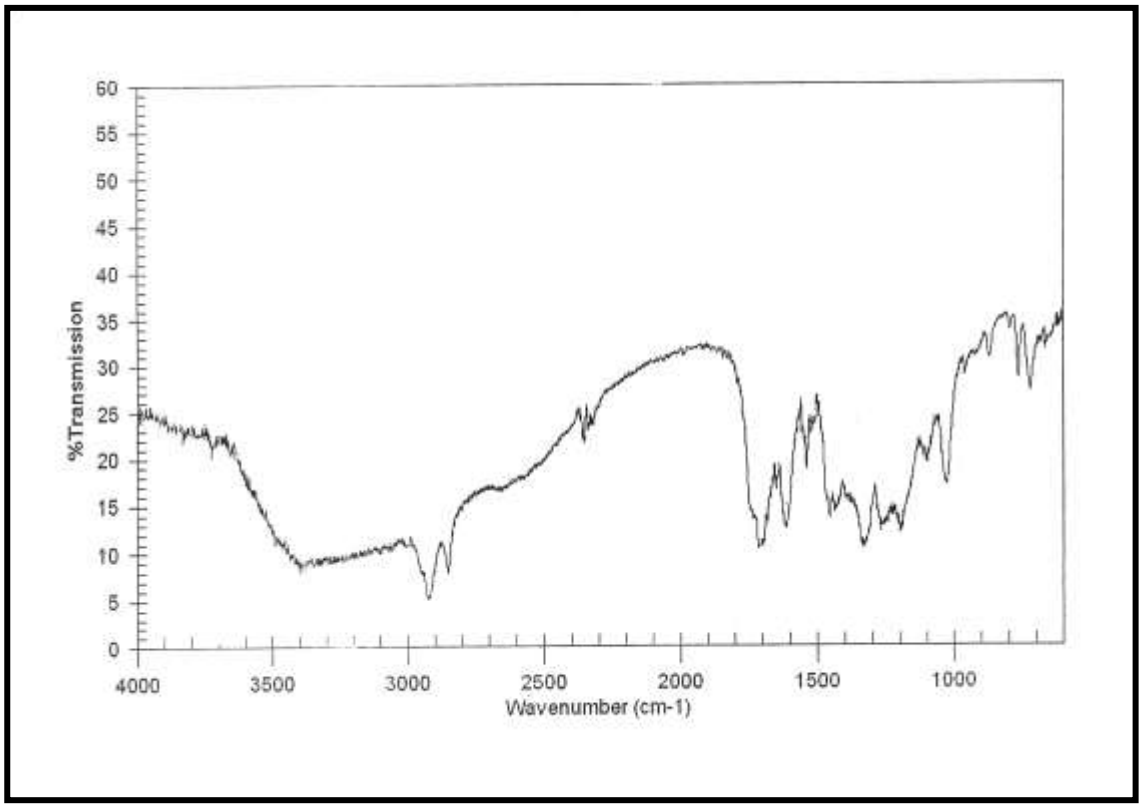


Fig. (1) FT – IR – spectrum of phenols isolated from *Matricaria chamomilla* flowers .

Table (5) Absorption bands and their related structural and functional groups in FT – IR – spectrum of phenols isolated from *Matricaria chamomilla* flowers .

Band frequency (cm^{-1})	Band	Band shape	Assignment of band	Structural and functional group
3400	O – H	Broad	Stretch	Phenols
2930 , 2870	C – H	Medium	Stretch	Aromatic compound
1715	C = O	Weak	Stretch	Keton
1610	C = C	Medium	Stretch	Benzene ring
1020	C – O	Medium	Stretch	Aryl ether
732	ph – x	Medium	Out of plane	Benzene ring substitution

The absorption bands and their structural or / and functional groups in FT – IR – spectrum of isolated alkaloidic compound , are indicated in figure (2) and table (6). Appearance of sharp band at (1670 cm^{-1}) belongs to stretching vibration of alkaloidic (C = N) group whereas the broad band at (3400 cm^{-1}) represents the stretching vibration of phenolic or alcoholic (-OH) group having the hydrogen bonding . Also the figure (2) and table (6) show presence of weak band at (3010 cm^{-1}) belongs to stretching vibration of aromatic (C–H) bond , while the two intensity medium bands at (2910 cm^{-1} and 2840 cm^{-1}) indicate aliphatic (C – H) bond . Appearance of the medium band at (1555 cm^{-1}) represents stretching vibration of aromatic (C = C) bond , also the sharp band at (1020 cm^{-1}) indicates the etheric (C – O) group which is due to the stretching vibration (23) .

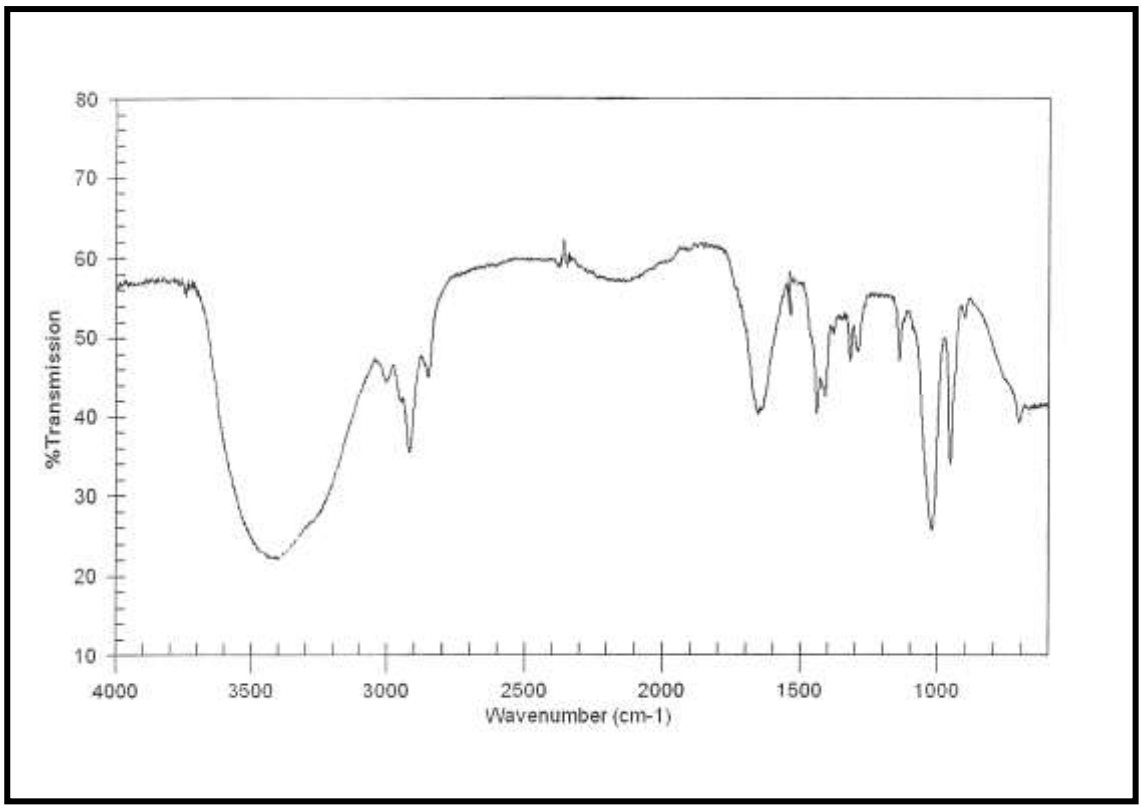


Fig. (2) FT – IR – spectrum of alkaloidic compound isolated from *Matricaria chamomilla* flowers .

Table (6) Absorption bands and their related structural and functional groups in FT – IR – spectrum of alkaloidic compound isolated from *Matricaria chamomilla* flowers .

Band frequency (cm^{-1})	Band shape	Band	Assignment of band	Structural and functional group
3400	Broad	O – H	Stretch	Phenols or Alcohols
3010	Weak	C – H	Stretch	Aromatic
2910 , 2840	Medium	C – H	Stretch	Aliphatic
1670	Sharp	C = N	Stretch	Alkaloids or Amines
1555	Medium	C = C	Stretch	Aromatic
1020	Sharp	C – O	Stretch	Etheric

The functional groups tests results of isolated phenols are shown in table (7) . It appeared that these isolated compounds contain phenol rings , carbonyl groups and the double bound . This ensures that the phenols isolated have phenolic compounds containing keton and aldehyde groups including double bond conjugation system .

Table (7) Functional groups tests results of phenols isolated from *Matricaria chamomilla* flowers .

Test	Test result	Indications	Conclusions
Br_2 / $KMnO_4$	+	disappearance of bromine and potassium permanganate colours	presence of double bond
$FeCl_3$ (1 %)	+	appearance of bluish – green colour	presence of phenols groups
DNPH	+	formation of orange precipitate	presence of aldehyde and keton groups

Table (8) indicates the functional groups tests results of isolated alkaloidic compound of *Matricaric chamomilla* flowers . The results ensure presence of double bound , alkaloids and (aldehyde or keton) groups , this indicates that the isolated compound is alkaloidic compound has carbonyl groups and double bond systems which are (C = C) and (C = N) bonds .

Table (8) Functional groups tests results of alkaloidic compound isolated from *Matricaria chamomilla* flowers .

Test	Test result	Indications	Conclusions
Br_2 / $KMnO_4$	+	disappearance of bromine and potassium permanganate colours	presence of double bond
Dragendroff	+	formation of orange precipitate	presence of alkaloids
DNPH	+	formation of yellow-orange precipitate	presence of aldehyde and keton groups

Table (9) indicates the results of antibacterial activity and minimal inhibitory concentration of isolated phenols of *Matricaria chamomilla* flowers by using different concentrations . It was noticed that the antibacterial activity increases with increasing of concentration , where the inhibition zone diameters were (10 , 12 , 19 and 22 mm) against *Staphylococcus aureus* bacteria and (8 , 10 , 16 and 20 mm) against *Escherichia coli* bacteria .

Some studies ensured that phenolic compounds (flavonoids and tannins) have high antibacterial activity because they contain hydroxyl group (- OH) in their chemical structure which it has ability to bonding with proteins hydrogen and this leads to break of sulphuric and hydrogen bonds abundant in the tertiary structure of proteins existing in bacterial cell (10,17) . Also the phenols are capable of destruction of cell wall then increase of its permeability for these compounds leading to denaturation of cell proteins (23) . Some studies indicated that the phenols have ability to bind with cell enzymes leading to inhibition of its biochemical activity (10,23) . The inhibition diameters values were greater towards positive bacteria than negative bacteria because *Escherichia coli* contains dense lipid layers in its cellular wall leading to resistance the entrance of the phenolic compounds into the bacteria cell . This biochemical case is opposite to *Staphylococcus aureus* which contains less lipid layers (10) . From table (9) the concentration of phenols (25 mg/mL) was the minimal inhibitory concentration to both types of bacteria . Some studies ensured that the phenolic compounds have a great antibacterial activity therefore they are used as anti – mutagenic and anti – carcinogenic agents (17,24) .

Table (9) Results of antibacterial activity and minimal inhibitory concentration of isolated phenols of *Matricaria chamomilla* flowers .

isolated extract	conc. (mg/mL)	inhibition zone diameter (mm)	
		Staphylococcus aureus	***Escherichia coli***
Phenols	25	10	8
	50	12	10
	75	19	16
	100	22	20

Table (10) shows the inhibition zone diameters which were recorded against positive and negative bacteria by using various concentrations of alkaloidic compound . It was found that the increase of concentration led to increase the antibacterial activity for both types of bacteria , also the concentrations (50 mg/mL and 75 mg/mL) were determined to be the minimal inhibition concentrations for *Staphylococcus aureus* and *Escherichia coli* bacteria strains respectively .

The studies represented that the alkaloids have a high inhibitory activity towards different microbes especially against positive and negative bacteria , because they inhibit metabolism of nucleic acids (DNA and RNA) by chemical bonding with both acids (12,25) . Also the medicinal activity of alkaloids , results from its antimicrobial role since they inhibit enzymes action and biosynthesis of proteins . Chemically alkaloids have heterogeneous nitrogenous rings contain imine group (– N = C ⟨) which is capable of decomposition and destruction of pathogenic bacteria cells (25,26).

Table (10) Results of antibacterial activity and minimal inhibitory concentration of isolated alkaloidic compound from *Matricaria chamomilla* flowers .

isolated compound	conc. (mg/mL)	inhibition zone diameter (mm)	
		Staphylococcus aureus	***Escherichia coli***
Alkaloid	50	9	0
	75	12	8
	100	16	10

Conclusions

This study proved that the phenols and alkaloidic compound which were isolated from *Matricaria chamomilla* flowers , have characteristic inhibition activity against pathogenic bacteria (*Staphylococcus aureus* and *Esherichia coli*) causing to skin infections , therefore these phenols and alkaloidic compound can be used as natural medicinal substituents instead of antibiotics having side effects , but this work demands further clinical studies .

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عـــزل وتشـــخيص الفينـــولات ومـــركب قلويـــدي مـــن أزهـــار نبـــات البـابونـــج (*Matricaria chamomilla*) ودراســة فعـاليتهـا الدوائيـة ضـد البكتريـا المرضيـة للالتهابات الجلدية

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الملخص :

تـم عـزل الفينـولات ومركـب قلويـدي مـن أزهـار نبـات البـابونج ودرسـت الصـفات الكيميائيـة والفيزيائية للمركبات المعزولة باستعمال الكشوفات النوعية وكشف المجاميع الفعالة وكروماتوغرافيا الطبقة الرقيقة ومطيافية الأشعة تحت الحمراء . أظهرت النتائج وجود مركبين فينوليين في مستخلص الفينولات ومركب قلويدي واحد في مستخلص القلويدات . تم قياس الفعالية الدوائية ضد نمو عزلتين من البكتريا المرضية للالتهابات الجلدية وهي الموجبة لصبغة كرام *Staphylococcus aureus* (NCTC6571) والسالبة لصبغة كرام (NCTC5933) *Escherichia coli* . وجد بأن التركيز (100 mg/ml) للفينولات المعزولة هو الأعلى فعالية تثبيطية حيث سجل قيم قطر تثبيط مساوية إلى 21 mm و 21 mm للبكتريا الموجبة والسالبة على التوالي . أما التركيز (100 mg/ml) كان الأعلى فعالية تثبيطية للمركب القلويدي المعزول حيث سجل قيمتي قطر تثبيط مساوية إلى 16 mm و 10 mm ضد البكتريا الموجبة والسالبة على التوالي .

تـم تحديـد التركيـز المثبـط الأدنـى (MIC) للفينـولات إذ كـان مسـاوياً لـ (25 mg/ml) لكـلا النوعين من البكتريا المرضية . كما تم قياس التركيز المثبط الأدنى للمركب القلويدي المعزول وكان مساوياً لـ (50 mg/ml و 75 mg/ml) للبكتريا الموجبة والسالبة على التوالي . وكنتيجة فأنه يوصى باستعمال المركب القلويدي والفينولات المعزولة كبدائل دوائية لعلاج الالتهابات الجلدية بدلاً من المضادات الحياتية المستعملة ولكن هذا العمل يتطلب المزيد من الدراسات السريرية .