

Isolation and Identification of Phenolic Compounds from *Elettaria cardamomum* Seeds and Study of their Medicinal Activity Against Pathogenic Bacteria of Prostate Gland

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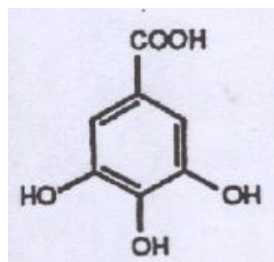
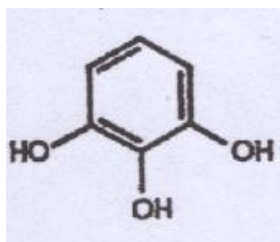
Abstract : Phenolic compounds were isolated from *Elettaria cardamomum* seeds by using organic and inorganic solvents. The chemical and physical properties of phenols were studied such as qualitative analysis, thin layer chromatography (TLC), functional groups test and infra-red spectroscopy. TLC results indicated presence of three compounds, two of them were flavonoids and the other was tanninic compound by using colour developers. The medicinal activity of phenolic

compounds was measured and determined against growth of two pathogenic bacterial strains of prostate gland are *Staphylococcus aureus* , positive towards Gram stain (NCTC 6571) and *Escherichia coli*, negative towards Gram stain (NCTC 5933). The concentration of (50 mg/ml) was recorded to be the most activity towards both types of the pathogenic bacteria with inhibition zone diameter equal to (25 mm and 15 mm) for positive and negative bacteria respectively.

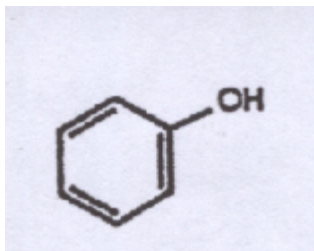
The minimal inhibitory concentration (MIC) was determined with value equal to (12.5 mg/ml) for both types of pathogenic bacteria. As a result, the phenolic compounds can be used as herbal drug substituent for treating diseases of prostate gland inflammatory instead of used antibiotics but this work demands further pharmaceutical and clinical studies.

Keywords: *Elettaria cardamomum*, Phenolic compounds, Pathogenic bacteria, Prostate gland, Medicinal activity

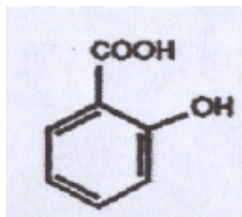
Introduction : Phenols are aromatic compounds contain one or more hydroxyl groups, and they are biosynthesid in secondary metabolism processes by shikimic acid pathway(1,2). Phenolic compounds are one of the largest groups of secondary plant constituents. In addition the aromatic benzene ring system, phenols may bear other substituent especially methyl groups. Simple phenols consist of aromatic ring in which a hydrogen is replaced by hydroxyl group (3,4). The simplest phenols are C_6 structures consisting of an aromatic ring with hydroxyl group attached. These include pyrogallol and hydroquinone (5). Their distribution is widespread amongst all classes of plants (6,7,8). The general properties of simple phenols are bactericidal, antiseptic and anthelmintic. Phenol itself is a standard for other antimicrobial agents. phenol, pyrogallol, gallic acid and salicylic acid are considered as simple phenols as shown in figure (1) (2, 9) .



pyrogallol



gallic acid



phenol

salicylic acid

Figure (1) some chemical structures of simple phenols

Salicylic acid is rarely found freely in plants, but usually occurs as glycosides (salicins), esters and salts. These derivatives are converted to salicylic acid in the human body (10, 11).

Many phenolic compounds were used as analgesic medicine, relief of head-a-ches, depressant on central nervous system, influence on prostaglandin metabolism, antipyretic, anti-inflammatory, anti-clotting agents, anti-coagulant, antispasmodic, and antifertility agents (9).

Phenolic compounds are chemically classified into many classes such as tannins, flavonoids, coumarins and

lignans. Tannins represent the largest group of polyphenols, they are widely distributed in the bark of trees, leaves, stem and fruits. Tannins are non-crystalline compounds which in water produce a mild acid reaction. Their ingestion gives rise to a puckering, astringent sensation in the mouth, the taste is sour and they often occur as glycosides. Their ability to precipitate proteins into insoluble complexes enables human to tan animals hides and convert them to leather. It is also the basis of their astringent effects. Due to protein precipitation, the tannins exert an inhibitory effect on many enzymes hence, contributing an anti-pathogenic protective function in bark and heartwoods of woody plant species (12,13).

Flavonoids are all structurally derived from the parent substance flavone. They are mainly water soluble compounds and they contain conjugated aromatic systems and thus show intense absorption bands in ultra violet and visible regions of the spectrum. Flavonoids are generally present in plants bound to sugar as glycosides and any one flavonoid aglycone may occur in single plant in several glycosidic combinations (14, 2).

Elettaria cardamomum (Cardamom) Heil, in Arabic, is one of perennial herbal medicinal plants of Indo-Malaya represented by a single species *E. cardamomum* Maton. The flowers of cardamom develop on leafless shoots which arise from the rhizome. It is very occasionally grown in the gardens, also the ripe fruits are picked up and dried and the seeds form a valuable spice (15). Cardamom (Heil) is an important commercial article, the seeds have pleasant aroma and characteristic, warm, slightly pungent taste. They are used as flavouring agent for many preparations like curries, cakes, bread and also for

flavouring coffee and tea. Medicinally it is an aromatic, stimulant and carminative. The plant also stomachic, and appears that notes of cultivation of this plant in Iraq are based on misidentifications. The oil extracted from the fruits of *Elettaria cardamomum* is used in pharmacy and perfumery (16, 15).

The aim of the current study is to isolate, identify and study of medicinal activity of phenolic compounds that isolated from *Elettaria cardamomum* seeds by using two pathogenic bacterial strains of prostate gland are *Staphylococcus aureus* and *Escherichia coli* bacteria.

Material & Methods

First: Materials:

Elettaria cardamomum seeds were gotten from local market of Abu Al-Khaseeb region in Basrah Governorate in south of Iraq. They were cleaned, dried, ground as powder and kept in plastic bottles in laboratory until to use.

Chemicals: All chemicals were of analytical grade and they were supplied as in the following:

Sulphuric acid, α -Naphthol, ethanol, sodium hydroxide, ammonium hydroxide, chloroform, hydrochloric acid, diethylether, ninhydrin, ferric chloride, lead acetate, copper sulphate, potassium hydroxide, potassium iodide, sub-nitrate bismuth, mercuric chloride, sodium citrate, sodium carbonate.

Culture medium: Muller Hinton medium was prepared according to information determining by Himedia company in India.

Bacterial strains: pathogenic bacterial strains of prostate gland were isolated, they are *Staphylococcus aureus* (positive towards gram stain) and *Escherichia coli* (negative towards Gram stain). These bacteria were grown well for measuring the medicinal activity against them.

Second: Methods

Isolation of phenolic compounds from *Elettaria cardamomum* seeds:

Fifty grams of seeds ground was mixed with 200 ml of hydrochloric acid (2%) and the mixture was placed in waterbath for one hour at 90°C. then the mixture was stirred on magnetic stirrer for two hours. Filtration was achieved by using Buchner funnel, the filtrate was treated with 200 ml of diethylether with the same volume of filtrate. The mixture was put other time in water bath for one hour, then it was evaporated by using rotary evaporator and finally crude phenols were gotten (4) with yield equal to 3.692 gm.

Preliminary qualitative analysis:

The phenolic compounds which were isolated were underwent to many detections such as:

1. Phenolic Compounds Detection:
It was carried out by using (1%) ferric chloride (17).
2. Flavonoids Detection:
It was achieved by using (5N) alcoholic potassium hydroxide (18).
3. Tannins Detection:
It was carried out by using (1%) lead acetate (19).
4. Carbohydrates Detection:
It was achieved by using Molish's reagent (2).

5. Alkaloids Detection:

It was tested by using Dragendroff 's reagent (20).

6. Saponin Detection:

It was carried out by using (5%) mercuric chloride (7).

7. Glycosides Detection:

It was achieved by using Benedict 's reagent (2, 14).

8. Amino Acids Detection:

It was tested by using (1%) ninhydrin (2).

Thin Layer Chromatography (TLC)

TLC technique was depended for separation of phenolic compounds presented in phenols extract and determination of their purity. Fifty microliters of phenols were toulencerced on glass plate (3×15 cm) coated by silica gel Butanol – Acetic acid – Water solvents were used as eluent with ratio (35: 5: 12). The separation time was 45 min, then the TLC was dried and the components were developed by iodine vapour, UV-lamp at 233 nm and (1%) ferric chloride. Rate of flow (R_f) values were measured for all spots (2).

Infra-red (IR) spectroscopy

FT-IR spectrum of phenolic compounds isolated, was recorded by using (FI-IR- 8400s-Japan) Spectrophotometer.

The phenols sample was mixed with potassium bromide (KBr) as a disk and spectral range for measuring was (600-4000 cm^{-1}).

Functional Groups Detection (21)

1. Double bond detection

It was achieved by using bromine and potassium permanganate reagents.

2. Phenols detection

It was carried out by using (1%) ferric chloride.

3. Aldehyde and keton groups detection

It was tested by using 2,4-dinitrophenyl hydrazine reagent.

Antibacterial activity study and determination of minimal inhibitory concentration.

Various several concentrations of phenolic compounds isolated (6.25, 12.5, 25 and 50 mg/ml) were carried out against growth of two pathogenic bacterial strains of prostate gland are *Staphylococcus aureus* and *Escherichia coli* in order to measure of inhibitory activity of phenols and to determine the minimal inhibitory concentration by using Muller-Hinton as a culture medium depending on diffusion method. The Petri dishes were placed in the incubator for 24 hr. at 37°C, then the inhibition zone diameters were calculated (22).

Results & Discussion

In this study phenolic compounds were isolated with extraction percentage equal to (7%). Preliminary qualitative analysis results of phenols isolated from *Elettaria cardamomum* seeds are shown in table (1). It was found that the phenolic compounds extract contain phenols, flavonoids and tannins whereas carbohydrates, glycosides, alkaloids, Saponin and amino acid were not present in phenols extract, this ensure that the phenolic compounds were isolated in high purity. Studies indicated presence of phenols as essential oils in *Elettaria cardamomum* (15)

Different medicinal plants were found to be contained the phenolic compounds such as *Picrorrhiza kurroa* which was used for treatment of urinary tract infection i.e. cystitis, urethritis, and prostatitis (23), *Curcuma longa* which have significant anti- inflammatory, hypertensive, and hepatoprotective properties (24), *Podophyllum peltatum* which was used as anticancer drug etoposide and teniposide (9), *Silybum marianum* which have hepatoprotective actions (5) and *Punica granatum* which was used for therapy of gastrointestinal disorders (25).

Table (1) Preliminary qualitative analysis results of phenolic compounds isolated from *Elettaria cardamomum* seeds

Reagents	Detection result	Indications	Conclusions
FeCl ₃ (1%)	+	Formation of bluish – green colour	Presence of phenolic compounds
Alcoholic KOH (5N)	+	Formation of yellow precipitate	Presence of flavonoids
Pb(Ac) ₂	+	Formation of brown – white precipitate	Presence of tannins
Molish	-	No violet ring	No carbohydrates
Benedicts	-	No red precipitate	No glycosides
Ninhydrin (1%)	-	No violet colour	No amino acids
HgCl ₂	-	No white	No Saponin

		precipitate	
Dragendroff	-	No orange precipitate	No alkaloids

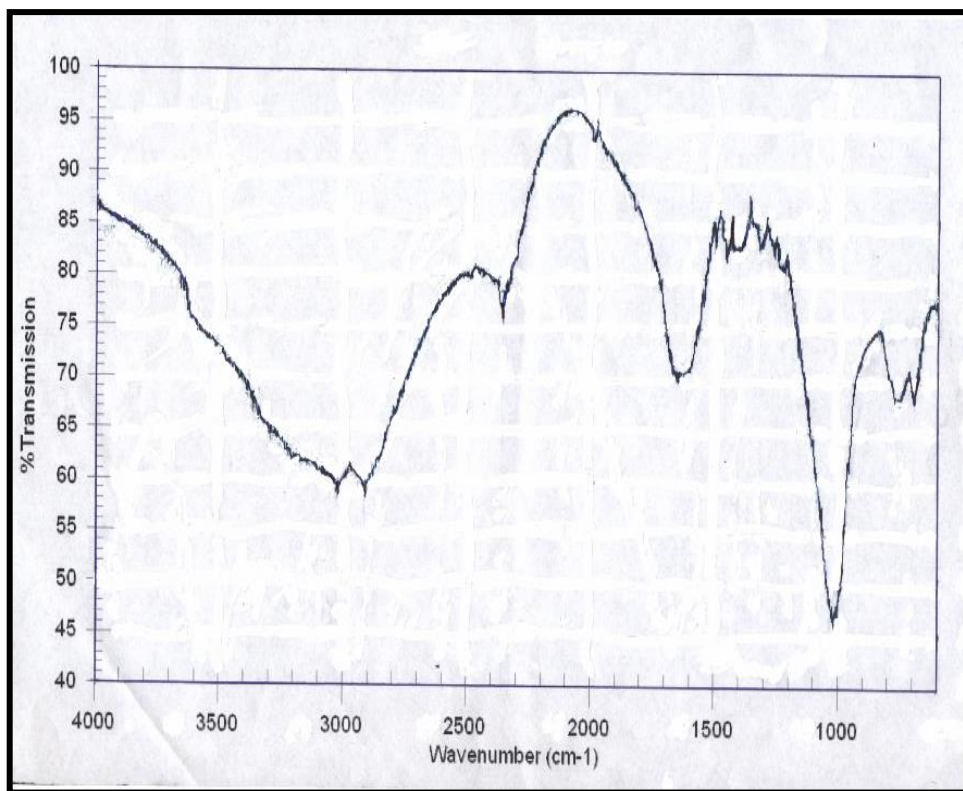
Table (2) indicates thin layer chromatography (TLC) results of phenols isolated from *Elettaria cardamomum* seeds. By using Butanol – acetic acid- water as eluent system, three spots were separated with rate of flow (R_f) values equal to 0.38, 0.46 and 0.72. Separation of three spots by TLC ensures presence of three phenolic compounds in phenols extract. These compounds separated are considered as organic compounds because they were tested by I_2 -vapour and also they have phenolic groups because of formation of green spots. The phenolic compounds contain double bond conjugation system since the spots appeared with light violet colour by using UV-lamp, also appearance of bluish-green spots by using ferric chloride as developer, ensures presence of phenols, therefore two of them are flavonoids and the third compound is tanninic one.

Table (2) Thin layer chromatography results of phenolic compounds isolated from *Elettaria cardamomum* Seeds

Eluent System	Reagent	Spot No.	Result
Butanol-acetic acid -water (35:5:12)	Eyes	3	Light green
	UV-lamp	3	Light violet
	I_2 -vapour	3	Brown
	$FeCl_3$ (1%)	3	Bluish-green

Rate of Rf value	Conclusion
0.38, 0.46, 0.72	Pure compound
0.38, 0.46, 0.72	Presence of double bond conjugation system
0.38, 0.46, 0.72	Presence of organic compounds
0.38, 0.46, 0.72	Presence of phenolic compounds

Results of furrier transformation – IR – spectrum of phenols isolated and absorption bands of functional and /or structural groups of this spectrum are shown in figure (2) and table (3) respectively. A broad band at 3030 cm^{-1} belongs to stretching vibration of phenolic hydroxyl group (-OH) which represent to hydrogen bonding in flavonoids and tannins. Appearance of broad band at wavenumber 2920 cm^{-1} indicates presence of vibration stretching of aromatic (C-H) group, also the medium band at 1660 cm^{-1} belongs to the aromatic keton group represents . Appearance of two medium and weak bands at 1615 cm^{-1} and 1500 cm^{-1} stretching vibration of aromatic (C=C) group but the sharp band at 1020 cm^{-1} belongs to etheric (C-O) group. Presence of intensity medium band at 670 cm^{-1} indicates stretching vibration of benzene ring containing aromatic substitution, also the medium band at 1420 cm^{-1} represents presence of bending vibration of (C-H) group belonging to methyl group (26).



**Figure (2) FI-IR-spectrum of phenolic compounds
isolated from *Elettaria cardamomum* seeds**

**Table (3) Structural and functional groups resulting
from absorption bands recorded in FI-IR-Spectrum
isolated from *Elettaria cardamomum* Seeds**

Band frequency (cm⁻¹)	Band	Band shape
3030	O-H	Broad
2920	C-H	Broad
1660	C=O	Medium
1615,1500	C=C	Medium, Weak
1420	C-H	Medium
1020	C-O	Sharp
670	Ar-X	Medium

B and assignment	Structural and functional groups
Stretching	Phenolic
Stretching	Aromatic
Stretching	Aromatic keton group
Stretching	Aromatic ring
Bending	Methyl group
Stretching	Phenyl etheric
Stretching	Phenyl ring substitution

Table (4) indicates functional groups detections results of phenolic compounds isolated from *Elettaria cardamomum* seeds. This table represents presence of phenolic rings, double bond and carbonyl groups therefore the phenolic compounds have rings of phenols, double bond conjugation system and aldehyde or keton groups. The flavonoids ring system is the structural unit of condensed tannins because this system consist of three rings, A-group contains hydroxylic phenolic groups, B- group contains catechol group and C- group which link between A and B groups together (27, 28).

Table (4) Functional group detections results of phenolic compounds isolated from *Elettaria cardamomum* Seeds

Reagent	Detection result	Indications	Conclusion
FeCl ₃ (1%)	+	Formation of bluish – green colour	Presence of phenols
Br ₂ /KMnO ₄	+	Disappearance of bromine & potassium permanganate	Presence of double bond system
DNPH	+	Formation of orange precipitate	Presence of aldehyde and/or keton groups

(+) positive detection

The medicinal antibacterial activity of phenolic compounds isolated from *Elettaria cardamomum* seeds is indicated in table (5). Various concentrations of these compounds were carried out against the positive and negative pathogenic bacteria concerning prostate gland . It was found that increase of concentration led to increasing in inhibition zone diameters for both pathogenic bacteria leading to increase antibacterial activity of phenolic compounds but this activity was greater against *Staphylococcus aureus* than *Escherichia coli*.

The inhibition zone diameters were recorded with values equal to (12, 18 and 25 mm) for *staphylococcus aureus* bacteria and (7, 12 and 15 mm) for *Escherichia coli* bacteria. Different studies indicated that medicinal plants phenols have high antibacterial activity, this means these compounds have ability to killing and inhibition of various pathogenic bacteria (29, 30, 31). The concentration of (50 mg/ml) showed the highest activity for both positive and negative bacteria.

Phenolic compounds contain hydroxyl group (-OH) in their chemical structure therefore this group has ability to bonding with proteins of pathogenic bacteria leading to break of sulphuric and hydrogen bonds existing in tertiary structure of cell proteins (31, 22). The phenols are capable of destruction of respiratory chain reactions and denaturation of proteins of living cell. Also the phenolic compounds inhibit the metabolism of protein biosynthesis by destructing the nucleic acid (DNA and RNA) which are responsible for production of different proteins in the cell (32, 33)

The phenols especially tannins have ability to form hydrogen bonds with carbohydrates and proteins by inhibition of some enzymes in the living cell leading to inhibit growth of microorganisms including pathogenic bacteria. Also the activity of phenolic compounds belongs to other chemical families abundant in phenols such as free phenols, tannins derivatives and flavonoids (34).

The minimal inhibitory concentration (MIC) was recorded with value equal to (12.5 mg/ml) for both positive and negative pathogenic bacteria of prostate gland, this concentration (MIC) is defined as the concentration which give lowest inhibition zone diameter. But it was noticed that the MIC against *Staphylococcus aureus* was highest compared with *Escherichia coli* because the positive bacteria contain less dense lipid layers in the cell wall, whereas negative bacteria has more lipid layers in the cell wall therefore the permeability of phenolic compounds towards the *Staphylococcus aureus* bacteria is more than *Escherichia coli* bacteria cell (30). Also phenols lead to disorder in carbohydrates, fats and proteins metabolism leading to death bacteria. Some studies ensured that plant phenolic compounds inhibit respiratory chain enzymes containing thiol group (-SH) by substituting it with carbonyl group existing in phenols after oxidation of hydroxyl group (-OH) by molecular oxygen then elimination of hydrogen molecule (6, 29).

Table (5) Antibacterial medicinal activity and minimal inhibitory concentration (MIC) of phenolic compounds isolated from *Elettaria cardamomum* Seeds

Extract	Conc. (mg/ml)	Inhibition zone diameters (mm)	
		<i>Staphylococcus aureus</i> (+)	<i>Escherichia coli</i> (-)
Phenolic compounds	50	25	15
	25	18	12
	12.5	12	7
	6.25	0	0

Conclusions

Phenolic compounds isolated from *Elettaria cardamomum* seeds proved a high medicinal activity against pathogenic bacteria represented by *staphylococcus aureus* and *Escherichia coli* which isolated from prostate gland inflammation. The concentration of (50 mg/ml) of phenols showed higher inhibitory activity against growth the both Gram positive and Gram negative bacteria and the concentration of (12.5 mg/ml) of phenolic compounds, was minimal inhibitory concentration for both pathogenic bacteria. As conclusion, the phenolic compounds isolated with concentration of (50 mg/ml) may be used as herbal therapy substituent instead of antibiotics used but this work demands further pharmaceutical and clinical studies.

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**عزل و تشخيص المركبات الفينولية من بذور نبات الهيل *Elettaria*
*cardamomum***

و دراسة فعاليتها الدوائية ضد البكتريا المرضية لغدة البروستات

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الملخص: تم عزل المركبات الفينولية من بذور نبات الهيل بإستعمال مذيبات عضوية ولاعضوية. درست الصفات الفيزيائية و الكيميائية و هي التحاليل النوعية و كروماتوغرافيا الطبقة الرقيقة (TLC). و كشف المجاميع الفعالة و مطيافية الأشعة تحت الحمراء لهذه المركبات. أشارت نتائج TLC إلى وجود ثلاث مركبات إثنين منها كانت فلافونيدات و الآخر هو مركب ثانيني و ذلك بإستعمال مظهرات لونية. تم قياس و تعيين الفعالية الدوائية للمركبات الفينولية ضد نوعين من العزلات البكتيرية المرضية المرافقة لخمج لغدة البروستات و هي الموجبة لصبغة كرام Staphylococcus aureus (NCTC 6571) و السالبة لصبغة كرام Escherichia coli (NCTC 5933). وجد بأن التركيز ٥٠ ملغم/مل الذي تم تسجيله هو الأعلى فعالية تجاه كلا النوعين من البكتريا المرضية بقطر منطقة تثبيط مساوٍ إلى ٢٥ ملم و ١٥ ملم للبكتريا الموجبة و السالبة على التوالي. تم تعيين التركيز المثبط الأدنى (MIC) بقيمة مساوية إلى ١٢,٥ ملغم/مل لكلا النوعين من البكتريا. كنتيجة فأن المركبات الفينولية المعزولة يمكن إستعمالها كبدايل دوائية عشبية لعلاج أمراض خمج إلتهاب غدة البروستات بدلاً عن المضادات الحياتية المستعملة ، لكن هذا العمل يتطلب المزيد من الدراسات الصيدلانية و السريرية.