Antibacterial activity of some plant extract against Escherichia coli

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Graduation requirements

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وَأَن لَّيْسَ لِلْإِنسَٰنِ إِلَّا مَا سَعَىٰ

﴿ الآية ٣٩ ﴾

وَأَنَّ سَعْيَهُۥ سَوْفَ يُرَىٰ

﴿ الآية ٤٠ ﴾

الاهداء

وصلت رحلتنا الدراسية إلى نهايتها بعد تعب ومشقَّة..

وها نحن نختم بحث تخرُّجنا بكل همَّة ونشاط،

و نمتنُّ لكل من كان له فضل في مسيرتنا،

وساعدنا ولو باليسير،

الأبوين، والأهل، والأصدقاء، والأساتذة المُبجَّلين..

ثُم لا ننسى كُليتنا بالذكر، الَتي كان لها منطلق كل ما أبتدئنا هذا، وما نحن اليوم عليه، هوَ نتاج ما تتلمذنا عليه داخل اروقتها.

نُهديكم ثَواب عِلمَنا
و أجر سقاية المرضى...

شكر و تقدير

اللهم لك الحمد حمداً كثيراً طيباً مباركاً فيه، ملء السموات وملء الأرض، وملء ما شئت من شيء بعد، أهل الثناء والمجد، أحق ما قال العبد، وكلنا لك عبد، أشكرك ربي على نعمك التي لا تعد، وآلائك التي لا تحد، أحمدك ربي وأشكرك على أن يسرت لي إتمام هذا البحث على الوجه الذي أرجو أن ترضى به عني..

ثم نتوجه بالشكر إلى من راعانا طلاباً في لنيل شهادة البكالوريوس، ومعداً هذا البحث مُشرفينا الافاضل (م.د عُلا عباس ، م.د طيف حسين) اللذين لهم الفضل- بعد الله تعالى- على البحث والباحثين منذ كان الموضوع عنوانا وفكرة إلى أن صار بحثاً فله منا الشكر كله والتقدير والعرفان.

وكذلك نشكر كل من ساعد على إتمام هذا البحث وقدم لنا العون ومد لنا يد المساعدة وزودنا بالمعلومات اللازمة لإتمام هذا البحث والذين كانوا عونا لنا في بحثنا هذا ونورا يضيء الظلمة التي كانت تقف أحيانا في طريقنا.

الباحثون

هـ ١٤٤٥

Abstract

Background: Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria. The aim of this study is to measure the effect of plant extracts on *E. coli* growth to determine the antibacterial activity of them .

Methods: Experimental, in vitro, College of pharmacy, evaluation of the activities of four plant extracts against *E. coli*. The activity was determined by well diffusion method at a concentration of 10%.

Result: The antibacterial activity of four aqueous plant extracts (*Peganum harmala, Piper nigrum*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*) has been evaluated against *E. coli* (Isolated from urinary tract infection). The concentration was used for each type of extract 10mg\ml. In this concentration, the aqueous extract was effective against *E. coli* with inhibition zones of 18 mm, 15 mm and 14 mm respectively, and extract of *Cinnamomum zeylanicum* shown no inhibition zone.

Conclusion: The aqueous extract of *Peganum harmala* against *E. coli,* showed the highest inhibition of 18mm with the concentration 10%. Whereas, aqueous extract of *Cinnamomum zeylanicum* has no antibacterial activity against *E. coli*.

Abstract

الخلاصة

المقدمة: تعتبر النباتات الطبية مصادر جديدة لإنتاج المواد التي يمكن أن تعمل كبدائل للمضادات الحيوية في علاج البكتريا المقاومة للمضادات الحيوية. الهدف من هذه الدراسة هو لتحديد التأثير التثبيطي للمستخلصات النباتية على نمو بكتريا *E. coli*.

طرق العمل : اجريت التجربة في مختبرات كلية الصيدلة ، لتقييم التأثير التثبيطي لبعض المستخلصات المائية للنباتات قيد الدراسة بطريقة النشر بالحفر بتركيز 10%.

النتائج: اوضحت نتائج الدراسة الحالية تأثير المستخلصات المائية للنباتات (الحرمل, الفلفل الأسود, القرنفل, الدارسين) ضد بكتريا القولون *E. coli* المعزولة من حالات التهاب المجاري البولية, حيث كان التركيز المستخدم ١٠٠ملغم/ لتر، اظهرت النتائج اعلى تأثير تثبيطي بقطر ١٨ملم، ١٥ ملم، ١٤ ملم على التوالي بينما لو يظهر اي تأثير تثبيطي لنبات الدارسين.

الاستنتاجات : اعلى تأثير تثبيطي للمستخلصات المائية النباتية لنبات الحرمل بينما لم يظهر نبات الدارسين اي تأثير على نمو البكتريا.

Chapter one
Introduction

1.1- Introduction

In recent years, the world has turned its attention to the study of medicinal plants, many of which have been found to have inhibitory effect against pathogens . They have been used in the treatment of many diseases, since they contain effective compounds that are inhibitory and free from side effects compared with the drugs used which have side effects on health with increased resistance towards it by time. This has called for urgent and continuous need to search for new antimicrobials as a result of increase in disease cases. The other reason is increasing resistance to antibiotics and on a continuous basis (Al-Hadidy, *etal.,* 2019).

Medicinal plants contain various types of natural active substances used in traditional medicine (alternative medicine) to treat diseases around the world, the efficiency of these medicinal plants or their extracts varies depending on the method of extraction, the type of solvent used in the extraction and the microscopic organism (Husein, 2010).

There are more than 20,000 species of all identified medicinal plants used internationally listed according to World Health Organization (WHO) (Maria *et al*., 2014).

Some of the compounds isolated from these plants proved to be a very effective preventive medicine and used to treat complex cases such as cancer diseases (Hassan, 2012). The critical necessities to use medicinal plants do not involving severe feature control concerning to safety and efficiency compared to the other drug types (Palhares, 2015).

1.2- Aime of study:

The aim of this study was to evaluate the antibacterial activity of aqueous plant extracts (*Peganum harmala, Piper nigrum*, *Syzygium aromaticum* and *Cinnamomum zeylanicum)* against *E. coli.*

2- Literatures review

2.1. The medicinal plants

Since the beginning of civilization, survival of the human race was dependent on plants, not only as a source of food and oxygen, but also as a source of natural remedies (Muthu *et al.,* 2010).

Medicinal plants have very strong relationship with the human society since the dawn of human civilization. According to the reports of world health organization (WHO), about 80% of the third world populations rely on traditional herbal therapies (Dubey *et al.,* 2012).

There are several studies that have been published to prove the efficacy of the antimicrobial potency and analyzed the therapeutic potential of the plants (Mustafa *et al.,* 2016).

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties. The screening of plant extracts has been of great interest to scientist for the discovery of new drugs effective in treatment of several diseases (Bhalodia and Shukla, 2011).

2.1.1. *Peganum harmala* (harmal)

Peganum harmala as a member of family Zygophyllaceae, also known as Harmal or Syrian rue, used in traditional medicine from ancient times, is considered an important medicinal plant for the treatment of a variety of human ailments (Asgarpanah and Ramezanloo, 2012). Peganum seed contain several alkaloids that are pharmacologically active and responsible of their effect (Diba *et al.* 2011).

There are various reports that *P. harmala* had different pharmacological activities including spontaneous effect, anti-tumor effect, insecticidal effect, caving malaria, anti-leishmanial, anti-spasmodic, anti-histaminic, vasorelaxant effect, wound healing, anti-oxidant activity, leukemic healing, hypoglycemic effect, immuno-modulator properties, analgesic and anti-inflammatory properties (Muhi-

eldeen *et al.,* 2008). Also, it has been reported that this plant had antibacterial, antifungal and antiviral effects (Shonoudam *et al.,* 2008).

2.1.2 *Cinnamomum zeylanicum* (cinnamon)

Cinnamomum zeylanicum (cinnamon) an evergreen tree, belongs to the family Lauraceae, which has been traditionally harvested in Asian countries. It is one of the oldest herbal medicines that have been mentioned in Chinese texts as early as 4,000 years ago. The bark of cinnamon possesses significant antiallergic, anti-ulcerogenic, antipyretic and antioxidant properties (Dhuley, *etal.,* 1999).

Water soluble cinnamon compounds in the diet could reduce risk factors associated with diabetes and cardiovascular disease because of its antioxidant effects (Roussel, *etal.,* 2009). Cinnamomum barks and leaves are widely used as spice and flavoring agent in foods (Schmid *et al.,* 2006).

Medicinally, cinnamomum is used for treating diarrhea, flatulent dyspesia, colic, colds, poor appetite, low vitality, kidney weakness, rheumatism. Oils and extracts from cinnamomum possess a distinct antioxidant activity, which is especially attributed to the presence of phenolic and polyphenolic substances (Jayaprakasha *et al.,* 2006).

The essential oil of plants such as cinnamon oil has both antibacterial and antifungal compounds that can be used for the prevention of food spoilage due to microbial contamination (Mahmoud, 2012; Al-Sahlany, 2017). In addition, some protein is also present in the bark. These substances are believed to play an important role in the antibacterial activity (Tung *et al.,* 2008).

There are some reports on antimicrobial activity of *Cinnamomum zeylanicum* against Gram positive and Gram negative bacteria, viruses, moulds and yeasts. The results have ranged according to the microorganism and assayed product (essential oil, extracts, decoct, plant powder). Phytochemicals are small organic biomolecules designated as naturally occurring antibiotics (Burt, 2004).

2.1.3 *Piper nigrum* (black pepper)

Piper nigrum (black pepper), which belongs to Piperaceae family , is commonly known as black pepper and has culinary and medicial uses (Roy, *et al.*, 2013). Medicinal properties of *Piper nigrum* have been comprehensively reviewed and documenetd by Ahmad *et al.* (2012) Damanhouri *et al.* (2014). This could be due to several organic chemicals including essential oil, alkaloids, flavonoids, phenols, lignans, acids, amides and other aromatic compounds it contains (Roy, *et al.,* 2013).

Piper nigrum used as a traditional medicine. There are many compounds are present in the *Piper nigrum* but the most important is piperidine. The biological role of piperine which is commonly known is antioxidant, anticancer, antipyretic, anti-inflammatory, anti-microbial agent and many more (Ahmad *et al.*, 2012).

Khan and Siddiqui in 2007 evaluated the antibacterial potential of aqueous decoction of *Piper nigrum*, against different bacterial isolates from oral cavity, black pepper (aqueous decoction) showed strongest antibacterial activity.

2.1.4 *Syzygium aromaticum* (clove)

Syzygium aromaticum commonly known as clove, is an median size tree (8-12 m) from the Mirtaceae family native from the east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Shan *et al.,* 2005)

The antimicrobial activities of clove have been proved against several bacteria and fungal strains. Sofia *et al.* 2007 tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sampled that showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli (E. coli), Staphylococcus aureus* and

Bacillus cereus was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

In another work published by Dorman and Deans 2000, the antibacterial activity of black pepper, geranium, nutmeg, oregano, thyme and clove was tested against 25 strains of Gram positive and Gram negative bacteria. The oils with the widest spectrum of activity were thyme, oregano and clove respectively. In addition to its anti-microbial, anti-fungal, and anti-viral properties, clove possesses anti-inflammatory, cytotoxic, and anesthetic properties (Chaieb *et al.* 2007).

2.2 Urinary tract infection and *E. coli*

Urinary tract infection UTI is one of the common infectious diseases that were with global distribution, caused by both Gram-negative and Gram-positive bacteria (Foxman, 2014).

E. coli is the common cause of many infections such as: urinary tract infections, diarrhea and bacteremia, can cause these infections because it have many virulence factors like adhesion ,iron uptake, toxins, capsular polysaccharides. *E. coli* is the most predominant pathogen causing 80-90% of community-acquired UTIs and 30-50% of nosocomially-acquired UTIs (Oliveira *et al.,*2011).

E. coli is is a gram-negative, rod-shaped a facultative anaerobe that can grow from 7ºC to 50ºC with an optimum temperature of 37ºC. (Xia, 2010).

In Iraq, urinary tract infection represent a public health problem with impact on quality of life (Alsamarai *et al.,* 2016). A urinary tract infection is caused by bacteria that enter the urinary tract; women are more likely than men to get UTI because of their urinary tract's design, (Shaaban *et al.,* 2012). Most of UTI are caused by gram-negative bacteria like *Escherichia coli, Proteus mirabilis, Proteus vulgaris Klebsiellasp, Pseudomonas aeruginosa, Acinetobacter, Serrati, and Morganella morganii.* Also UTI are caused by Gram positive bacteria include

Enterococcus, Staphylococcus, and Streptococcus agalacticae (Tangho and Mcaninch, 2004).

3. MATERIALS AND METHODS

3.1: Instruments

The following instruments were used in this study as listed in table (3-1).

Table (3-1): Instruments, their manufacturer companies and its origins.

No.	Instruments	Manufacturer company and Origin
1	Centrifuge tube	NURE /turkey
2	Incubator	Biomerieux/USA
3	Autoclave	Hirayama/japan
4	oven	Biomerieux/USA
5	Sensitive balance	Precia/swesra
6	Petri dish	Al-hanoof/jordon
7	Loop	Al-rawan/china

3.2: Chemicals and Biological Materials

The chemicals and biological materials used in this study are mentioned in the table (3-2).

Table (3-2): The chemicals and biological materials as well as their suppliers and origin.

No.	Name of material	Supplier / Origin
1	Muller Hinton agar	Oxoid /united kingdom
2	Nutrient agar	Oxoid /united kingdom

3.3. Samples

The plant materials were purchased from a local market, in Babylon Province, which include

Table (3-3): Scientific name of plant

No	Scientific name of plant
1	*Peganum harmala* (Harmal)
2	*Piper nigrum* (Black pepper)
3	*Syzygium aromaticum* (Clove)

4	*Cinnamomum zeylanicum* (Cinnamon)

The plant material were washed with distilled water and dried in shade separately at room temperature. Most of the moisture has been removed, the plant material grounded in a mill to produce fine powdered.

3.4. Study design

All plants were convert to fine powder

↓

5 gm of plant powder

↓

50 ml D.W

↓

Concentrated the extract by using an oven at 40 c ^{o}

↓

1g\ 10ml of each plant extract against *E.coli*

↓

By the well diffusion method 50µ

3.5. Extract preparation

We prepared the plant extracts of (*Peganum harmala, Piper nigrum*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*) according to Harbone, 1984 that include the following : All plants were convert to fine powder and freshly prepared at the day of experiment using distilled water . In this method, the dried powder plants (5g) were soaked in 50 ml of distilled water. The solvent was evaporated by using an oven at 40 c ^{o} and then stored the dry powder in the

refrigerator for further use to test the effectiveness against the bacteria used in the experiment.

3.6 Antibacterial activity

The antibacterial activities of the plant extracts (*Peganum harmala, Piper nigrum*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*) were tested using Muller-Hinton plats by the well diffusion method. The medium was prepared according to the manufacture company instructions, and it was sterilized by autoclave at 121ºC (1.5 psi/inch 2) for 15 min. Wells were cut into the agar and filled with 50 uL of the plants extract. After that the plates were incubated at 37ºC for 24h.The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

3.7. Determination of antibacterial activity (Agar Well Diffusion Method)

Muller Hinton agar plates were prepared and inoculated with test organisms by spreading the bacterial inoculum on the surface of the media with the help of sterile swab. Wells were punched in the agar by using cork borer. Extracts with concentrations (10 mg/ml) were added. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and recorded in mm.

4. Results and Discussion

Results of antibacterial activity of four aqueous plant extracts (*Peganum harmala, Piper nigrum*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*) against *E. coli* (Isolated from urinary tract infection).

Table (4-1): The activity of aqueous extracts against *E.coli.*

Name of plant	Inhibition zone of diameter
Peganum harmala	18 mm
Piper nigrum	15 mm
Syzygium aromaticum	14 mm
Cinnamomum zeylanicum	No inhibition zone

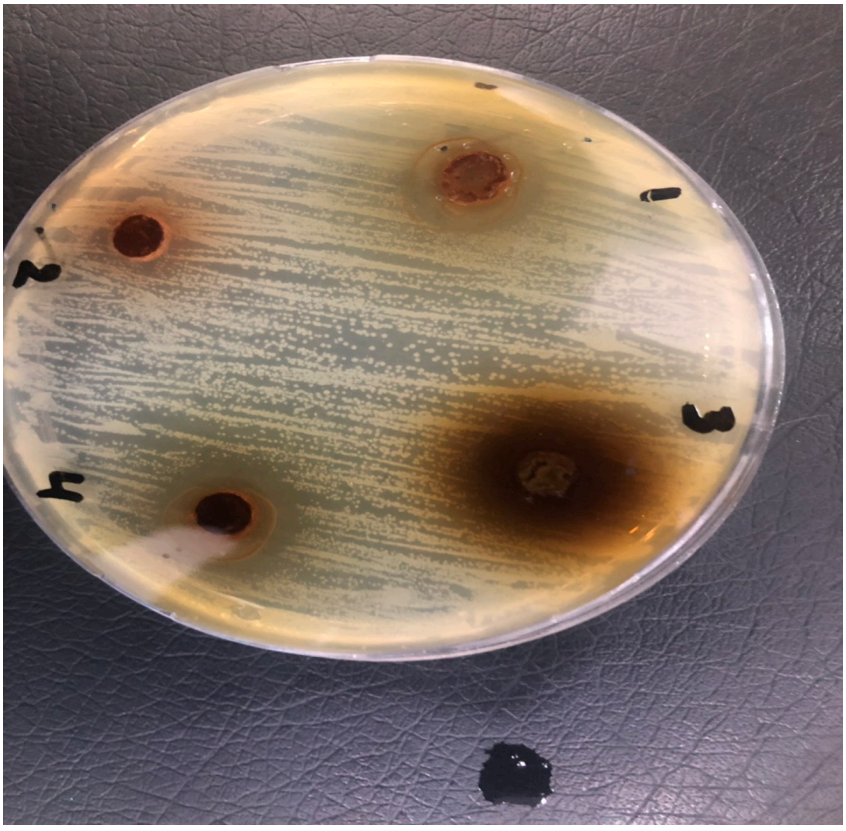

Figure (1): Inhibition zones of *E.coli* growth on Mueller-Hinton agar produced by (1) black pepper (2) Cinnamon (3) clove (4) harmalcontained extract concentrations (10 mg/ml), whereas the central well contained 50 µl.

These results in table(1) revealed that this bacteria was sensitive to the concentrations of 10% in aqueous extract of harmal *(Peganum harmala*) with inhibition zone 18 mm, that agree with Abid, etal., 2008 which revealed the highest effect on bacterial growth since the hot extract reduced growth of most of bacterial isolates (*E.coli*). Voravuthikunchai *et al.*,2004, which obtained good antibacterial activity in harmal and pomegranate against *E.coli and Klebsiella* using aqueous extract.

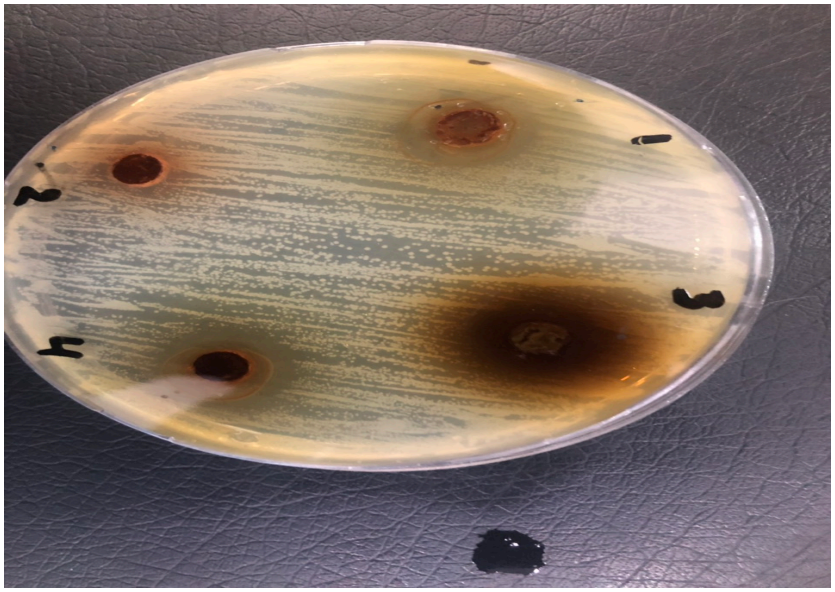

Figure (2) Inhibition zones of *E.coli* growth produced by harmal contained extract concentrations (10 mg/ml), whereas the central well contained 50 µl.

And aqueous extract of black pepper (*Piper nigrum*) showed in Table1 that antibicrobial activity against *E. coli* with inhibition zone 15 mm, that agree with Shiva Rani*, etal.,* 2013 reported the antibacterial effect of piperine against *E. coli* with inhibition zone (8mm). Also with Khan, 2007 which he mentioned that black pepper (aqueous decoction) showed strongest antibacterial activity and in research against different bacterial isolates from oral cavity of two hundred individual volunteers.

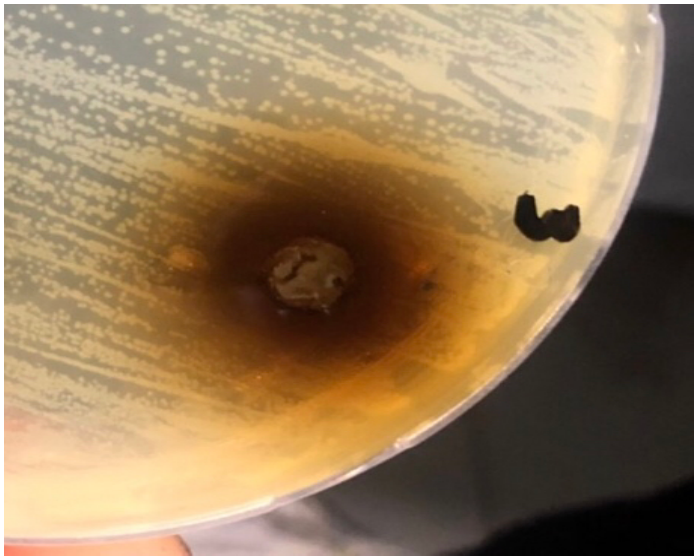

Figure (3) Inhibition zones of *E.coli* growth produced by black pepper contained extract concentrations (10 mg/ml), whereas the central well contained 50 µl.

Our results of clove *(Syzygium aromaticum*) revealed antibacterial activity against *E. coli* with inhibition zone 14 mm, that agree with, Hussein and Hanon, 2017, which reported the result of antibacterial susceptibility to *S.aromatic* in aqueous extract the average diameter zone of inhibitions was 31mm, 8mm, 14.33 to the *S.aureus*, *E.coli* and *P.aeruginosa* respectively. These results are also evidenced through the work done by Michael, *etal.*, 2010.

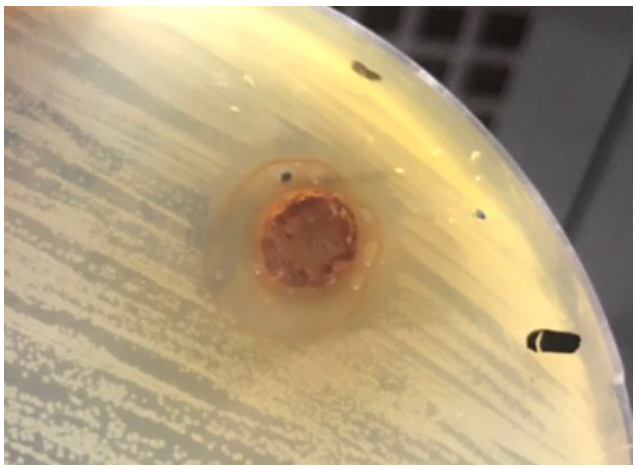

Figure (4) Inhibition zones of *E.coli* growth produced by clove contained extract concentrations (10 mg/ml), whereas the central well contained 50 µl.

While *Escherichia coli* showed resistance against aqueous concentration extract of Cinnamon *(Cinnamomum zeylanicum*) as showed in table (1).

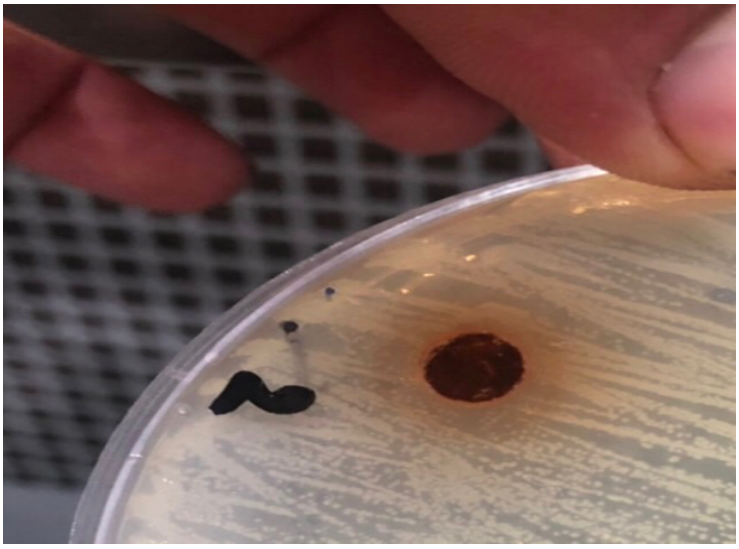

Figure (5) Inhibition zones of *E.coli* growth produced by Cinnamon contained extract concentrations (10 mg/ml), whereas the central well contained 50 µl.

Conclusions

1- Plant extracts contained a very complex structure with the active ingredients present in the form of natural organic compounds.

2- The process of extraction for a particular compound is dependent on the solubility of the component in the solvent (water or organic solvent).

3- The extracts of the tested plants demonstrated good potential antibacterial activities. The potential to develop antimicrobial compounds from higher plants appears rewarding as it will propel to the expansion of a phytomedicine to turn against multidrug resistant microbes.

4- The aqueous extract of *Peganum harmala* against *E. coli,* showed the highest inhibition of 18mm with the concentration 10%. Whereas, aqueous extract of *Cinnamomum zeylanicum* has no antibacterial activity against *E. coli.*

Recommendations

1- The extracts of these plants should be further analyzed to isolate the specific antibacterial principles in them.

2- Toxicity studies of the effective plants should also be done to determine the safety indices of the extracts. Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of these infectious diseases.

-3 Determine the activity of these plant extracts on the types of fungi as *Candida albicans*, in addition to the synergistic activity of these medicinal plants with antibiotics and Non-antibiotics.

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Antibacterial activity of some plant extract against Escherichia coli

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