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Effects of *Coriandrum sativum*, *Thymus vulgaris*, *Borago officinalis* and *Pimpinella anisum* on biofilm *Escherichia coli*

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Abstract

The aim of this study was to evaluate the efficacy of *Coriandrum sativum, Thymus vulgaris, Borago officinalis* and *Pimpinella anisum* against clinical isolates of biofilm *Escherichia coli*. These herbs are an important medical herbs used traditionally for their antimicrobial properties. Crude water and ethanol extracts of *Coriandrum sativum*, *Thymus vulgaris*, *Borago officinalis* and *Pimpinella anisum* were tested for inhibition of biofilm *E. coli* in vitro by the microtitre plates method. Results revealed that the isolates showed different potential to form biofilm under the same conditions of experimentation. The highest biofilm formation strains were *E. coli* numbers 3, 19, 23 and 24 isolates, absorbance of them (OD) were: 1.641, 1.107, 1.118 and 1.013, respectively. We selected these isolates to investigate the *In vitro* antibiofilm activity of water and ethanol extracts of *Coriandrum sativum*, *Thymus vulgaris*, *Borago officinalis* and *Pimpinella anisum*, the ethanol plant extracts were less effected than watery plant extracts .The effect of watery and ethanol plant extracts was more effect when plant extracts were added before formation of biofilm(incubation of bacterial isolates for 48h. at 37°C) than in the case of adding the plant extracts after formation of biofilm(incubation of bacterial isolates for 72hr. at 37°C). The most effected plant extract in inhibition of biofilm formation was *Thymus vulgaris* while the most non effected plant extract in inhibition of biofilm formation was *Borago officinalis*. In conclusions, the present study shows differences in results observed when slime forming *E. coli* clinical isolates treated with watery and ethanol plant extracts. *Thymus vulgaris* can be used as natural products to treated biofilm *E. coli*

Keywords*: Escherichia coli*, Biofilm, Plant extracts,

Introduction

Escherichia coli is an important bacterial species, belonging to the family Enterobacteriaceae, large heterogeneous group of Gram negative rods whose natural habitat is the intestinal tract of human and animal (Atlas, 1984). *E. coli* is a gram-negative, facultative anaerobic, rod-shaped bacterium (Brooks *et al.*, 2007). Most *E. coli* isolates are harmless to hosts, some are useful for producing sources of B and K vitamins for the host but some isolates can cause illness once they leave their natural habitat. It may cause urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia (Fratamico and Smith, 2006). Biofilms have great importance for public health because of their role in certain infectious diseases and importance in a variety of device-related infections (Characklis, 1973). Biofilm communities of microbes is different from their planktonic counterparts in very important ways. First, when microbes live as a community, they become much less susceptible to antibiotics, even if highly susceptible as individual cells. Thus, when microorganisms form a community, they are protected against a variety of antibiotics that clinicians commonly prescribe for their patients. Second, and more the focus of this perspective, these communities of microorganisms resist attack and killing by the host immune system (Leid, 2009).

Plant-derived compounds such as tannins, phenolic compounds and so on, are usually found in various parts of the plants like roots, leaves, shoots and bark. Many plants have therefore gained widespread interest in the search to identify the alternatives for microbial control and treatment of various infections and diseases as some chemically synthesized drugs have undesirable side effects (Sandasi, *et al.*, 2010). Medicinal plants have been investigated to possess antibiofilm activities (Chusri *et al.*, 2013). This study was undertaken to

investigate the in vitro anti-biofilm activity of water and ethanol extracts obtained from four types plants (*Coriandrum sativum, Thymus vulgaris, Borago officinalis* and *Pimpinella anisum*) on biofilm producing by *E. coli.* These plants used traditionally in our country to prevent different infections and diseases in children. To our knowledge, this study is the first to demonstrate the effects of crude plants extracts on biofilm formation *E. coli* strains isolated from blood and urine of neonates in Iraq.

Materials and Methods

Isolation and identification of *Escherichia coli*: A total of 30 clinical isolates of *Escherichia coli* were isolated from urine and blood samples of children inpatients age ≤5 months in three separate children hospitals in Baghdad/Iraq . The isolates were received at a period of three month (from 1 October till 31 December 2013). Bacterial isolates were identified depending on morphological characteristics and Vitek-2 system (Bio-Merieux , France) using ID-GNB cards according to the manufacturer's instructions.

Antibiotic Susceptibility Test: Antibiotic susceptibility test towards ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobbactam, cefazolin, ceftriaxon, ceftazidime, cefepime, ciprofloxacin, gentamicin, imipenem, meropenem, nitrofurantoin, levofloxacin, tobramycin and trimethoprim/ sulfamethoxazole was done by Vitek-2 system (Bio-Merieux, France) using AST cards according to the manufacturer's instructions

Detection of Biofilm Formation: All the 30 *E. coli* isolates were subjected to biofilm production. The methods used for testing biofilm production in *E. coli* include qualitative Congo red agar plate test (CRA test) and quantitative (tube method (TM) and microtitre plate assay (MTP)).

Congo red agar (CRA) method: This method is based on the characteristic cultural morphology of biofilm-forming bacteria on Congo red medium. Qualitative evaluation of biofilm producers using the Congo red agar method to detect slime production was performed as follows: The medium is comprised of brain heart infusion broth (Hi media /India) 37 g/l, sucrose (BDH / England) 50 g/l, Congo red (Fluka) 0.8 g/l and agar (Bio life /Italy) 10 g/l. Inoculated plates were incubated at 37°C for 24hr. Slime producing strains presented black colonies while non-producing strains developed red colonies. The scale of colony color evaluation was assessed as follows: very black and black colonies were biofilm producing strains, almost black colonies were weak biofilm producers while very red to Bordeaux colonies were considered as non-biofilm producing (Saising *et al.*, 2012).

Tube method(TM): The quantitative assay for biofilm formation was performed as follows: Two sets of glass tubes filled with 3ml of trypticase soy broth (Hi media/ India) were inoculated with a loopful of a pure culture of a strain of *E. coli* grown overnight from blood agar plate. After 24 and 48 hr. incubation at 37°C, the content of each tube was decanted. The tubes were then stained with 1% crystal violet (1 gram crystal violet in 100 ml D. W.) for 7 min. Then the tubes are washed with distilled water for 5 min. A positive result was indicated by the presence of an adherent film of stained material on the inner surface of the tube. Presence of stained material at the liquid-air interface alone was not regarded as indicative of slime production. Tubes containing trypticase soy broth only were included in the test as negative controls (Dheepa *et al.*, 2011).

Microtitre-plate (MTP) method: Biofilm formation was determined as follows: Studied *E. Coli* isolates cultured in Brain Heart Infusion (BHI) broth (Hi media /India) incubated at 37°C for 18hr, after that bacterial culture was diluted in BHI broth and adjusted in comparison to MacFarland tube no. 0.5. Two hundred microliters of this bacterial culture were used to inoculate pre-sterilized 96-well polystyrene microtiter plates and later incubated for 48 hours at 37°C. After incubation, all wells were washed with sterile physiological saline for the elimination of unattached cells. Afterward, 200 µl of 1% crystal violet was added to each well, shaking the plates three times to help the colorant to get the bottom of the well. After 15min. at room temperature, each well was washed with 200µl sterile physiological saline. This process was repeated three times. The crystal violet bound to the biofilm was extracted later with 200µl of ethyl alcohol, and then absorbance was determined at 540nm in an ELISA reader (Human). Controls were performed with crystal violet binding to the wells exposed only to the culture medium without bacteria. All the assays were performed in triplicates (Maldonado *et al.*, 2007).

For the purpose of comparative analysis of test results, the adherence capabilities of the test strains were classified into the following under four categories: non-adherent (0), weakly (+), moderately (++), or strongly (+++) adherent, based upon the ODs of bacterial films. The cut-off optical density (ODc) for the microtitre-plate is defined as three standard deviations above the mean OD of the negative control. Strains were classified as

follows: OD ≤ ODc non-adherent, ODc < OD ≤ 2 x ODc weakly adherent, 2 x ODc < OD ≤ 4 x ODc moderately adherent, 4 x ODc < OD strongly adherent (Dheepa *et al.*, 2011).

Plant materials: Four plant species including: *Coriandrum sativum, Thymus vulgaris, Borago officinalis* and *Pimpinella anisum* were selected for inhibitory activity of biofilm formation by *E. coli*.

Preparation of hot watery extracts: Purchased dry each plant species were ground into powder in an electric blender. 20g of each plant powder was dissolved in 180ml of distilled water in a glass bottles, heated to 85°C in a water bath and kept at this temperature with shaking for 8hr. After cooling, the liquid was filtered through the Whatman No. 1 filter paper. The filtrates were then condensed and dried in smaller glass bottles at 37°C for 48hr. Then, 1g of dried extracts was dissolved in 5mL of distilled water to obtain concentrations 200mg/ml (Wojnicz *et al.*,2012).

Preparation of cold ethanol extracts: Forty grams of powdered sample were added to 250 ml of 95% ethanol in flask for 24hr. on magnetic stirrer at room temperature then the mixture was precipitated by centrifuge at 3000rpm/15min, after that the supernatant was collected and further filtered through filter paper Wattman No.1, the filtrate were evaporated by rotary vacuum evaporator at 40°C then dried at 37 °C for 48-72hr. Then, 1 gram of dried extracts was dissolved in 5ml of D.W. to obtain concentrations 200mg/ml (Abbas, 2011).

Inhibitory effect of plant extraction on biofilm: To study the inhibitory effect of plant extracts on biofilm of *E. coli*, the highest biofilm producing isolates of *E. coli* (E3, E19, E23 and E24) were selected to be assayed. Two procedures were used to study the inhibitory effect of plant extracts on biofilm *E. coli* .

Biofilm inhibition assay procedure (1): Biofilm inhibition carried out in 96 wall plates adopting modified method of biofilm inhibition spectrophotometric assay (Al-Mathkhury, and Abed Assal, 2012): Studied *E. coli* isolates cultured in Brain Heart Infusion (BHI) broth (Rashmi, India) incubated at 37°C for 18 hour, after that bacterial culture was diluted in BHI broth and adjusted in comparison to MacFarland tube no. 0.5. Two hundred microliters of this bacterial culture were used to inoculate pre-sterilized 96-well polystyrene microtiter plates and later incubated for 48hr. at 37°C. After incubation, all wells were washed with sterile physiological saline for the elimination of unattached cells. Then, before the staining step, the previously prepared plant extracts were added to the biofilm containing wells: Subsequently, the tray was incubated for another 24hr. at 37°C, after incubation period all wells were washed and stained as the same procedure described above.

Biofilm inhibition assay procedure (2): Biofilm inhibition carried out in 96 wall plates adopting modified method of biofilm inhibition spectrophotometric assay (Namasivayam and Roy, 2013). 100μl of cell suspension of *E. coli* was added into 96 well microtitre plate then 100μl of plant extracts was added, the plates were incubated at 37°C for 3days. After the incubation, the liquid suspension was removed and 100μl of 1% w/v aqueous solution of crystal violet was added. Following staining at room temperature for 30 minutes the dye was removed and the wells were washed thoroughly, 95% ethanol was added and the reaction mixture was read spectrophotometrically at 540nm.

Results and discussion

Isolation and Identification of *E. coli*: A total of(30) *E. coli* clinical isolates were isolated from three separated children´s hospitals in Baghdad (Fatima-AL-Zahra Hospital, Ibn–Albaldi Hospital and Central Teaching Hospital of the child). At a period from October to December 2013. Routinely, all isolates were cultured on MacConkey agar and Blood agar plates. Bacterial isolates were identified as *E. coli* depending on morphological characteristics and Vitek-2 system (Bio-Merieux, France) using ID-GNB. The date of isolation, type of specimen, probability of identification by Vitek-2 system as well as age and sex distribution of the infected patients was summarized in Table (1). Out of 13(43.33%) isolates were from blood and 17(56.67%) isolates were from urine. Among the infected children patients which their ages ranged from 1 day to 5 months, the highest incidence was among females 18(60%) than that of males 12(40%) (Figure 1).

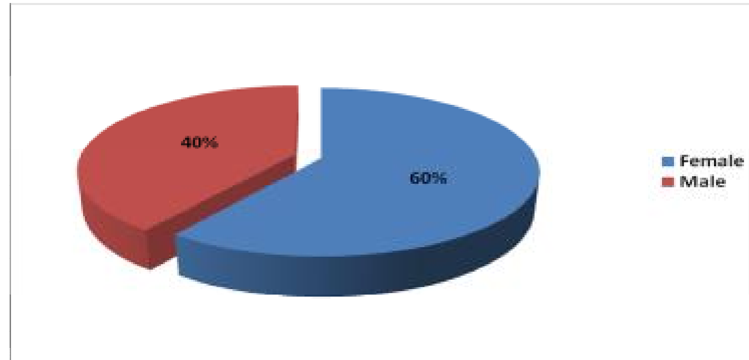

Figure (1): The percentage of male and female babies infected with *E. coli*

Table (1): Isolation date, type of specimen, probability of identification by Vitek-2 system and distribution of *E. coli* according to age and sex.

Isolate No.	Date	Specimen	Age	Sex	Probability of identification
1	11/2013	Urine	2 month	♀	96%
2	10/2013	Urine	40 day	♀	97%
3	10/2013	Urine	33 day	♀	96%
4	10/2013	Urine	4 month	♀	96%
5	10/2013	Blood	7 days	♂	99%
6	10/2013	Urine	30 day	♀	96%
7	10/2013	Urine	3 month	♀	99%
8	10/2013	Urine	3 month	♀	95%
9	10/2013	Urine	7 days	♂	97%
10	12/2013	Urine	3 month	♂	99%
11	10/2013	Urine	5 month	♀	99%
12	10/2013	Blood	44 day	♂	95%
13	12/2013	Blood	5 days	♀	95%
14	12/2013	Blood	1 month	♀	96%
15	10/2013	Blood	11 day	♂	97%
16	12/2013	Blood	5 days	♂	95%
17	12/2013	Blood	9 days	♂	96%
18	10/2013	Blood	50 day	♂	99%
19	11/2013	Blood	3 month	♂	99%
20	10/2013	Blood	5 month	♂	97%
21	11/2013	Urine	2 month	♀	96%
22	11/2013	Blood	7 days	♂	99%
23	11/2013	Urine	35 days	♀	95%
24	11/2013	Urine	40 days	♀	96%
25	11/2013	Urine	7 days	♀	96%
26	12/2013	Blood	3 month	♀	96%
27	12/2013	Urine	5 month	♀	99%
28	12/2013	Urine	3 month	♀	95%
29	12/2013	Blood	10 days	♂	96%
30	12/2013	Urine	25 days	♀	99%

♀= female, ♂= male.

Antibiotic Susceptibility Test: The overuse and the misuse of therapeutic agents readily led to the emergence and dissemination of antibiotic resistance bacteria, over the last several decades, which implies enormous health concerns, including the increased number of patients at risk, the increased severity of hospital or community-acquired infections and also the failure of treatments. The emergence of bacterial resistance is intensified when bacteria form biofilms.

Our results show a high level resistance of *E. coli* clinical isolates to most of the antibiotics under test. *E. coli* clinical isolates had (100%) resistance to ampicillin, the resistance to cefazolin were (93.33%), to ceftazidime (86.67%) and to ceftriaxone (86.67%). *E. coli* isolates were completely sensitive (100%) to imipenem and meropenem, 25(83.33%) isolates showed sensitivity to nitrofurantoin while 5(16.67%) isolates were intermediate to this antibiotic. *E. coli* isolates showed variable level of sensitivity and resistance to other antibiotics as shows in Table (2).

Our results are in agreement with the result of Sandeep *et al.* (2012) revealed that 100% isolated *E. coli* were resistance to penicillin G, erythromycin, streptomycin and oxacillin; 90% of isolated *E. coli* were resistance to ampicillin and kanamycin; 70% were resistance to gentamycin and ciprofloxacin; 60% of isolates were resistance to tetracycline and norfloxacin; 40% of isolated strains were resistant to cephotaxime. Some isolates were resistance to amikacin and chloramphenicol, confirmed that all the isolates were multi drug resistant (MDR). High pattern of resistance in *Klebsiella* and *E coli* species was reported by Eslaminezhad *et al.* (2010) ,who stated that isolates of *E. coli and Klebsiella* were multidrug resistant. Al-Hashash *et al.* (2013) found high levels of extended spectrum beta lactamase (ESBL) carriage and multidrug resistance in *E. coli* isolates that cause bacteremia. A comparison with urine isolates provided evidence that ESBL mediated drug resistance appears to be the selective pressure in the emergence of bacteremia. However, as previous studies have shown that biofilm formation is higher in MDR strains (Rao, *et al.*, 2008), the ability of a strain to develop biofilms may have an important, yet not fully understood role in the development of multidrug resistance. Biofilms are far more difficult to treat as of bacteria living within are vastly resistant up to 1000 fold higher to potent antibacterial agents which are used as a last resort, including Methicillin and vancomycin compared with planktonic forms (Suzana and Robert 2006).

There are six interesting hypothesized mechanisms that help to explain the increased resistance of biofilms to antimicrobials: the direct interaction between the biofilm substances and antimicrobials, affecting diffusion and availability; the existence of an altered chemical microenvironment within the biofilm leading to areas of reduced or no growth (dormant cells); the development of biofilm- specific phenotypes; the ability of microorganisms in biofilms to express specific resistance genes; the possibility of damaged bacterial cells undergoing programmed cell death; the existence of persister cells (Simões, 2011).

Detection of Biofilm Formation:

Congo red agar method: In the Congo red agar method, slime production was investigated, 12(40%) of *E. coli* were slime production, while 18(60%) of *E. coli* were non slime production Figure (2) and Table (3).

Tube method: In tube method, result after 24hr. and 48h. incubation were screening for biofilm formation, screening showed 20 (66.67%) isolates were positive for biofilm production from which 12 strongly adherent and 8 weakly adherent while other10 (33.33%) isolates were negative for biofilm production after 24hr. of incubation at 37°C. After 48hr. of incubation at 37°C, 23(76.67%) isolates were positive for biofilm production from which 14 strongly adherent and 9 weakly adherent while 7(23.33%) isolates were negative for biofilm production (Table 3). In the quantitative assay for the biofilm production, they were classified as strongly adherent, weakly adherent and non-adherent (Figure 3).

Microtitre-plate method: In microtitre plate method the strains assayed for production of biofilm (Figure 4). The results indicate that each strain shows a different potential to form biofilm under the same conditions of experimentation. The highest biofilm strains were *E. coli* numbers 3, 19, 23 and 24 isolates which absorbance of them (OD) in an ELISA reader were ; 1.641, 1.107, 1.118 and 1.013, respectively. The OD of control was 0.116 and all strains absorbance were compared with control. We noticed that the highest biofilm formation strains were isolated from urinary tract infections and the lowest biofilm formation strains were isolated from blood. The overall results for biofilm detection are given in the Table (3).

Table (2): Antibiotic sensitivity of 30 *E. coli* clinical isolates.

Isolate No.	AM	AMC	TPM	SAM	MEM	IMP	FEP	CRO	CAZ	CZ	TMP	NF	LVX	CPI	TB	CN
1	R	I	S	R	S	S	R	R	R	R	R	S	R	R	R	R
2	R	I	S	R	S	S	R	R	R	R	R	I	R	R	R	S
3	R	R	S	R	S	S	R	R	R	R	R	I	S	S	S	R
4	R	R	R	R	S	S	R	R	R	R	R	I	S	S	S	R
5	R	S	R	S	S	S	R	R	R	R	R	S	R	S	S	R
6	R	S	R	S	S	S	S	R	R	R	R	S	R	S	S	S
7	R	S	R	S	S	S	R	R	R	R	R	S	R	R	I	S
8	R	I	R	S	S	S	R	R	R	R	R	S	R	R	R	R
9	R	I	R	S	S	S	R	R	R	R	R	S	S	R	R	R
10	R	S	R	S	S	S	R	R	R	R	R	S	R	S	S	S
11	R	I	R	S	S	S	R	R	R	R	R	S	R	R	R	R
12	R	S	S	S	S	S	R	R	R	R	S	S	S	S	S	S
13	R	S	R	S	S	S	R	R	R	R	R	S	S	S	S	S
14	R	I	R	S	S	S	S	R	R	R	R	S	R	S	S	R
15	R	I	R	S	S	S	R	R	R	R	S	S	S	S	S	R
16	R	S	R	I	S	S	R	R	R	R	R	S	R	S	S	S
17	R	S	R	S	S	S	S	R	R	R	R	S	R	S	S	R
18	R	S	R	S	S	S	R	R	R	R	S	S	R	S	S	S
19	R	S	S	S	S	S	S	I	S	R	R	S	S	S	S	R
20	R	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S
21	R	I	S	R	S	S	R	R	R	R	R	S	R	R	R	R
22	R	R	R	S	S	S	S	S	S	S	S	I	S	S	S	S
23	R	I	S	R	S	S	R	R	R	R	R	S	R	R	I	R
24	R	R	R	R	S	S	R	R	R	R	S	S	S	S	S	R
25	R	R	R	R	S	S	R	R	R	R	R	S	S	R	R	R
26	R	S	S	R	S	S	R	S	S	R	S	S	S	S	S	S
27	R	S	S	I	S	S	R	R	R	R	R	S	R	R	I	S
28	R	I	S	R	S	S	R	R	R	R	R	S	R	R	I	R
29	R	R	R	S	S	S	R	S	S	R	S	I	S	S	S	S
30	R	R	S	I	S	S	R	R	R	R	R	S	R	R	I	S

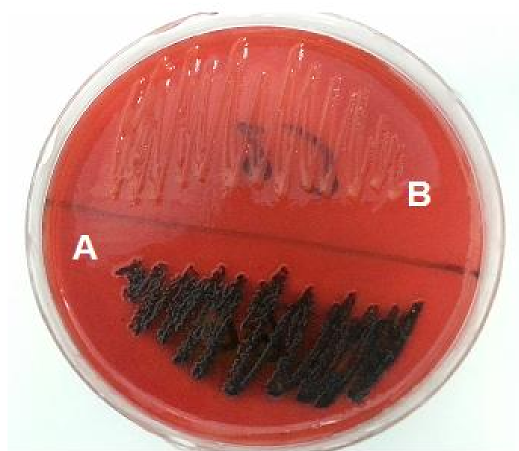

Figure (2): Congo red agar after 24 hours of incubation at 37°C, in which shown A - slime production *E. coli* and B - non slime production *E. coli*

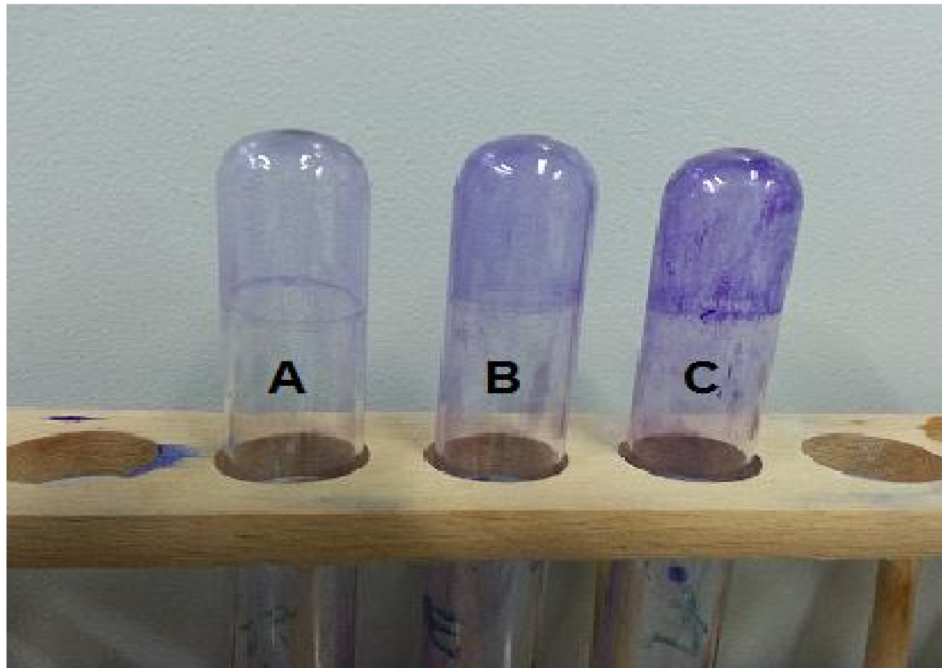

Figure (3): Biofilm formation on glass tube after 48hr. of incubation at 37°C in which shown: tube A- non adherent, tube B- weakly adherent and tube C- strongly adherent.

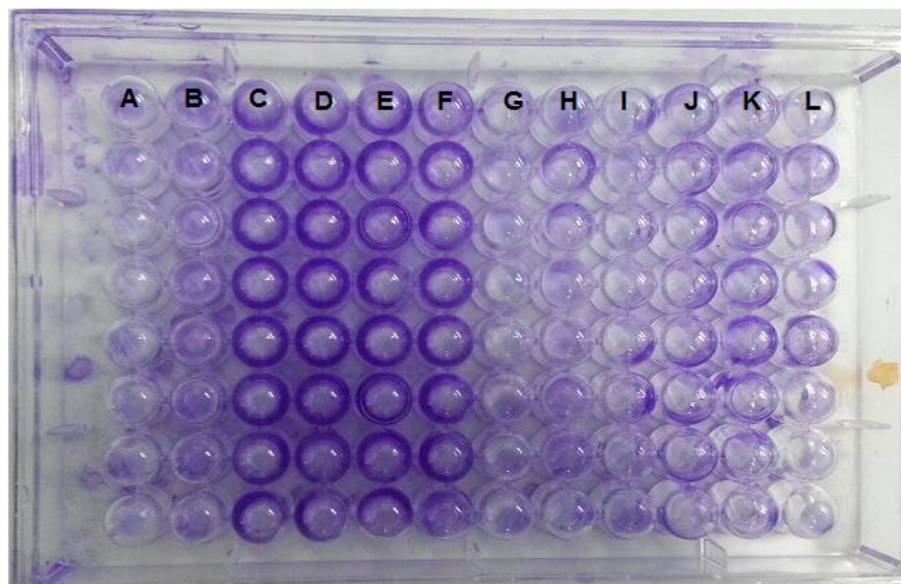

Figure (4): Biofilm formation on microtitre plate after 48h. of incubation at 37°C in which shown: A, G, I and L: non adherent, B, H, J and K: weakly adherent and C, D, E and F: strongly adherent.

Table (3): Biofilm formation by tube, Microtiter plate and congo red methods.

Isolate No.	Tube method after 24 hour	Tube method after 48 hour	Congo red	Microtiter after 48 hour	Absorbance (OD)
1	+ve	+ve	+ve	+ve	0.365
2	++ve	++ve	-ve	++ve	0.981
3	++ve	++ve	+ve	++ve	1.641
4	-ve	-ve	-ve	-ve	0.147
5	-ve	-ve	+ve	-ve	0.128
6	-ve	+ve	+ve	+ve	0.336
7	+ve	+ve	-ve	+ve	0.241
8	++ve	++ve	-ve	++ve	0.853
9	++ve	++ve	-ve	++ve	0.699
10	++ve	++ve	-ve	++ve	0.783
11	-ve	+ve	-ve	+ve	0.546
12	++ve	++ve	-ve	++ve	0.766
13	-ve	-ve	-ve	-ve	0.133
14	-ve	-ve	-ve	-ve	0.180
15	++ve	++ve	-ve	+ve	0.590
16	-ve	+ve	+ve	+ve	0.267
17	+ve	+ve	-ve	+ve	0.504
18	+ve	+ve	+ve	+ve	0.360
19	++ve	++ve	+ve	++ve	1.107
20	-ve	-ve	-ve	-ve	0.162
21	++ve	++ve	+ve	++ve	0.520
22	++ve	++ve	-ve	++ve	0.651
23	++ve	++ve	+ve	++ve	1.118
24	++ve	++ve	+ve	++ve	1.013
25	+ve	+ve	+ve	+ve	0.458
26	-ve	-ve	-ve	-ve	0.176
27	+ve	++ve	-ve	+ve	0.263
28	+ve	++ve	-ve	+ve	0.287
29	-ve	-ve	-ve	-ve	0.087
30	+ve	+ve	+ve	+ve	0.351

As biofilm formation has been shown to be a mechanism for evading host-defenses and resisting the effect of antimicrobials, it has been suggested that strains capable of forming biofilms may persist within the host contributing to relapsing/chronic infections (Hall-Stoodley *et al.*, 2004). Urinary infections often provoke bacteremia, especially in patients that are hospitalized because of catheter contamination by *E. coli* biofilms (Martinez *et al.*, 2006). ExPEC strains, such as uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and SEPEC, typically share many virulence factors that promote the colonization of host surfaces, avoidance and/or subversion of host defense mechanisms, invasion and/or injury of cells and tissues and the initiation of inflammatory responses (Croxen and Finlay, 2010).

Inhibitory effect of plant extracts on biofilm: In the present study, we focused on use of seed and leaf extracts of some plants used traditionally to treat different diseases like diarrhoea,liver and respiratory tract diseases of neonates in Iraq ,as anti-bacterial adhesion and biofilm growth.

We selected four isolates of *E. coli* ; 3, 19, 23 and 24 which produced the thickest biofilm; with absorbance 1.641, 1.107, 1.118 and 1.013, respectively. Inhibitory effect of four plant extracts (watery and ethanol extracts) on biofilm formation of these isolates were screening by two procedures including: added plant extracts before biofilm formation in the first experience and added plant extracts after biofilm formation in the second experience. Results of inhibitory effect after treatment with plant watery extracts before biofilm formation are shown in Table (4).

In regard to Table (4), differences were observed when treated with watery plant extracts, we noticed that the most effected watery plant extract in inhibition of biofilm formation was *Thymus vulgaris* while the most non effected watery plant extract in inhibition of biofilm formation was *Borago officinalis* and the other two watery plant extracts *Coriandrum sativum and Pimpinella anisum* showed good result in inhibition of biofilm formation.

Data presented in table (5), also showed differences in inhibition of biofilm formation when treated with ethanol plant extracts. We showed that the most effected ethanol extracts in inhibition of biofilm formation were *Thymus vulgaris* and *Pimpinella anisum*, while the most non effected ethanol plant extract in inhibition of biofilm formation was *Borago officinalis* but the ethanol plant extract *Coriandrum sativum* showed moderate effect in inhibition of biofilm formation.

When we added the plant extracts after formation of biofilm by incubation of bacterial isolates for 72hr. at 37°C, the biofilm showed more resistance to plant extracts than in the case of added plant extracts from the first time with bacterial isolates and then incubated 48h. at 37°C.

Table (6) showed bacterial isolates when treated with watery plant extracts, also the most effected watery extract in inhibition of biofilm formation was *Thymus vulgaris* followed by *Coriandrum sativum* and the most non effected watery plant extract in inhibition of biofilm formation was *Borago officinalis* followed by *Pimpinella anisum*.

From Table (7), we notice that the biofilm formatted by *E. coli* isolates showed most resistance to all ethanol plant extracts which added after formation of biofilm by incubation of bacterial isolates for 72hr. at 37°C.

From all those results we noticed that the ethanol plant extracts were less effected than watery plant extracts and the effect of watery and ethanol plant extracts was more effect in the case of added the plant extracts before formation of biofilm (by incubation of bacterial isolates for 48hr. at 37°C) than in the case of added the plant extracts after formation of biofilm(by incubation of bacterial isolates for 72hr. at 37°C). The most effected plant extract in inhibition of biofilm formation was *Thymus vulgaris* and the most non effected plant extract in inhibition of biofilm formation was *Borago officinalis*. The reduction of biofilm biomass using plant extracts individually and in combination shows potential in the development of medicinal products that will prevent microbial adhesion thus reducing the incidence of infections associated with biofilm formation. Although a lot of research on plants and the active constituents is currently underway, the focus is mainly on the antimicrobial properties against planktonic bacteria(Essawi and Srour, 2000 ; Gulcin *et al.*, 2003; Al-Bayati, 2008; Osman *et al.*, 2009). The resistant biofilms remain largely unexplored although they have been shown to be more resistant to antimicrobial agents than their planktonic counter parts (Aboaba *et al.*, 2006).

Many plants contain nontoxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens .The study of Wojnicz *et al.* (2012) suggest that anti-bio-film effect of plant extracts can be caused by modifications in the bacterial surface structures responsible for binding to the occupied surface. The activity of plant extracts on significantly reduced or inhibited biofilm production by E. coli rods can be explained by the presence of flavonoids such as quercetin, kaempherol, naringenin and apigenin that reduce biofilm synthesis because they can suppress autoinducer-2 importer genes of *E. coli* O157:H7 biofilm cells activity which is responsible for cell-to-cell communication (Lee *et al.*, 2011).

Table (4): The inhibitory effect after treatment with plant watery extracts on biofilm formation (before biofilm formation)

E. coli isolates Number	Before treatment (OD)	(OD) After treatment with			
		Coriandrum Sativum	*Thymus vulgaris*	*Borago officinalis*	*Pimpinella anisum*
3	1.641	0.375	0.114	0.761	0.252
19	1.107	0.298	0.227	0.507	0.466
23	1.118	0.303	0.211	1.004	0.238
24	1.013	0.511	0.279	1.113	0.264

Table (5): The inhibitory effect after treatment with plant ethanol extracts on biofilm formation (before biofilm formation).

E. coli isolates Number	Before treatment (OD)	(OD) After treatment with			
		Coriandrum Sativum	*Thymus vulgaris*	*Borago officinalis*	*Pimpinella anisum*
3	1.641	0.534	0.114	1.061	0.252
19	1.107	0.676	0.227	0.907	0.466
23	1.118	0.806	0.211	1.004	0.238
24	1.013	0.648	0.279	1.113	0.264

Table (6): The inhibitory effect after treatment with plant watery extracts on biofilm formation (after 72hr. biofilm formation)

E. coli isolates Number	Before treatment (OD)	(OD) After treatment with			
		Coriandrum sativum	*Thymus vulgaris*	*Borago officinalis*	*Pimpinella anisum*
3	1.641	0.578	0.331	1.218	0.606
19	1.107	0.462	0.370	0.829	0.823
23	1.118	0.721	0260	0.940	0.590
24	1.013	0.316	0.231	0.958	0.462

Table (7): The inhibitory effect after treatment with plant ethanol extracts on biofilm formation (after 72hr. biofilm formation)

E. coli isolates Number	Before treatment (OD)	(OD) After treatment with			
		Coriandrum sativum	*Thymus vulgaris*	*Borago officinalis*	*Pimpinella anisum*
3	1.641	0.969	1. 561	1.106	1.090
19	1.107	0.718	0.962	0.785	0.690
23	1.118	1.011	0.917	1.006	0.869
24	1.013	0.978	0.901	0.912	0.607

Conclusions

The results appeared that most isolates were resisting to most antibiotics and each strain shows a different potential to form biofilm under the same conditions of experimentation. The most effected plant extract in inhibition of biofilm formation was *Thymus vulgaris* and the most non effected plant extract in inhibition of biofilm formation was *Borago officinalis*, therefore *Thymus vulgaris* could be a source to Prepare new and effective herbal medicines to treat infections caused by multi-drug resistant strains of *E. coli* from community as well as hospital settings. However, it is necessary to determine the toxicity of the active constituents, the side effects and pharmaco-kinetic properties.

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