Anti-Adhesion Effect of Some Medical Plant Extracts on Some Bacteria Causing Urinary Tract Infection

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ABSTRACT

Urinary tract infection (UTI) is among the commonest human health problems, which may require antibiotic treatment. Recently, bacterial resistance to antibiotic is detected in most of UTIs patients. The aim of the present study was to examine the anti-adhesion properties of five medicinal plant extracts (*Nasturtium officinale, Petroselinum crispum, Trigonella foenum-graecum, Nigella sativa, Pimpinella anisum*) on some enterobacteriace causing UTI (*Escherichia coli, Proteus mirabilis, Klebsiella spp., Pseudomonas aeruginosa*).The results indicated that the plants' alcoholic extracts had significantly higher anti adhesion effect than the watery one. N. officinale had the highest anti-adhesion effect at 5mg/ml. The inhibition rates for each of *E.coli, proteus, kelbsiella* and *pseudomonas* were 51.2, 45.1, 58.5, and 57.9 % when watery extracted; while alcoholic extracts scored 90.2, 91.4, 82.9, 71.1% for the same species, respectively. The lowest inhibition rate was reported in watery extract of *T. foenum-graecum*. High anti-adhesion effect was noted with *N. sativum* in both watery and alcoholic extracts. Low inhibition rate was resulted in *P. anisum* watery extracts while the highest effect resulted from its alcoholic extracts. Variable inhibition rates, moderate to low, were noted in both watery and alcoholic extracts of P. crispum. The conclusion of the investigation was that the alcoholic extracts of *N. officinale* and *T. foenum-graecum* have high anti-adhesion effect at 5mg/ml against *E.coli*, *Proteus mirabilis*, *Klebsiella spp* and *Pseudomonas aeruginosa*. The consequences of this investigation suggest that the extracts of *N. officinale* and *T. foenum-graecum* could be used as prophylaxis for preventing the bacterial UTI especially in female with recurrent infection.

Key Words: Anti- adhesion, Bacteriuria, Plant extracts, Uroepithelial cells.

INTRODUCTION

Urinary tract infection (UTI) is a common human health problem that may require medical treatment (Drekonja and Johnson, 2008). The incidence of UTIs appears to increase with age with high percentage of elderly females have bacteriuria (Foxman, 2002). Interactions between the uropathogen and the host occur during UTIs process as the bacteria adhere first to the epithelial cells then colonize and disperse, causing damages to the surrounding tissues (Mulvey, 2002). After colonization in the urinary bladder, the bacteria causes symptomatic or asymptomatic bacteriuria. Pyelonephritis and renal impairment may result from further progression of the pathogen (Anderson *et al*., 2004). Bacteria cause most cases of UTIs. The most common bacteria are *Escherichia coli* and *Staphylococcus saprophyticus*. *E. coli* is responsible for 80-90% of urinary tract infections while *Staphylococcus saprophyticus* causes about 10-20% of UTIs. Other bacteria including *Proteus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and *Pseudomonas spp*. and *Enterococcus faecalis* cause about 5% of the cases. Other less common microorganisms, such as *Chlamydia* and *Mycoplasma*, can cause UTIs (Stamatiou *et al*., 2005).

Specific virulence factors on the bacterial membrane are thought to be responsible for bacterial resistance to host defense mechanisms (Hull *et al*., 2002). The bacterial binding sites and their anti-adherence mechanisms have been studied (Duke, 2007). The main traditional treatment for UTI is chemotherapy. However, anti-bioresistant strains among bacterial population are increasing. Therefore, therapy with antibiotics especially for β-lactamase bacteria may be ineffective (Jadhav *et al*., 2011). The need for different type of vaccines and new drugs generations against pathogenic bacteria is increase (Dormigny *et al*., 2005). The use of plant- origin

compounds as anti-adhesive for bacterial attachment is necessary. Recently, researches are exploring the inhibition of pathogen virulence factors (VFs) using medicinal plant products. Number of studies for different microorganisms VFs mainly bacteria and yeast have been developed (Defoirdt *et al*., 2011, and Orlando and Guillermo, 2012). Considerable types of plant compounds are available to be examined as new mechanisms for pathogens' anti-virulence factors. High number of medicinal plant products appeared to have antimicrobial activity (Pinzon *et al*., 2009 and Jepson and Craig, 2008). The use of medicinal plant for treating urinary tract diseases was well known in different regions and ancient cultures. During the last few years, studies focused on the interference between the adhesion-receptor and medicinal plants components. A number of *in vitro* and *in vivo* investigations on anti adhesion effect of plant extracts reported such type of action (Jepson and Craig, 2008, and Koo *et al*., 2010). Repeated treatment with antibiotic results in bacterial resistance and may lead to vaginal and intestinal dysbiosis. Therefore, it is necessary to seek alternative methods to prevent and treat simple UTIs. The present study aims to examine the anti-adhesion effect of some medicinal plant extracts (*Nasturtium officinale, Petroselinum crispum, Trigonella foenum-graecum, Nigella sativa,* and *Pimpinella anisum*) on some bacteria causing urinary tract infection namely *E. coli*, *Proteus mirabilis Klebsiella spp,* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Pathogenic Bacteria:

Bacterial isolates (*E. coli, Klebsiella spp., Proteus mirabilis,* and *Pseudomonas aeruginosa*) were obtained from culturing urine of patients attended Azadi Teaching Hospital in Kirkuk city in year 2010 and diagnosed with UTI. The bacteria were isolated and identified by microbiology department staff in the hospital using routine clonal characteristics, staining, and biochemical methods (Colle *et al*., 1996), then supplied for testing. All experiments were conducted in the microbiology section of Azadi Teaching Hospital.

Bacterial Suspension:

Two to three colonies from each isolated bacteria were taken by loop and suspended in normal saline in plane test tube, centrifuged for 10 min at 250*g*, then triple washed with saline using repeated centrifugation. Finally, the deposited bacteria was diluted in normal saline to a concentration of 1.5x10 cell/ml by calibrating it with McFarland tube number 4 (Geerlhngs *et al*., 2002).

Uroepithelial Cells Collection:

Thirty ml of urine from healthy females (with negative urine culture result) were collected in clean sterile disposable container to obtaining urine epithelial cells (uroepithelial cells). For this purpose, the urine was centrifuged for 10 min at 250*g*, the supernatant was discarded. The urine deposit was triple washed with saline using repeated centrifugation and finally suspended in about 8 ml of saline. The prepared uroepithelial cells suspension was resuspended, then added to petri dishes containing Whatman filter paper No.1 to retain the epithelial cells on the filter paper. The retained epithelial cells on the filter paper were transported to the surface of cover slips. This was achieved by applying cover slips with gentle pressure for 10 min on the filter paper, to allow the epithelial cells to attach to the cover slips. The cover slips was then removed from the filter paper and left for air-drying to be used in adherence assay (Geerlhngs *et al*., 2001).

Plant Extracts Preparation:

Five medicinal plant (*Nasturtium officinale* (watercress), *Petroselinum crispum* (parsley) whole plant, in addition to *Trigonella foenum-graecum* (fenugreek), *Nigella sativa* (black cumin), and *Pimpinella anisum* (anise) seeds; were bought from the local markets. The plant parts were dried in dark place and crushed to powder. 10g of each plant powder was weighted and put in a thimble of Soxhlet extraction apparatus (boiler and

reflux). 100 ml of distilled water or absolute ethanol alcohol was put in the heating flask of the apparatus. The solvent was boiled by refluxing method until the refluxing solvent become colorless. The solvent was then removed under reduced pressure to give a crude extract that was covered and kept in refrigerator until used. Four concentrations (0.63, 1.25, 2.5, 5 mg/ml) were prepared for anti-adhesion assay by diluting the obtained extracts in distilled water or ethanol alcohol to get the exact concentrations (Adonizio *et al*., 2006).

Adherence Assay of Epithelial Cells:
For each experiment, the air dried cover slips containing the uroepithelial cell were incubated with 0.5 ml of bacterial suspension and 200µl of each plant concentration with shaking 350 motilities/min for 1 h at 37°C. After incubation, the cover slips were washed repeatedly with phosphate buffer solution to remove any unattached bacteria. The covers were then fixed for 15 min in absolute methanol, washed with saline, stained with 3% Geimsa stain for 20 min, washed with saline to remove excess stain, air dried, then placed on clean microscope slides. The margin of the cover slips were fixed by applying small drops of DPX (Di-n-butyl phthalate in Xylene). In order to see the adhered bacteria to each uroepithelial cells, the cover slips were examined under high power magnification microscope (oil immersed lens, 1000x). The number of adhering bacteria to 50 epithelial cells were counted for each bacteria and the mean number of adhered bacteria to each single epithelial cell was calculated. Overlapped epithelial cells were excluded from calculation. All experiments were performed in duplicate (Geerlhngs *et al*., 2002). For controls, the experiment was repeated by adding 200µl water or alcohol with 0.5 ml of bacterial suspension to the cover slips without plant extracts, this was used as a positive control, or 200µl of plant extracts without bacterial suspension which was used as a negative control.
The inhibition rate of each extracts was estimated by this equation

Inhibition rate= Control adhesion mean × Adhesion mean of bacteria × 100/Control adhesion mean

Statistical analysis: Statistical analyses were performed using Chi-square test to compare categorical variables. A p-value less than 0.05 is considered significant.

RESULTS

The alcoholic anti-adhesion effect of the plant extracts was significantly more potent than the watery extracts ($p< 0.05$). Both watery and alcoholic extracts of *N. officinale* as shown in tables 1 and 2 significantly had the highest anti-adhesion effect when compared with other plant extracts and with controls particularly at 5mg/ml with all bacteria under investigation. The most anti-adhesion effect was for alcoholic extracts with inhibition rate of 91.4, 90.2, 82.9, and 71.1 % for each of *Proteus, E. coli, Klebsiella* and *pseudomonas,* respectively at 5mg/ml (table 2). Fig. 1 shows the adhered bacteria to the uroepithelial cells.

Table 1: Anti-adhesion effect of watery *N. officinale* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	39	4.9	32	22.1	27	34.1	20	51.2
Proteus mirabilis	35	32	8.6	27	22.9	22	37.1	19	45.1
Klebsiella spp.	41	30	26.8	24	41.5	20	51.2	17	58.5
P. aeruginosa.	38	32	15.8	29	23.7	21	44.7	16	57.9

Table 2: Anti-adhesion effect of alcoholic *N. officinale* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	37	9.8	18	56.1	10	75.6	4	90.2
Proteus mirabilis	35	25	28.6	15	57.1	8	77.1	3	91.4
Klebsiella spp.	41	37	9.8	26	36.6	18	56.1	7	82.9
P. aeruginosa.	38	35	7.9	31	18.4	26	31.6	11	71.1

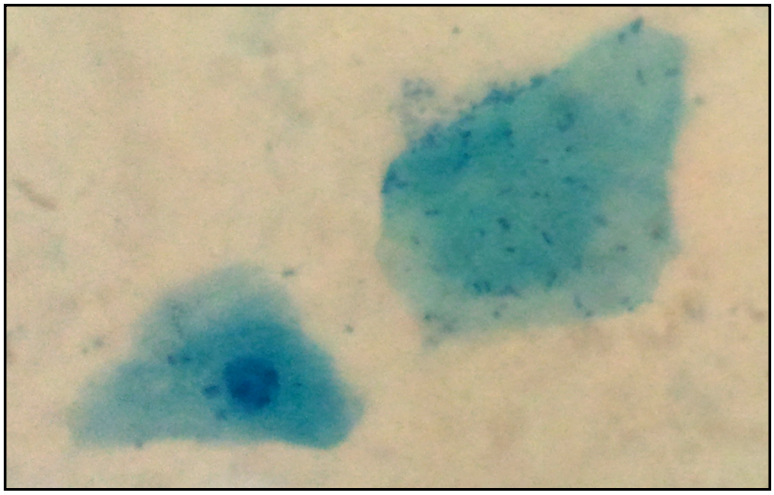

Fig.1: The adhered bacteria to the uroepithelial cells.1000x.

The lowest anti-adhesion effect was for fenugreek watery extracts especially with *E. coli and Proteus* bacteria (table 3). The results in table 4 of alcoholic extract of fenugreek indicated high anti-adhesion effect with rate of 87.8, 91.4, 87.8, and 89.5% for *Proteus, E. coli, Klebsiella*, and *Pseudomonas* bacteria, respectively.

Table 3: Anti-adhesion effect of watery *T. foenum-graecum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	42	-2.4	45	-9.8	42	-2.4	48	-17.1
Proteus mirabilis	35	38	-8.6	37	-5.7	40	-14.3	38	-8.6
Klebsiella spp.	41	34	17.1	31	24.4	28	31.7	24	41.7
P. aeruginosa.	38	35	7.9	33	13.2	33	13.2	30	14.3

Table 4: Anti-adhesion effect of alcoholic *T. foenum-graecum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	42	-2.4	45	-9.8	42	-2.4	48	-17.1
Proteus mirabilis	35	38	-8.6	37	-5.7	40	-14.3	38	-8.6
Klebsiella spp.	41	34	17.1	31	24.4	28	31.7	24	41.7
P. aeruginosa.	38	35	7.9	33	13.2	33	13.2	30	14.3

Moderate to high inhibition rates were recorded with watery and alcoholic black cumin extracts, respectively for each solvent. Inhibition rates of 53.7, 40, 57.9 % at 5mg/ml in watery extracts for *E. coli*, Proteus and Pseudomonas, respectively. For alcoholic extracts effect on *E. coli, proteus, Klebsiella,* and *Pseudomonas,* inhibition rate were 82.9, 71.4, 80.5, 81.6% at 5mg/ml, respectively (tables 5 and 6).

Table 5: Anti-adhesion effect of watery *N. sativum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	35	14.6	30	26.8	27	34.1	19	53.7
Proteus mirabilis	35	35	0	37	14.3	29	17.1	21	40
Klebsiella spp.	41	37	9.8	30	9.8	35	14.6	36	12.2
P. aeruginosa.	38	30	10.5	30	21	22	42.1	16	57.9

Table 6: Anti-adhesion effect of alcoholic *N. sativum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	33	19.5	25	39.1	18	56.1	7	82.9
Proteus mirabilis	35	30	14.3	24	31.4	16	54.3	10	71.4
Klebsiella spp.	41	32	22.1	21	48.8	16	60.9	8	80.5
P. aeruginosa.	38	30	21.1	20	47.4	15	60.5	7	81.6

As shown in tables 7 and 8, low to high anti-adhesion effect were recorded with watery and alcoholic extracts of *P. anisum* for each solvent compared *to* other plant extacts.

Table7 :Anti-adhesion effect of watery *P. anisum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	38	7.3	35	14.6	30	26.8	27	34.1
Proteus mirabilis	35	31	11.4	30	14.2	25	28.6	22	37.1
Klebsiella spp.	41	36	4.9	36	12.2	31	24.3	28	31.7
P. aeruginosa.	38	39	4.9	30	21.1	27	28.9	23	39.5

Table 8: Anti-adhesion effect of alcoholic *P. anisum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	32	21.9	16	61.1	15	63.4	9	78.1
Proteus mirabilis	35	31	11.4	29	17.1	16	54.2	9	74.3
Klebsiella spp.	41	31	24.4	30	26.8	23	43.9	10	75.6
P. aeruginosa.	38	38	0.0	25	34.2	12	68.4	5	86.8

The inhibition rate of parsley watery extracts did not differ significantly from that of alcoholic one, as it clear in tables 9 and 10. The highest effect was for *Pseudomonas* bacteria

with rate of 42.1, 47.4% at 5mg/ml for watery and alcoholic extracts, respectively.

Table 9: Anti-adhesion effect of watery *P. crispum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	39	4.9	37	9.8	35	14.6	35	14.6
Proteus mirabilis	35	34	2.9	35	0.0	29	17.1	30	14.3
Klebsiella spp.	41	38	7.3	35	14.6	30	26.8	29	29.3
P. aeruginosa.	38	31	18.4	24	36.8	24	36.8	22	42.1

Table 10: Anti-adhesion effect of alcoholic *P. crispum* extracts

Bacterial type	Adhesion mean of bacteria without plant extract (control)	Adhesion mean of bacteria							
		Concentration mg/ml							
		0.63	In.%	1.25	In.%	2.5	In.%	5.0	In.%
E. coli	41	40	2.4	35	14.6	33	19.5	31	24.4
Proteus mirabilis	35	32	8.6	30	17.1	29	17	29	17
Klebsiella spp.	41	39	4.9	37	9.8	31	24.4	28	31.7
P. aeruginosa.	38	33	13.2	28	26.3	22	42.1	20	47.4

DISCUSSION

Many host-bacteria interactions and managing methods have been developed. However, too much information are still needed in this aspect such as natural signaling among microbes and their environments, including hosts including new clinical strategies and drug candidates. As alternative to chemical treatment, in the current study, five medicinal plant extracts were investigated for their anti-adhesion effect against some bacteria causing UTIs. According to the results of this study, alcoholic extracts were significantly more effective than watery extracts, this is probably because the high efficiency of alcoholic solvent in extracting active component from plant parts. Identical results were recorded by Uma *et al*. (2009). He found that alcoholic extracts were significantly more potent than watery one. Among plants under the study, watercress had the highest significant effect. This may be due to the active component it contain like glocon-sfurtin, raphanol, nasturtiosied (Gill *et al.*, 2007). Both watery and alcoholic extracts of parsley had low inhibition rates comparing to the control and to the other plant extracts. No significant anti adhesion rate was noted with watery extracts of *T. foenumgraecum*. However, high inhibition rates were recorded with the alcoholic extracts of the same plant due to the explanation of Uma *et al.* (2009). In addition, The extracted active compounds effect on preventing bacteria from getting adhered to cells. The active components of the plants may bind to the receptors on the epithelial cells or may block the bacterial fimbria. Mechanism of fimbrial adhesion inhibition can be related to different effects of plant metabolites. Anti-fimbrial activity is similar to some antibiotics mechanism (Orlando and Guillermo, 2012). This mechanism had been proposed for berberine alkaloid (Sun *et al*., 1988). Results of adhesion-receptor interaction blockade by plant extracts, suggest that it can be mediated by glycoprotein (like lectins) with peptidic sequences similar to PapG, PapG-PapF, or by compounds that subvert spatial configuration of Gal-Gal receptors. (Coutino *et al*., 2001). Recently, the interactions and chemical signal between the pathological microorganisms and host have been understood, and seem to be very common in nature. The host consumed plant product

could compete for specific receptors with the infectious microbes. Plant products are now known to be involved in a microorganism molecular mechanism of adhesion, human carbohydrate food compounds are believed to be involved in adhesion, invasion, and infection of microorganism, Glycoproteins (lectins) and a broad range of non-nutrient components of food plants (phytochemicals) may be active in this way (Dixon *et al.*, 2005). There is evidence that a type of coordination between host defense mechanisms and plant metabolites that can inhibit different (virulence factors) VFs expressions may assist the host to overcome an infection. Fimbrial adhesion interference in UTI is fundamental to avoid bacterial attachment and colonization. This may explain the bacteriostatic/ bactericides activity of medicinal plant extracts traditionally used in managing urinary complaints (Dobrindt and Hacker, 2008 and Defoirdt *et al*., 2011).

More than 60 plant species from diverse families were used for their potency against different VFs on a number of Gram negative, Gram positive and *Candida albicans* (Orlando and Guillermo, 2012). Most of references related to anti-adhesion activity, or inhibition of toxins of enteric pathotypes were on *E. coli*, the most causative agent of UTI. The expression of fimbrial subunits on uropathogenic *E. coli* was described to be inhibited by berberine alkaloids (Sun *et al*., 1988). Plant compounds can neutralize several VFs, and like in other biological activities, the search for antivirulence factor novel drugs or herbal medicine can afford success in ethnomedical knowledge based criteria. Anti adhesion activity of *A. aspera, L. virginicum, Ageratum conyzoides* (*Asteraceae*), *Zingiber officinale* (*Zingiberaceae*), *Curcuma longa* (*Zingiberaceae*) and *Costus speciosus* (*Costaceae*) extracts was determined by monoclonal antiserum assays in *E. coli.* Anti adhesive effect was detected in all plant species except *C. longa* extracts (Barreto *et al*., 2001). The antibacterial properties of human food and medicine (Cranberry) is widely used for women in prophylaxis of UTI infection (Jepson and Craig, 2008). Cranberry fruit mainly contains organic acids and polyphenols like flavonoids, and anthocyanin pigment glycosides of cyanidin and paeonidin (Foo *et al*., 2000a). Trimeric type A proanthocyanidines characterized in cranberry by (Foo *et al.,* 2000b), is of particular interest. *V. macrocarpon* is among the most phytochemically studied *Vaccinium* species. Polyphenolic compounds have been the most researched in *Vaccinium spp.* because of its anti-oxidant and anti-UTI activity, mainly in *V. macrocarpon* and *V. mirtillus* fruits (Abreu *et al*., 2005).

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التأثير المضاد للالتصاق لمستخلصات بعض النباتات الطبية على بعض أنواع البكتيريا المسببة للالتهاب المجاري البولية

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الملخص

التهاب المجاري البولية من بين أكثر المشاكل الصحية للإنسان التي في أغلب الأحيان تحتاج إلى تدخل علاجي بالمضادات الحيوية. وفي الآونة الأخيرة ظهرت مقاومة البكتيريا للمضادات الحيوية في نسبة كبيرة من المصابين بالتهاب المجاري البولية، لذلك هدفت الدراسة الحالية إلى التحري عن مضادات الالتصاق في مستخلصات خمسة نباتات طبية (الحرف، الكرفس، الحلبة، الحبة السوداء واليانسون) ضد بعض أنواع البكتيريا (الإيشريشيا كولاي، بروتيس ميرابيلس، كليبسيلا والسيدوموناس أيروجينوسا) المسببة لالتهاب المجاري البولية.

أشارت النتائج إلى أن المستخلص الكحولي أكثر كفاءة معنويا من المستخلص المائي. سجل المستخلص الكحولي لنبات الحرف أعلى نسبة تثبيط لالتصاق البكتريا وبنسبة 90.2، 91.4، 82.9، 71.1 %، بينما سجل المستخلص المائي لنفس النبات تأثيرات متوسطة ونسبة 51.2، 45.1، 58.5، 57.9 % لكل من بكتيريا الإيشريشيا، بروتيس، كليبسيلا والسيدوموناس على التوالي عند تركيز 5ملغ/مل والنسبة الأقل للتثبيط كانت للمستخلص المائي للحلبة. ولوحظت تأثيرات معنوية عالية عند استخدام المستخلص الكحولي والمائي لنبات الحبة السوداء. ولوحظت نسب تثبيط منخفضة للمستخلص المائي لليانسون، بينما أظهر المستخلص الكحولي لها تأثيرات عالية. وسجلت نسب تثبيط متفاوتة (متوسطة إلى منخفضة) لمستخلصات نبات الكرفس.

واستنتج من هذه الدراسة أن للمستخلص الكحولي لنباتي الحرف والحلبة تأثيرات عالية مضادة لالتصاق بكتيريا الإيشريشيا، بروتيس، كليبسيلا والسيدوموناس عند تركيز 5ملغ/مل ويقترح البحث إمكانية استخدامه كمادة وقائية لمنع التصاق البكتيريا وخاصة في النساء اللاتي يعانين من الالتهابات المتكررة للمجاري البولية.

الكلمات المفتاحية: بكتيريا بولية، خلايا ظهارية بولية، مستخلصات نباتية، مضاد الالتصاق.