Efficiency of some plant extracts, carbohydrates and inorganic salts as anti-adhesion agents against the adhesion of *Staphylococcus* strains to HEp-2 cells

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Abstract: Some Staphylococcus strains are well adapted to humans. It can live as a commensal bacterium but it can initiate severe infection at various body sites. Its structural components secrete products which can efficiently target human tissues and evade host defense mechanisms. So it continues to cause invasive, life-threatening infections despite the availability of effective antimicrobial agents. *Staph. aureus* produce variety of diseases like soft tissue infection (wound infection, boil, eczema, blister and scalded skin syndrome), pneumonia and osteomyelitis. The present study was conducted to the screening of different substances (plant extracts, carbohydrates and inorganic compounds) as anti adhesion agents of Staphylococcal strains to human epithelial cells and trials for improvement of the anti adhesion characters of positive compounds. Clinical samples were collected regardless the type of infection and the sex of patients. This study was carried out over a period of 7 months from September 2009 to May 2010. Pus, urine, sputum and stool were collected to isolate samples. The obtained results showed that; among the plant extracts tested for their anti-adhesion potency the highest effect was recorded to the extract of *Nigella sativa* followed by the extract of *Trigonella fosnum* and *Eucalyptus globules*. Clear antiadhesion potencies were recorded also to glucose, arabinose, galactose, xylose and fructose (monosaccharides), sucrose, maltose and lactose (disaccharides), starch and cellulose (polysaccharides) and the inorganic salts NaCl and $CaCl_2$.
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Key word: Staphylococcus**,** adhesion, antiadhesion, plant extracts, carbohydrates

1. Introduction

Staph. aureus is a common human colonizer and pathogen that causes infections ranging from skin and soft tissue to invasive diseases such as pneumonia, osteomyelitis, and endocarditic (**Allison *et al.*, 2010**). *Staph.s aureus and Staph epidermidis* are major causes of infections associated with wounds, indwelling catheters, and cardio-vascular and orthopedic implant devices (**Eidhin *et al.*, 1998**).

Bacterial adhesion to human epithelial cells (HEp-2) is a key step in infections, allowing subsequent colonization, invasion and internalization of pathogens into tissues. Antiadhesive agents are therefore potential prophylactic tools against bacterial infections (**Janecki and Kolodziej, 2010**).

Anti-adhesion therapy and anti-adhesin immunity are meant to reduce contact between host tissues and pathogens, either by prevention or reversal of adhesion of the infectious agent. It is well established that adhesion of enteric, oral and respiratory bacteria is required for colonization and for subsequent development of disease (**Ofek *et al.*, 2003**).

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In the developing countries, they used as a primary source of medicine (**Chitme *et al.*, 2003**). About 80% of the people in developing countries use traditional medicines for their health care (**Kim, 2005**). The natural products derived from medicinal plants have to be a source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals (**Enzo, 2011**).

Sugars are vital components of infecting microbes and host cells, and are involved in cell signaling associated with modulation of inflammation. Indeed, sugars are the molecules most commonly involved in cell recognition and communication. In skin, they are essential to epidermal development and homeostasis. They play important roles in microbial adherence, colonization and biofilm formation, and in virulence (**David *et al.*, 2007**).

Inorganic antimicrobial agents are being increasingly for control of microorganism in various areas, especially in dentistry. Particle size of mental oxides had an impact on their anti-microorganism activity. There is growing interest in nanoscale particles since materials exhibit unique properties which offer considerably from those of macroscopic materials. Inorganic nano mental oxides including MgO, ZnO and CaO have been shown anti-microorganism activity (**Shi *et al.*, 2010**).

2. Material and Methods

1. Material

A) The tested bacterial strains

The study involved 3 *Staphylococcal* strains, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus aureus* NCTC 6571 that were obtained from El Nasr pharma company, Cairo; *Staphylococcus epidermids* and *E. coli* were obtained from Tanta Cancer Center and Faculty of Medicine, Tanta University respectively.

B) Plants tested

Extracts of three plants namely *Nigella sativa* (family: Ranunculaceae), *Trigonella fosnum* (family: Fabaceae) and *Eucalyptus globules* (family: Myrtaceae) were used in the study.

C) Media used for tissue culture cultivation:

(1): Rosewell park memorial institute medium (RPMI-1640 medium) (**Moore and Woods, 1976**), the medium was used for culturing and maintenance of the human tumor cell lines.

(2): Dulbecco's modification of Eagle's medium (DMEM) (**Dulbecco and Freeman, 1959**), medium was used for cultivation of human epithelial cells

(3): Transport Medium, (Dulbecco's modification of Eagle's medium (DMEM)) supplemented with fetal bovine serum 10 %, gentamicin sulphate (Sigma co.) 100 µg/ml and amphotericin B 10 µg/ml (Aliquot in 10 ml lots and store at - 20 ºC)

D) Media used for isolation and cultivation of bacteria:

(4): Nutrient broth medium (Shirling and Gottlieb, 1966).

It contains beef extract (3 gm), peptone (5 gm, for nutrition), NaCl (5 gm) and distilled water (1000 ml).

(5): Nutrient agar medium (Shirling and Gottlieb, 1966).

It composed of nutrient broth plus 20 gm agar.

(6): MacConkey 's agar(**Oxoid Manual,1981**), Composed of peptone (20 gm, for nutrition), Lactose (10 gm as test sugar), neutral red (0.05gm, indicator that changes pink in the presence of acid which is produced as a result of lactose fermentation), bile salts (1.5 gm, sodium taurocholate to inhibit the growth of non intestinal bacteria) and agar (20 gm, solidifying agent) and crystal violet (0.001 gm); it used to differentiate between Lactose fermenters (L.F) group which give rose pink colonies include the coliform group and Non-lactose fermenters (Non L.F) group which give pale yellow and include the *Salmonella* and *Shigella*.

(7): Manitol salt agar (**Chapman, 1945**) used as a selective and differential medium as the growth of *Staphylococcus aureus* appears yellow while *Staphylococcus epidermidis* appears white.

(8): Blood agar (**Oxoid Manual, 1981**)**,** 100 ml of sterilized nutrient agar media in a flask was cooled to 40°C – 45°C before adding 5% defibrinated blood.

(9): Tryptic Soy Broth. (**Smith and Dell, 1990**), it contains casein peptone (17 gm), dipotassium hydrogen phosphate (2.5 gm), glucose (2.5 gm), sodium chloride (5 gm) soya peptone (3 gm)

2. Methods

Identification of *Staph. epidermids* and *E. coli:*

Three *Staphylococcal* strains were obtained in addition to twenty five bacterial isolates isolated from Tanta Cancer Center (10 samples) and the Faculty of Medicine, Tanta University (15 samples) from different human infected tissues during the period of 3 months from March 2010 to May 2010, the medical samples included pus, sputum, urine and stool.

The twenty five bacterial isolates were identified through studying their morphological, physiological and biochemical characteristics.

Morphological identification (Gram staining) revealed that, the isolates under study were classified as fifteen isolates belonging to Gram positive bacteria, while the other ten bacterial isolates were belonging to Gram negative bacteria and with concern to the best grown isolates; it was found that the isolate no. 7 (Gram positive cocci, from pus) and isolate no. 22 (Gram negative bacilli, from stool) were subjected to further studies concerning their identification into the species level.

They were tested for growth on mannitol salt agar as a specific and differential media for *Staphylococcus* ; they exhibited a heavy growth for isolate No. 7 and no growth for isolate No. 22 this may refers that isolate No. 7 might be *Staphylococcus epidermdis* as small white colonies.

Analytical profile index (API) 20 E: (bio-Merieux SA, Montalieu Verica, and france).

It is an identification system using standardized and miniaturized biochemical tests, used for biotyping to delineate different species.

Preparation of Human laryngeal Epithelial cells (HEp-2 cells).

Human epithelial cell lines used for testing anti-adhesion activity, were obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The cell lines were maintained in the Tissue Culture Unite (TCU), The Holding Company for Production of Vaccines Sera and Drugs (VACCERA) Cairo, Egypt by serial sub culturing (**Andrei *et al*., 2000; Leivo *et al*., 2000**).

Cultivation of Human Epithelial cells:

HEp-2 cells were maintained in 10 ml (DMEM) containing 2.5 % fetal bovine serum (FBS) with 1% antibiotics (Ampicillin or chloramphencal or gentamicin). The culture were incubated in humidified

atmosphere with 5% CO_2 incubator, sub-cultivated once every 4 days and harvested from sub-confluent monolayer by washing with 5 ml Hank's balanced salt solution (HBSS). 1 ml of 0.25 % trypsin EDITA were added, incubated for 15 minutes then washed with DMEM and only used adherent cells (**El silk *et al*., 2003**)

Investigation of the adhesion of bacterial strains on eukaryotic human epithelial cell lines (HEp-2) (Adhesion assay).

Adherence of the Staphylococcus strains to human epithelial Cells (HEp-2) was studied using [3-(4, 5- Dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide] MTT assay. The principle of this assay is depend on cleavage of MTT by bacterial enzymes to formazan that was used to measure the adherence of bacterial strains to a fixed eukaryotic cell lines at 492 nm. (**Elsilk *et al*., 2003**)

Evaluation of the efficiency of different substances as anti-adhesion agents for the pathogenic bacterial strains.

From confluent HEp-2 cells in a 96-well microtiter plate the medium was removed and cells were fixed by adding 100 µl of 3.7 % paraform aldhyde at room temperature for 30 min. After washing three times with 50 µl saline solution (0.9 % NaCl), 2 ml of this fresh culture were used.

a) Evaluation of plant extracts as anti-adhesion agents.

Fresh leaves and aerial parts of the three different plant species *Nigella sativa, Trigonella fosnum and Eucalyptus globules* were collected during spring 2010 from different localities. The identification of the plant material was confirmed at Pharmacognosy Department, Faculty of Pharmacy, and University of Tanta. The plants were dried at room temperature and were then milled by electric miller. 100 gm was added to 500 ml of 70% ethanol, left in room temperature for 24 hrs. The mixture was then filtered, kept overnight and then evaporated until dryness. (**Al-Bakri *et al*., 2010**).

b) Evaluation of carbohydrates and inorganic compounds as anti-adhesion agents.

Different carbohydrate compounds including; monosaccharides (glucose, fructose, xylose, arabinose and galactose); disaccharides (sucrose, maltose and lactose), polysaccharides (cellulose and starch) and inorganic compounds including (NaCl & $CaCl_2$) were tested for acting as anti-adhesion agents as the following:

Two ml fresh culture of the tested bacterial strains were centrifuged and suspended in 1ml broth medium containing 1% of the tested compound, compared with control sample (without compound addition).

The tubes were incubated at 37° C for one hour, centrifugation for 5 minutes and then wash the pellet by 100µl HBSS. Centrifuged again and the pellet was resuspended in broth medium. The plates were inoculated with 100µl of cells treated by the compounds; the plates were then centrifuged for 10 minutes at 700 rpm and incubated for one hour at 37°C. The plates were washed five times with 100 µl 0.9 % NaCl and 50 µl DMEM/MTT (1mg/1ml) was added, after 3 hours at 37°C, the medium was removed and the violet crystals were dissolved with 100 % methanol (50 µl /well),after shaking horizontally, plates were read by ELISA reader at 492 nm.

C) Evaluation of the different concentrations of the best anti-adhesion agents.

For the purpose of detection the best concentration of the best anti-adhesion substances (Starch and $CaCl_2$), different concentrations (0.5, 1, 1.5 and 2 %) of the two substances were tested for their activity as anti-adhesion agents through mixing with two ml of the freshly prepared bacterial culture then added to HEp-2 cells.

Investigation of the adhesion, invasion and anti-adhesion of *Staphylococcus epidermidis* using Scanning Electron Microscope (SEM) was performed as the following: two ml fresh culture of *Staph. Epidermidis* were added to 1 ml of human epithelial cell culture, incubated at 37°C for three hours then centrifuged. The bacterial pellet was fixed and examined by (SEM) for observation of cell adherence. For the purpose of investigation of cell invasion, the cells were incubated for six hours then investigated by (SEM).

Anti- adhesion of *Staphylococcus epidermidis* was investigated through suspension of the bacterial culture in 1 ml of the best concentration (Starch and $CaCl_2$) of the most anti-adhesive effect of the tested substances then investigated by (SEM).

3. Results

The work in this study included three standard *staphylococcal* strains obtained; *Staph. aureus* ATCC 25923, *Staph. aureus* ATCC 6538, *Staph. aureus* NCTC 6571 and twenty five bacterial isolates isolated from different human infected tissues which were identified as *Staph. epidermids* from pus and *E. coli* from stool.

1) Determination of Adhesion of freshly and overnight prepared bacterial cultures on (HEp-2):

The results in figure (1) explained that, the maximum value of the bacterial adherence was recorded in the case of fresh cultures in *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* and *E. coli* while *Staph. aureus* NCTC 6571 only showed more adherence in the case of overnight culture rather than fresh culture.

2) Effect of plant extracts as anti-adhesion agents on HEp-2 cells.

Trigonella fosnum, *Eucalyptus globules* and *Nigella sativa* were tested for their anti-adhesion activity. It was found that all plants affect on adhesion of all strains. The effect changed from strain to another one. The results indicated that the maximum effect was on ***E. coli, Staph. aureus NCTC 6571, Staph. epidermidis*** and ***Staph. aureus ATCC 25923*** respectively with *Nigella sativa* while ***Staph. aureus ATCC 6538*** only gave the best result with *Eucalyptus globules*.

So *Trigonella fosnum* was found to have the minimum effect as anti-adhesion agent.

Results of anti-adhesion activities of the tested plant extracts were represented in figure (2).

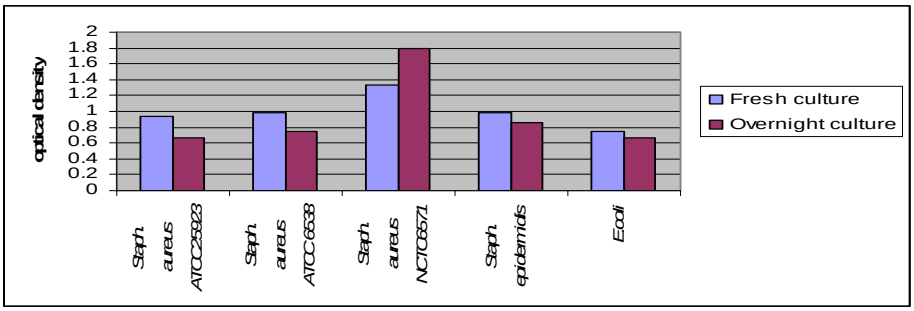

Figure (1): Adhesion of freshly and overnight prepared bacterial culture on (HEp-2) cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator to the degree of adhesion in fresh and overnight cultures.

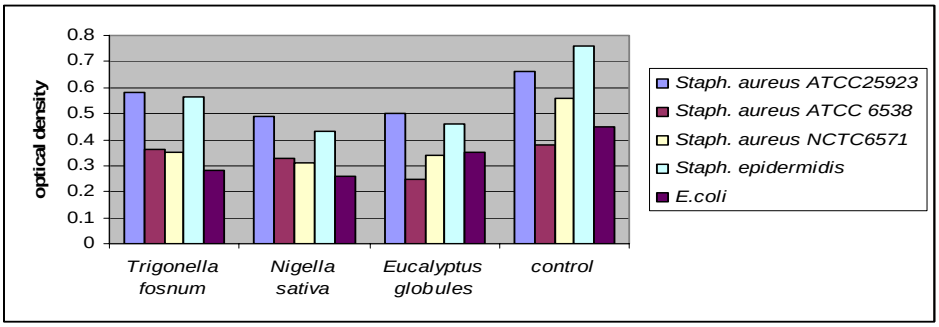

Figure (2): Effect of plant extracts as anti-adhesion agents on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different plant extracts in comparison to a control (free from plant extracts).

3) Effect of monosaccharide as anti-adhesion agents on HEp-2 cells.

Different monosaccharide sugars including (glucose, fructose, xylose, arabinose and galactose) were tested for their activity as anti-adhesion compounds on HEp-2.

It was found that all monosaccharide affect on adhesion of all strains. The effect changed from strain to another one. The maximum effect on all strains was with glucose except ***Staph. aureus ATCC 25923*** which recorded maximum effect with galactose in comparison with control which contained no sugars.

Results of anti-adhesion activities of the tested monosaccharide were represented in figure (3).

4) Effect of disaccharide as anti-adhesion agents on HEp-2 cells.

Different disaccharide sugars including (sucrose, maltose and lactose) were tested for their activity as anti-adhesion on HEp-2 cells.

Finally it was found that the maximum effect on all strains were with sucrose except ***Staph. aureus ATCC 25923*** which recorded maximum effect with lactose in comparison with control .

Results of anti-adhesion activities of the tested disaccharide were represented in figure (4).

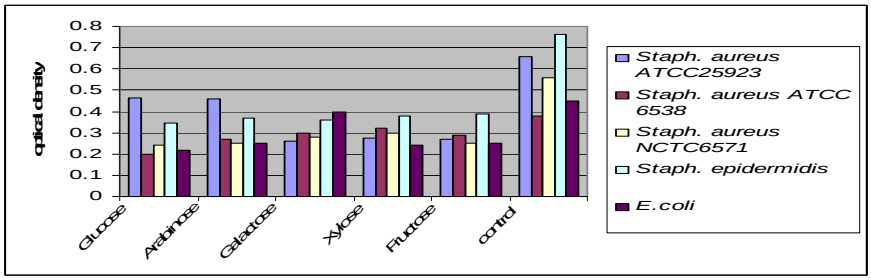

Figure (3): Effect of monosaccharide as anti-adhesion agents on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different monosaccharide in comparison to a control (free from monosaccharide).

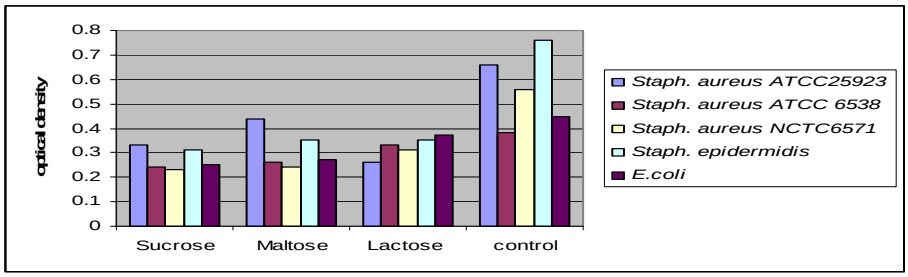

Figure (4): Effect of disaccharide as anti-adhesion agents on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different disaccharide in comparison to a control (free from disaccharide).

5) Effect of polysaccharide as anti-adhesion agents on HEp-2 cells.

Also Different polysaccharide sugars including (starch and cellulose) were tested for their anti-adhesion activity on HEp-2 cells.

It was found that the maximum effect on all strains ***Staph. aureus ATCC 25923***, ***Staph. aureus ATCC 6538***, ***Staph. aureus NCTC 6571, Staph. epidermidis*** and ***E. coli*** were with starch which gave best result rather than cellulose in comparison with control which contained no sugars.

Results of anti-adhesion activities of the tested polysaccharide were represented in figure (5).

6) Evaluation of inorganic compounds on HEp-2 cells as anti-adhesion agents.

Different inorganic compounds including (NaCl & $CaCl_2$) were tested for their activity as anti-adhesion agents.

It was found that the maximum anti-adhesion of the tested inorganic compounds was recorded in $CaCl_2$ with **Staph. *aureus ATCC 25923***, ***Staph. aureus ATCC 6538*, Staph. *aureus NCTC 6571 and Staph. epidermidis***, while NaCl was found to give the maximum anti-adhesion result with only ***E. coli*** on HEP-2 cells in comparison with control which contained no sugars.

Results of anti-adhesion activities of the tested inorganic compounds were represented in figure (6).

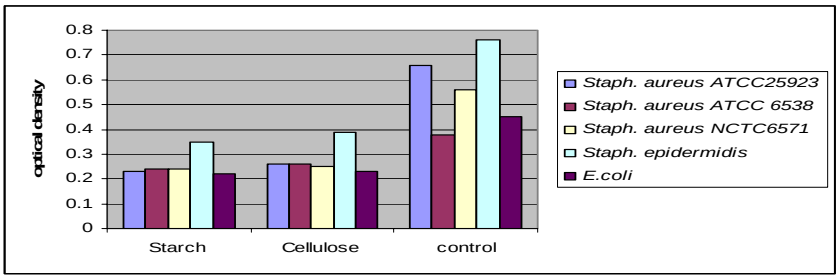

Figure (5): Effect of polysaccharide as anti-adhesion agents on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different polysaccharide in comparison to a control (free from polysaccharide).

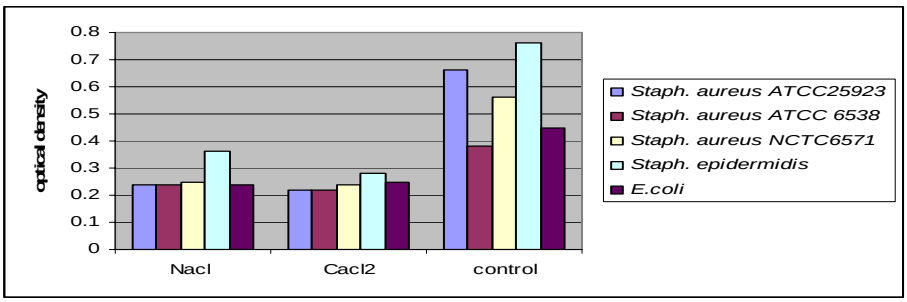

Figure (6): Effect of inorganic compounds as anti-adhesion agents on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different inorganic compounds in comparison to a control (free from inorganic compounds).

7) Effect of different starch concentrations on HEp-2 cells.

Different concentrations of starch (0.5, 1, 1.5 and 2 %) were tested as anti-adhesion agents.

It was found that the maximum anti-adhesion was found by increasing the concentration of starch, so the maximum anti-adhesion of starch was recorded at 2 % for all strains followed by 1.5 %, 1 gm and then 0.5 %.

So by increasing concentration the effect increases on all strains under study.

Results of anti-adhesion activities of starch were represented in figure (6).

8) Effect of different $CaCl_2$ concentrations on HEp-2.

Different concentrations of $CaCl_2$ (0.5, 1, 1.5 and 2 %) were tested as anti-adhesion agents on HEp-2 cells.

It was found that the maximum anti-adhesion was found by increasing the concentration of $CaCl_2$, so the maximum anti-adhesion of $CaCl_2$ was recorded at 2 % for all strains followed by 1.5 %, 1 % and then 0.5 %.

So by increasing concentration the effect increases on all strains under study.

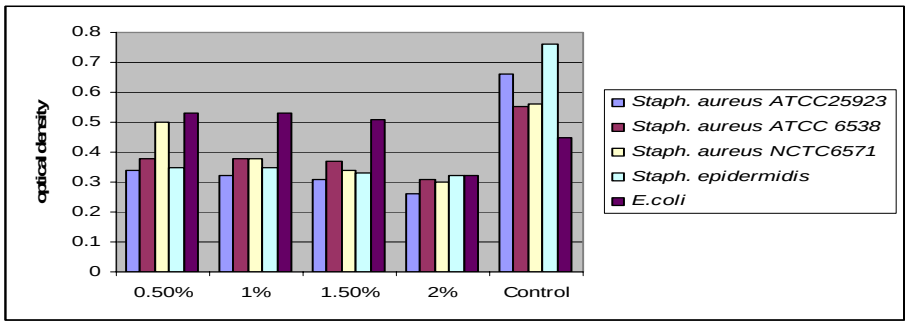

Figure (7): Effect of different starch concentrations on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different concentrations of starch in comparison to a control (free from starch).

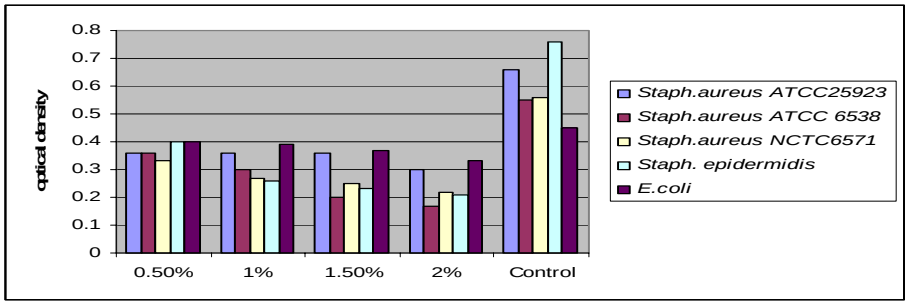

Figure (8): Effect of different $CaCl_2$concentrations on HEp-2 cells showing values of optical density at 492 nm of different tested bacterial strains as an indicator of the degree of adhesion in the presence of different concentrations of $CaCl_2$in comparison to a control (free from $CaCl_2$).

Investigation of adhesion, invasion and anti-adhesion of *Staph. epidermidis* by scanning electron microscope.

For the purpose of investigate cell adhesion the cells were further incubated for three hours then investigated by (SEM), and also incubated for six hours to investigate cell invasion while anti-adhesion of *Staphylococcus epidermidis* was investigated through suspension of the bacterial culture in 1 ml of the 2 % starch then investigated by (SEM). Results of Adhesion, invasion and anti-adhesion of *Staphylococcus epidermidis* by scanning electron microscope was illustrated in plates No. 1, 2, 3&4.

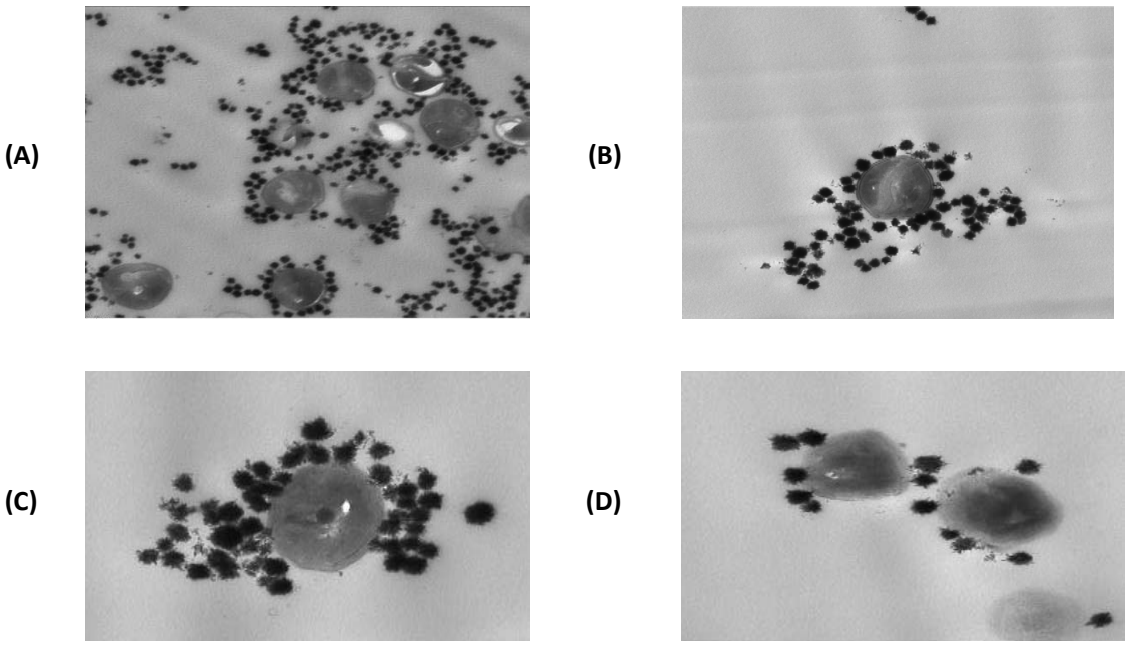

Plate no. 1: Scanning electron microscope photographs showing attachment of *Staph. epidermidis* cells to HEp-2 cells at different magnifications A(X 6000), B(X 8000), C&D(15000)

The previous photos (A, B, C & D) showed bacterial cells *Staph. epidermidis* and human epithelial cells in the first stage of microbial infection (adhesion)and the arrow showed the place of attachment.

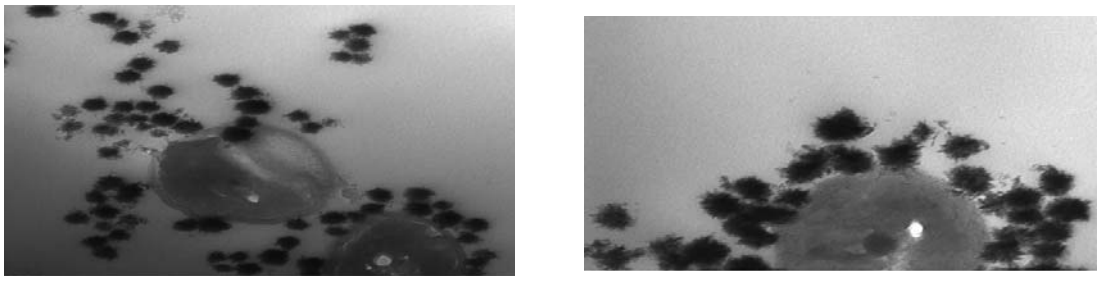

Plate no. 2: Scanning electron microscope photographs showing *Staph. epidermidis* cells invade to HEp-2 cells at magnification x 15.000

The previous plate showed *Staph. epidermidis* and human epithelial cells in the stage of invasion and the arrow showed the place of invasion.

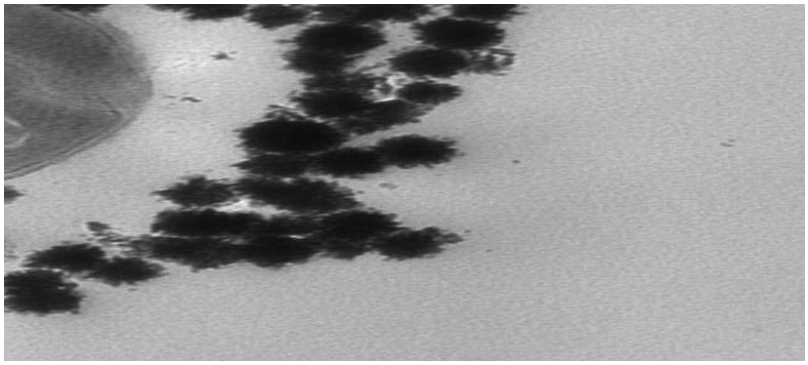

Plate No. (3): Scanning electron microscope photograph showing prevention of adhesion of *Staph. epidermidis* to HEp-2 by starch used as antiadhesion agents magnification was (× 20.000)

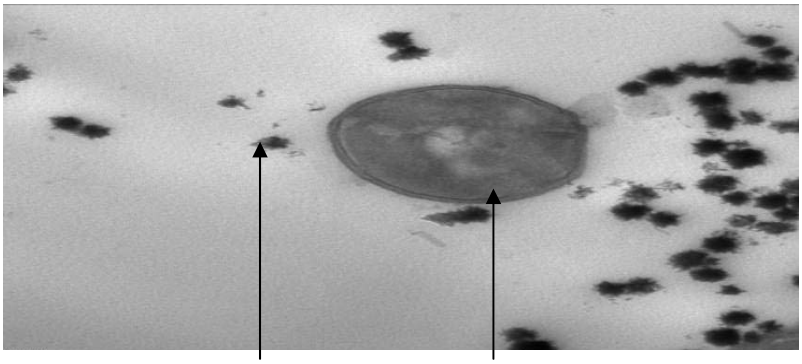

Plate No. (4): Scanning electron microscope photograph showing prevention of adhesion of *Staph. epidermidis* to HEp-2 by $CaCl_2$ used as anti adhesion agents magnification was (× 20.000)

Plate showed ***Staph. epidermidis*** away from human epithelial cells by the action of **$CaCl_2$** as anti-adhesion

4. Discussion

The strategy for using adhesion analogs to prevent infections is based on the assumption that the isolated adhesion molecule, or an active synthetic or recombinant fragment, binds to the receptor and competitively blocks adhesion of the bacteria (**Ofek *et al.*, 2003a**).

Bacterial adhesion to epithelial cells is a key step in infections, allowing subsequent colonization, invasion and internalization of pathogens into tissues. Antiadhesive agents are therefore potential prophylactic tools against bacterial infections. The range of anti-adhesive compounds is largely confined to carbohydrate analogues (**Aneta and Herbert, 2010**).

This study have been aimed at providing a basis for the developments of methods showing new means of controlling the emergence and adhesion of pathogenic *Staphylococcus* strains, through screening of different substances (plant extracts, organic and inorganic compounds) as control agents to prevent their colonization. (**Ehsanollah *et al.*, 2009**) reported that one of the key steps in controlling nosocomial infectious by MRSA could be through preventing their colonization.

Adhesion of pathogenic organisms to host tissues is the prerequisite for the initiation of the majority of infectious diseases. Five bacterial strains (*Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 6538, Staphylococcus aureus NCTC 6571, Staphylococcus epidermidis & Escherichia coli*) were tested for their adherence activity through adding of freshly prepared and overnight incubated cultures to the HEp-2 in order to determine the optimum state for the bacterial adherence; it was found that the freshly prepared culture recorded maximum values in comparison with values of overnight incubated cultures, This may be return to the cell wall of fresh culture as it have higher water content (**Soto and Hultgren, 1999**). At the same time the maximum value of the bacterial adherence was recorded in the case of the strain of *Staph. Aureus NCTC 6571*, followed by *Staph. epidermidis*. Similar results were obtained by **Blickwede *et al.*, 2000.**

During the infectious process, inflammatory stimuli activate vascular endothelial cells to express adhesion molecules and chemokines that physically engage circulating leukocytes. A coordinated sequence of adhesion and locomotion steps, including (i)

leukocyte rolling, (ii) cell activation, (iii) firm cell adhesion, and (iv) transendothelial migration, requires that adhesion receptors on leukocytes and endothelial cells are up-regulated and activated (**Springer, 1994**).

There is evidence that the receptor analogs work as agents for anti-adhesion therapy would be practical primarily against pathogens that bind to animal cells via carbohydrate-specific adhesins (i.e. lectins). In these cases, the receptor analogs are saccharides that are structurally similar to receptors for the adhesion and therefore, act by competitive inhibition. It was less than three decades ago that mannose was first shown to be a receptor for enterobacteria (**Ofek *et al.*, 1977**). Since then, the sugar specificities of many bacteria have been determined, leading to the development of receptor-like carbohydrates, which inhibit the adhesion of pathogens to host cells and tissues (**Ofek *et al.*, 2003a**).

Although plant lectins are well represented in the human diet and many of these lectins are well characterized (**Nachbar and Oppenheim, 1980; Liener, 1986; Cowan, 1999**). Their application to anti-adhesion therapy is very limited. Theoretically, these lectins could interact with animal cell surface saccharides to block adhesion mediated by lectin-carrying bacteria and they may enhance clearance of bacteria from the host (**Slifkin and Doyle, 1990**).

Because of their ready availability, plant materials possessing anti-adhesin activities are attractive candidates for antibacterial agents. There is, however, a relative paucity of information regarding the anti-adhesive properties of most plant materials. It was found the maximum anti-adhesion activity of the tested plant extracts was recorded in the case of *Trigonella fosnum* extract, followed by the extract of *Eucalyptus globules* then *Nigella sativa*; compared with control values**.Ehsanollah et al., 2009 ,** reported that, the oil based di-herbal extract formulated in this study shows a very good antimicrobial, anti-adhesive and anti-invasive activity against MRSA.

Thus, it appears only reasonable that much attention has been paid to carbohydrates as anti-adhesive agents of potential medicinal value that block the surface adhesions. Bearing in mind the well-known capabilities of proanthocyanidins to interact with macromolecules, including carbohydrates and proteins, members of this class of compounds may be another group of promising anti-adhesive compounds (**Aneta and Herbert, 2010**).

It was found the maximum anti-adhesion activity of the tested monosaccharides sugars was recorded in the case of glucose, followed by fructose and arabinose in comparison with control values. Sucrose and glucose are dietary sugars commonly consumed in the western world and the results reported here imply that diets rich in these sugars may facilitate adhesion and colonisation of the oral mucosa (**Samaranayake *et al.*, 1980; Samaranayake and MacFarlane, 1980**) and these findings have been confirmed by (**McCourtie and Douglas 1981**) Blocking or inhibition of these lectins by suitable carbohydrates or their analogs for the prevention and treatment of microbial diseases is the aim of anti-adhesion therapy of such diseases (**Kahane and Ofek, 1996; Zopf and Roth, 1996;Karlsson, 1998; Kelly and Younson, 2000; Sharon and Ofek, 2001; Ofek *et al.*, 2003**), while the maximum anti-adhesion activity of the tested disaccharides sugars was recorded in the case of sucrose, followed by maltose and lactose in comparison with control values. Also the maximum anti-adhesion activity of the tested polysaccharide sugars was recorded in the case of starch, followed by cellulose in comparison with control values.

Very significantly, lectin-inhibitory saccharides have been shown to protect mice, rabbits, calves and monkeys against experimental infection by lectin-carrying bacteria (**Nathan, 2006**).

Different inorganic compounds including (NaCl & $CaCl_2$) were tested for their activity as anti-adhesion agents.

It was found the maximum anti-adhesion activity of the tested inorganic compounds was recorded in the case of $CaCl_2$, followed by NaCl in comparison with control values.

But it was revealed that calcium ions significantly increased the binding of tested lactobacilli to IPEC-J2 cells; and therefore, added calcium may be useful in enhancing the adhesion of normally weakly adhesive probiotic cultures. The increase of adhesion in the presence of Ca^{2+} is due to the creation of additional Ca^{2+} mediated bonds which shield more hidden length (**Nadja *et al.*, 2007**)

Different concentrations of starch were tested for their activity as anti-adhesion agents. And the maximum anti-adhesion activity of the tested concentrations was recorded.

These results imply that exogenous or endogenous carbon sources may affect the oral and vaginal carriage of *C. albicans*, by modifying their adhesive properties.

Different concentrations of $CaCl_2$ were tested for their activity as anti-adhesion agents. And the maximum anti-adhesion activity of the tested concentrations was recorded.

It has thus far been impractical to use analogs of adhesions for anti-adhesion therapy, because they are typically macromolecules that are not readily available and because they must be employed at relatively high concentrations. In addition, careful consideration must be given to their toxicity and immunogenicity. Nevertheless, modern proteomics and recombinant biotechnology have permitted the development of

unique types of relatively small peptides for anti-adhesion therapy, as reported by (**Kelly *et al*., 1999**).

Many extracts not only has antibacterial activity which will be very useful in the treatment, but also has anti-adhesive and anti-invasive property that adds the value of the extract to be a colonization inhibitor, hence the extract could be used for treatment and prophylaxis (**Ehsanollah *et al*., 2009**).

Investigation the adhesion, invasion and anti-adhesion of *Staphylococcus epidermidis* were carried out, investigation the adhesion of *Staphylococcus epidermidis*, was carried out after incubation.

Also investigation of cell invasion was carried out from six hours incubated bacterial culture. While anti-adhesion of *Staphylococcus epidermidis* was investigated through suspension of the bacterial culture.

Surface modification is an effective way to decrease bacterial adhesion. In this study, we prepared surfaces with different wettability on titanium surface based on TiO_2 nanotube to examine the effect of bacterial adhesion. Observed by SEM and contact angle measurements, the different surfaces have different characteristics (**Tang *et al*., 2011**).

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