



## Antibacterial Activity of Aquatic Zea Mays L. Hairs Extract against Different Bacteria in Babylon Province: An In-Vitro Study

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### Abstract

Background Zea Mays L. has been frequently used as a therapeutic agent used for treatment of various diseases. Objective A evaluation the antibacterial, anti-biofilm, adherence and swarming in hibitory activities of Zea Nujm mays L. hair extract against different bacteria. Methods: Well diffusion method, bio film inhibition test using a tissue culture plate, adherence and swarming inhibition assays were done for estimation and evaluation the anti-bacterial activity of Zea mays L against a number of bacteria. Results: The result showed that Zea Mays L hair extracts the vying potent antibacterial activity. It's also inhibiting adherence to epithelial cells and biofilm formations of the bacteria. The result shows that the extract has high anti-swarming activity. Conclusions: The extract has marked antibacterial activity. It can inhibit biofilm formation, swarming to ility, and bacterial cell adherence to oral epithelial cells.

**Keywords:** *Biofilm, Bacterial adherence, Swarming.*

### Introduction

In spite of the major advance made in the microbiology field, infectious diseases remain an important cause of mortality worldwide, where multi –drug resistant bacteria and fungi are increasingly prevalent (1), because of indiscriminate use of drugs that traditionally utilized a for treating in factious diseases (2).

In addition, microbial bio film infections have been increasing at a high rate. National Institutes of Health (NIH) have mentioned that (70%) of microbial infections are related to biofilm(3). Biofilm is an aggregation of cells associated to surface surround with complex exopolymeric substance (EPS).

The biofilm making bacteria the resistant because of prevent penetration of antimicrobial agents. The biofilm has been found to cause a variety of infections, such as urinary tract infections, of it is media, catheter infections, gingivitis, dental plaque formation and coating contact lenses (4).

Bacterial motility plays inessential role in microbial colonization, spreading of bacteria and contributes to the biofilms formation. Bacteria have many types of motility depending on the bacteria included a swimming, twitching, swarming, sliding and gliding. Many organisms colonize a surface via using a flagellum to swim on the surface and associated by bacterial adhesions, like type IV pili (5). Bacterial adhesion to the cells is critical step for colonization and infection of bacteria, mainly in a system of continuous urinary flow. Many pathogenic bacteria have the ability to express the number of adhesive factors (6).

Chemical drugs are usually expensive, also undesirable side effects of most antibiotics and the emergence of uncommon infections led the scientists to find new antimicrobial agents from medicinal herbs which are active against plank tonic microorganisms and microbial associated biofilms (7). Natural plants represent an important medicinal agent for treating of bronchial, respiratory

and gastrointestinal, infections caused by infectious microorganisms. It is acceptable to the human with few side effects and safe compared with synthetic chemical drug (8). There are two important strategies in using medical herbs; the choice of herb depending on its action, and the appropriate therapeutic dosing that will determine the efficacy of herbal treatment and decrease the need to intervene with antibiotics (9).

Several plants with antimicrobial effects are widely used in medicine, like corn silk (*Zea mays* L.) refers to the female flower stigmas of maize belongs to Gramineae family. Fresh corn silk like soft silk threads that are either yellow-brown or slightly green. It is an available waste substance from corn cultivation (10). Its commonly grown in warm climates like tropical regions such as North American, India, China and various places of the world (11).

Silk of corn has spooning, alkaloids, vitamins, carbohydrates, proteins Na, Mg, K, and Ca salts; steroids like sitosterol and stigma sterol; volatile oils, flavonoids and Tannins. There are many reports demonstrated the biological activeness of corn silk components. Corn silk extract is being applied commonly for treatment of some medical conditions such as cystitis, edema, gout, kidney stones, prostates' and nephritis, and urinary system irritation (12). Its exhibited antioxidant activity, anti-diabetic activity, anti-fatigue activity, anti-depressant activity, antitumor activity and antibiotic activity towards corn earworm (13, 37).

The objectives of the present study were to identify the antibacterial activities of *Zea mays* L hair extract, and to compare the activities with standard antibiotic like gentamycin. In addition, evaluating the anti-biofilm, anti-adherence and swarming inhibitory effect against bacteria.

## Material and Methods

### Preparation of Aquatic Extracts

The dried *Zea mays* L. hair powder was used and obtained from the pharmacy in Al-Hila city, Iraq. Aqueous extract was prepared by soaking 30 ml of corn of silk in 100 ml distillate water and mixed, then filtration the

mixture (by using of 0.45 Millipore filter paper) and sterile the juice by filtration. This extract was regarded as the 30% concentration of the silk of corn extract and for screening of antimicrobial activity (14).

### Bacterial Isolates

In this study, 11 Gram negative, and 8 Gram positive bacterial isolates (isolated from clinical samples) were investigated.

The bacterial isolates represented by; *S. aureus*, *S. epidermidis*, *S. pyogenes*, *S. pneumoniae*, *E. faecalis*, *P. aeruginosa*, *P. fluorescens*, *E. coli*, *S. typhi*, *E. aerogenes*, *K. pneumoniae*, *Proteus mirabilis*, *P. vulgaris*, *Acinetobacter*. These bacterial isolates were activated and cloned three times and stored on the slant of nutrient agar slant at 4 °C. The bacterial identification was confirmed by using traditional biochemical tests (15).

### In-Vitro Antimicrobial Activity Test (Well Diffusion Test)

A nutrient broth tube were inoculated with a loop full of bacterial isolates growths and incubated at 37 °C for 18 hours. Then, diluted these bacterial suspensions with normal saline, adjust the turbidity and compare with standard McFarland tube (0.5) to obtain bacterial suspension has  $1.5 \times 10^8$  CFU/ml.

Dip cotton swab into adjustment suspension and streaks the entire Mueller-Hinton agar surface of the plates and left the plates for 5-15 minutes to dry at room temperature. Media were cut into four wells (5 mm diameter) by cork borer and add 0.1ml of the extracts. Then, incubated the plates at 37 °C for overnight. The inhibition zone size was measured from edge of well to the growth inhibition edge (16).

### Biofilm Formation Assay

Tissue culture plate method (TCP) (semi quantitative Microtiter plate test) developed by Christensen et al., (1985) (17) was most commonly used and was represented as a standard test of biofilm formation as follows:

- Inoculated TSB has 1% glucose with isolates from fresh agar plates and incubated at 37°C for 18 hours, then diluted 1:100 using TSB.
- Individual wells of sterile, polystyrene, 96 well-flat bottom plate of tissue culture was filled

with 150µl aliquots of the diluted cultures and a broth alone represent as a control. Each isolate was inoculated in triplicate.

- Incubated the tissue culture plates at 37°C for 24 hours. After the incubation, the content of each well was removed by tapping of the plates. Then, washed the wells with phosphate buffer saline (PBS, pH 7.2) four times to remove free-floating 'plank tonic' bacteria.
- Biofilms formed by adherent 'sessile' organisms and plant extracts in plate were fixed by placing in oven at 37C° for 30min.

- All wells stained with crystal violet (0.1% w/v). Excess stain was rinsed off by deionized water was hang and kept the plates for drying.
- 150 µl of acetone/ethanol (20:80 v/v) mixture were added to dissolve bounded crystal violet.
- The optical density (O.D.) at 630 NM was recorded and in turreted the results according to the Table (1).
- This method was repeated and modified by adding the extracts in stage 2 to inhibit formation of biofilm by extracts.

**Table 1: Classification the adherence and biofilm formation of bacterial by TCP method (18)**

Mean of OD value at 630nm	Adherence	Biofilm formation
<0.120	Non	Non
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

### Adherence Test

- The adherence of bacteria to oral epithelial cell is one of important virulence properties of this bacteria and detected as following steps:-
- Prepare the bacterial broth and in cubated for 72 hrs.
- Proper dilution of bacterial broth by use phosphate buffer (PBS) then take  $10^8$  (CFU/ cm3).
- Take the oral epithelial cells by swabbing the epithelial layers of the oral cavity by cotton swabs, then transferred directly into sterile tubes contain PBS (PH 7) after that wash the epithelial cells by PBS by using centrifuge 5000 RPM (10 minute) for three times.
- Filtered the PBS contain epithelial cells by filter paper and place the epithelial cells on the cover slide by press the cover on the surface of filter paper then lifted to be dry.
- Place the cover slides the cover slide on the sterile

glass plate, then add 5ml of previously prepared bacterial broth and extract and place the plate contains the epithelial cells and bacterial broth on an incubator for 1hr at 37c.

- Wash the cover slides by PBS to remove an adherent bacteria.
- Fixed the epithelial cells by ethanol for 15 minutes.
- Stain with giemsa stain (30%) for 20 minutes, then wash the cover slides by DW and lifted to dry by air.

The cover slides were placed on glass slides by inverted position, then tested under light microscope. (Mataveki et al (19) and Avila-Compose, et al (20).

This method was repeated and modified by adding the extracts in stage 3 to inhibit formation of biofilm by extracts.

**Table 2: Classification adherence of bacteria by TCP method**

Mean OD values	Adherence	Biofilm formation
<0.120	Non	None / Weak
0.120 – 0.240	Moderately	Moderate
>0.240	Strong	High

### Inhibition of Motility (Swarming) by Plant Extract

The method of I walokunet al., (21) which contain the next steps was used:

- Bacterial isolate was cultured on nutrient agar and incubated at 37°C for 24 hr. as a control.
- Plant extract was added separately in concentration of (10%, 20%, 30%) respectively and incubated for 24 hr at 37°C.
- Added few drops of ethanol (90%) were in a petri dish, cover and cultured with bacterial isolate and incubated for 24 hrs at 37°C.
- After that determined the influence of plant extracts by measuring swarming diameter.

### Antibacterial Activity Assessment

Agar disc diffusion was used to determine the antibacterial activity (15). Inoculated the agar plates with (0.1) ml broth culture of examining organisms and spreader with an L-shaped glass spreader. The antibiotic disks of ciprofloxacin were adding an agar plate

center (performed the plates in triplicates). Then, incubate the plate for overnight at 37°C. After 24 hr, the zone of inhibition of the extract was noted for all isolates. The inhibition zone diameters were measured in millimeter (mm) (38, 39, 40).

### Result and discussion

The antibacterial effect of aquatic Zea mays Extracts against bacteria was carried out using the well diffusion method. The results show that Zea mays Extract was found to be active in inhibiting the growth of testing bacterial pathogens according to the inhibition zone. Its in hibita wide range of bacteria, including gram positive and gram negative.

These results revealed that these extract gives a high inhibition effect against gram-positive bacteria including *S. aureus*, *S. saprophyticus*, *S. epidermidis*, *S. pneumoniae*, *S. progenies*, *S. agalactia*, *S. mutants* and *E. feacalis* with inhibition zone ranging between 26 and 32 mm (Figure1)

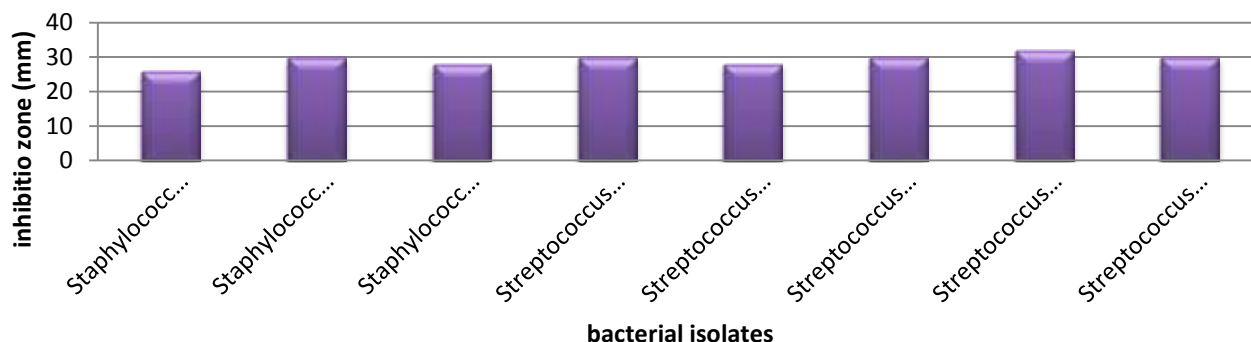


Figure 1: The antibacterial effect of Zea mays L. hair extract on different Gram positive bacterial isolates

Among gram- negative bacterial isolates, The maximum inhibition zone of the extract was observed against *S. typhimurum* (35 mm),

followed by *S. typhi* (34 mm) and the minimum inhibition zone was seen against *K. pneumoniae* (17mm) (Figure 2).

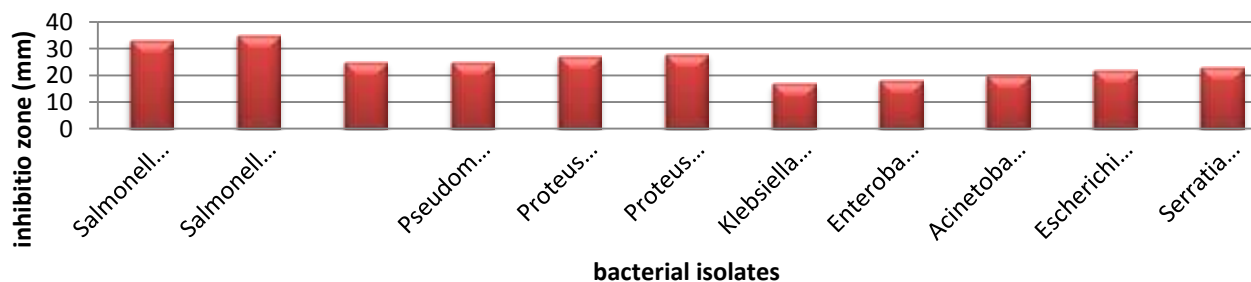
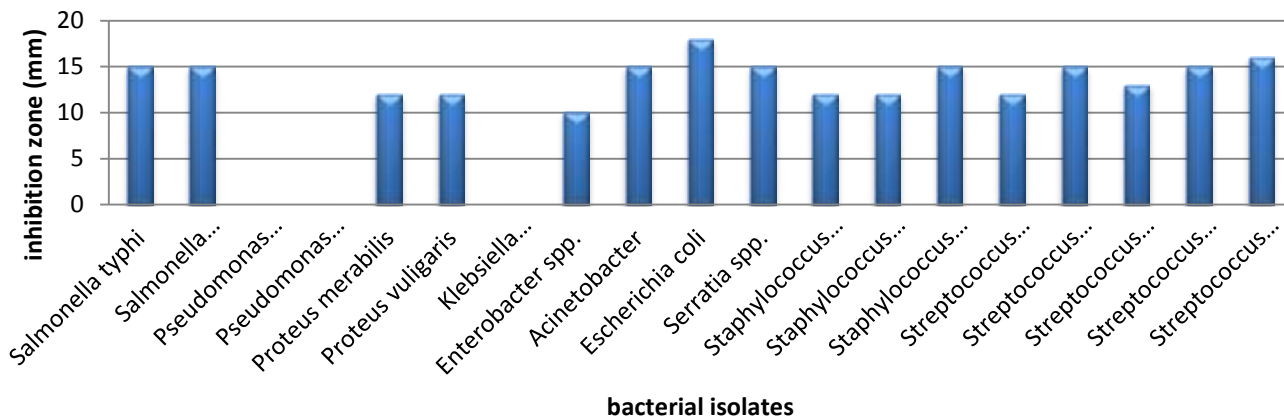


Figure 2: The antibacterial effect of Zea mays L. hair extract on different gram negative bacterial isolates



**Figure 3: Effect of ciprofloxacin against different bacterial isolates**

The antibacterial efficacy of extract was compared a ciprofloxacin, Figure (3) showed that bacterial isolates from different site of infection were sensitive to antibacterial ciprofloxacin except *Pseudomonas* spp., and this ciprofloxacin antibacterial effect was less than the extract effect.

The results in this study were compatible with other studies, like (22) who reported that the extract of corn silk has antibacterial activity; it's in hi bit the growth of *Staphylococcus aureus* and *Staphylococcus epidermis* bacteria.

Similar results reported by (23) who found that *Zea mays* L. has been known to be effective against pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli* K. pneumoniae, *P. aeruginosa* *Shigella* spp. and *Salmonella* spp.

Surjee and Zwain, (24) found in his study, that all bacterial species isolated from infections of the urinary tract that included in the test showed effected toward aqueous extracts of *Zea mays* L. hairexcept *P. Lutela*. It was found that the extract possesses strong activity toward *S. aureus*, *S. capitis*, and *P. aeruginosa* while moderate against *S. albus*, *M. Morganii*, *S. epidermis*. *E. coli*, *K. Pneumonia*, *Micrococcus* and *C. frenu dii* and weak against *Proteus*. Spp, *Klebsiella*. spp., the extracts were less or no antibacterial action at concentrations of 25 and 12.5%.

Nessa et al, (13) stated that different corn silk solvent extracts exhibited a board range of antimicrobial activity .It was found that administration of aqueous xtract of corn silk significantly reduces the symptoms in patients have urinary tract infections, also

decrease in the values of routine urine examination such as pus cells, RBCs, and crystals, without any reported side effect which indicate its efficacy and safety of the extract (9). In contrast, Al-Saddi et al. (25) found in his study that corn extracts don't have antibacterial action against the tested bacteria when using well diffusion assay. It may be due to that the in habitation zone size may be influenced by diffusion rate of the extract, the extract concentration on the well, the density or viscosity of the culture medium, the sensitivity of the microorganism to the extracts, and the reaction between the extract and the medium. The reason for the different sensitivity of the Gram-negative compared to that of Gram-positive bacteria ascribe to differences in their cell wall structure. Gram- negative bacteria contain a peptidoglycan layer and outer phospholipidic, which is permeability barrier, whereas Gram-positive contain an outer peptidoglycan layer barrier. Biological and pharmaceutical action of *Zea mays* L. may contribute to the active compounds found in these plant such as phenolic compounds, particularly flavonoids, proteins, vitamins, carbohydrates and minerals, volatiles oils and steroids, alkaloids, and spooning that possess antifungal and antibacterial activities (26). Phenols of the plant are groups of antioxidant that inhibit stages of the cancer process. Phenols protected from disease of cardiovascular system and lipoprotein oxidation. It is exhibiting board range of biological effects including antibacterial, antiviral, anti-inflammatory, land anti-allergic (11). Phenolic compound was causing protein denaturation of microbes through the pause of the enzymes action of metabolic reactions, and dead the microorganism (27).



Flavonoids were considered as the greater constituent of the phenolic composites and it had antimicrobial activities. The flavonoids compound in the corn hair possesses ant oxidative activity, in which the cells protected from oxidation process damages by free radicals in the body. Flavonoids disrupt the integrity of the cell membrane in a way denature bacterial proteins and bacterial cell membrane damage (28). Corn silk also contains saponin and tannins, tannins are water soluble polyphones. It is traditionally referred as tannic acid. Tannins have antimicrobial activity Tannins are toxic for bacteria and fungi. Its bind with the organism cell wall and cause inhibition of the growth. Saponin can be found as steroidal saponin, triterpenoid spooning or steroidal alkaloids. It is the essential constituents of many drugs. Spooning has antibacterial, antiviral activity, ant parasitic, antifungal

and antitumor, capacities (11). The hair of Zea mays world widely utilized as a therapeutic agent and as classical medicine in many regions of the world such as China, Turkey, France and United States (11). It has been consumed as a therapeutic remedy for various illnesses, particularly renal diseases, including chronic nephritis, cystitis, benign prostate hyperplasia, edema and gout. It helps to pass a stone from kidney and urinary tract and inhibit the in flammation (29).The results of the biofilm and the adherence inhibition effect of aquatic extract of Zea mays L. hair against gram negative bacteria were shown in (Table 3). The results displaced that the extract having a high inhibition capacity of bacterial adherence and the formation of biofilm and for most bacterial isolates. The extract having potential anti-biofilm and anti-adherence properties

Table 3: Biofilm and adherence inhibition effect of aquatic corn extract against gram negative bacteria

Bacteria	Biofilm formation of corn	Adherence of corn
<i>S. typhi</i>	High**	High
<i>P. aeruginosa</i>	High	High
<i>P. fluresence</i>	High	High
<i>P. vulgaris</i>	High	High
<i>P. mirabilis</i>	High	High
<i>K. pneumoniae</i>	*Moderately	High
<i>E. aerugenes</i>	*Moderately	*Moderately
<i>Acinetobacter</i>	*Moderately	*Moderately
<i>E. coli</i>	High	High

The growing of bacteria on the surfaces usually develop biofilms, which appear as complex style of living adaptation that gives protection from environmental conditions. The cells of biofilm are higher resistant to antimicrobial agents than plank tonic cells. Biofilm formation associated with the capacity of bacteria to produce disease. Its play an important role in

pathogenesis of many bacterial species. Despite the importance of biofilms, few studies have concentrated on the fining of compounds capable to specifically target and inhibit this type of bacterial growth. Instead, most research has been aimed at the discovery of anti-infective agent skill a board range of resistant plank tonic bacteria (30).

Bacterial adhesion is an essential initiation step in bacterial colonization and pathogenesis, both for pathogenic and commensalism bacteria. Bacteria have many adhesive surface factors like capsule, pili or fimbriae, and other surface proteins (31). In bacterial species like *E. coli*, *V. cholerae*, *P. aeruginosa* and *S. enteritidis*, adhesive factors are presented in the tips of cell-surface construction which stretch out from the cell membrane call edgily. Alternatively, nonplus adhesive factors may be directly presented from the cell surface of bacteria (32).

In the current study, anti-swarming activity of aquatic *Zea mays* L. hair extract at different concentration (10%, 20%, and 30%) against gram negative bacteria were investigated (Table 4). The extracts at 10% concentration could inhibit only the swarming of *K. pneumonia* and *E. aerogenes*, while the extracts could inhibit the swarming activity of all tested bacteria when increased concentrations up to 10% (20 and 30%). The inhibition of swarming motility were extrusive proportioning with increase of extract concentration as a result of increase of active corn silk components concentration

**Table 4: Anti-swarming activity of aquatic corn extract against gram negative bacteria at 10% 20% 30% concentration**

Bacteria	Swarming of corn		
	10%	20%	30%
<i>S. typhi</i>	Motile	No motile	No motile
<i>P. aeruginosa</i>	Motile	No motile	No motile
<i>P. fluresence</i>	Motile	No motile	No motile
<i>P. vulgaris</i>	Motile	No motile	No motile
<i>P. mirabilis</i>	Motile	No motile	No motile
<i>K. pneumonia</i>	No motile	No motile	No motile
<i>E. aerogenes</i>	No motile	No motile	No motile
<i>Acinetobacter</i>	Motile	No motile	No motile
<i>E. coli</i>	Motile	No motile	No motile

The swarming phenomenon occurring on solid surfaces and mediated by flagella and at some time the short vegetative cells transform into elongate hyper flagellated cells. Flagella swarming motility led to biofilm formation (33), and its also associated with the expression of virulence genes, the ability of bacteria to invade human tissue and increased antibiotic resistance (34).

The swarming phenomena has been studied in different bacteria including *Bacillus* spp *Serratia* spp. *Aeromonas* spp. *Pseudomonas*

spp., *Salmonella* spp., *Yersinia* spp., *E. coli*, *Vibrio* spp., and *P. mirabilis* (35). *P. aeruginosa*, can undergo the flagellum-dependent swimming motility and the surface-associated twitching and swarming motilities, which are traditionally mediated by hyper flagellation and type-IV pili, respectively(5).

Swarming motility of *Proteus* is a characteristic phenomenon, which enable the bacteria to colonize surfaces and invade the host (33). Its happen on (1.5%) of agar surface and describes flagellum-associated movement across the surface, resulting a

characteristic bull's eyes pattern *P. mirabilis* is a main cause of catheter-associated urinary tract infections, *P. mirabilis* initiates the colonization of the urinary system by colonizing the perurethral area. Then, this microorganism moves across the urethra and reach the bladder. The contact with these sites is facilitated by motility (36).

## Conclusion

From this study *Zea mays* L. hair extract showed strong antibacterial activities to

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