

RESEARCH ARTICLE

Effect of Tea Extract on Few Bacterial Species Isolated from Tonsillitis by Adding Some Concentrations of NaCl

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ABSTRACT

Several bacterial species associated with tonsillitis were isolated from the samarra general hospital, including *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The extract of alcohol and water tea was used to observe its effect in inhibiting the growth of bacteria with the addition of 3 concentrations of NaCl (5, 7.5, 10 g/L) to the medium used (Muller hinton agar, Nutrient agar), the results showed that the effect of *S. aureus* on the alcohol and water extracts and all the concentrations used in addition to the use of the muller hinton agar and the use of nutrient agar the result showed that the bacteria were affected by alcohol extract and all the concentrations used however when adding the water extract the effect of bacteria was observed when adding the third concentration of NaCl, The effect of *E. coli* was observed by alcoholic and water extract of tea with the addition of the third concentration of NaCl using the muller hinton agar and the use of the nutrient agar the growth of *E. coli* was observed by alcoholic extract only and by the addition of the third concentration of NaCl, The effect of the bacteria was observed with alcohol extract only for the tea and all the concentrations using the muller hinton agar and using the nutrient agar the effect of bacterial growth was observed by the extract when adding the third concentration of NaCl to the medium, we conclude are differences in the effect of extract on isolated bacterial species

Keywords: Bacteria, Concentration, Inhibition, NaCl, Tea extract.

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INTRODUCTION

Inflammation of the tonsils and pharynx is one of the most important infections of the upper respiratory tract and is one of the most common diseases around and affects all age groups and both sexes and is most frequently in children between the ages of 5 to 15 years, the incidence of tonsillitis increases while in adults it decreases due to the immunity generated against many serotypes.¹⁻⁶

The tonsils are composed of lymphatic tissue and are a component of Waldeyer's ring along with the adenoids (nasopharyngeal tonsil), tubal tonsil, and lingual tonsil.¹⁻³ They serve as an important defense against inhaled or ingested pathogens by providing the initial immunological barrier to insults; many aerobic and anaerobic bacteria are endemic to the tonsils and pharyngeal areas where appropriate conditions such as temperature 35–37°C, high humidity and heterogeneity of oxygen are present, bacterias especially *Streptococcus* and *Staphylococcus* are first found specifically *S. aureus*.⁴

Bacteria are also a major cause of tonsillitis and pharyngitis infection in infants and young children are characterized by pharyngitis accompanied by swollen lymph nodes surrounding the pharynx. In older children and adults the infection is more severe, manifested as tonsillitis and pharyngitis; although they are found naturally in those areas of the tonsils but also tend to move causing inflammation of the middle ear and upper and lower respiratory infection and acute glomerulonephritis and meningitis and scarlet fever and rheumatic heart disease.⁵ The immune system is an important defensive device in cases of infection as the host's different defense mechanisms play an important role in dealing with infection. The task of protections the body form various infections, including tonsillitis, and the various medical preparations have an important role in eliminating many sources of infection. Sometimes have different side effects, and many microbes have acquired resistance against a wide spectrum of these preparations, which called for a medical conference to return to the natural pharmaceutical sources of plant extracts and medical herbs

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for containing the active substances and duck for the growth of microorganisms and as safe sources for the pharmaceutical industry.³

One of these medically important herbs is tea, the most frequently consumed beverage worldwide, besides water. All the three most popular types of tea, green (unfermented), black (fully fermented), and oolong (semi-fermented), are manufactured from the leaves of the plant *Camellia sinensis*. Tea possesses significant antioxidative, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering, and thermogenic properties.⁷⁻⁹

This study aims to isolate and diagnose some species of bacterial from tonsillitis patients from samarra general hospital and statement the effect of alcohol and water extracts tea in inhibiting isolated microbial and addition to some NaCl concentrations.

MATERIALS AND METHODS

Collection of Specimens

A 25 samples were collected from different ages and both sexes of people with tonsillitis and cotton swabs, and in cooperation with a group of specialists of the nose, ear, and throat in samarra general hospital, bacterial samples were identified *S. aureus*, *E. coli*, *K. pneumonia*.

Bacterial Isolates

Samples were planted on the following media.

- Blood agar
- MacConkey's agar
- Nutrient agar
- Muller hinton agar
- Mannitol Salt Agar to diagnose bacteria *S. aureus*.

All media were attended and dissolved in distilled water and then sterilized by autoclave at a temperature of 121°C and pressure of 15 pounds for 15 minutes. The dishes were incubated aerobically and at 37°C for 24 hours.

Diagnosis of Bacterial Isolates

The morphological and chemical properties of developing colonies were observed.

Microscopy and Agricultural Characteristics

The bacteria were first identified by observation of agricultural characteristics of the developing colonies on the media used form where size, height, and colonial color and attended thin swabs and a pigment with a gram stain to observe cell shapes, arrange and their susceptibility to pigmentation.¹⁰

Bacteriological Test

IMVIC tests were conducted, including indol test, methyl red, vog's proskauer, and cimmon citrate.¹⁰ As well as tests such as oxidase, catalase, coagulase to confirm isolated bacterial species.¹¹

Preparation of the Plant Extract Tea

Ahmed's method adopted,^{12,16} in the preparation of water and alcohol extracts added 40 m from tea powder both on my

own to 160 mL from distilled water. In the case of alcohol extract the same quantity has been added to ethyl alcohol at a concentration 95%, then leave the mix for 24 hours in the refrigerator for the purpose of soaking and then filtered through several layers of sterile gauzo. Then the resulting extracts were concentrated by a rotary evaporator at a temperature of 40°C until a heavy liquid was obtained. The solution is filtered with microbial filtration membranes (Micropipette) with minute openings and a diameter of 0.22 µL each sample is placed in sealed glass bottles and marked and stored in the refrigerator until use.

Test the Sensitivity of Bacteria Isolated to Plant Extract Tea

Effectiveness of the plant extract of the tea and the addition of known NaCl concentration were tested using the swabbing method in the dish, If both arms are prepared nutrient agar, Muller hinton agar according to the manufacturer's instruction when preparing the center of muller hinton agar 3 NaCl concentration were used in the first concentration case 5 g/L was weighed and added to the media in the second concentration 7.5 g/L NaCl was weighed and added to the media in the third concentration 10 g/L NaCl was weighed and added to the media, when using the nutrient agar media the percentage of NaCl contained in the components of the media was adopted as the first concentration because it contains a ratio of 5 g/L NaCl in the second concentration 7.5 g/L NaCl was weighed and added to the media in the third concentration 10 g/L NaCl was weighed and added to the media and then sterilization of the agricultural communities using autoclave and then put the circles in the dishes of petri and after the hardening of dishes was planted with the newly developed bacteria using cotton swab and then the work was drilled with diameter 5 by sterile fine puncture and add the extract of alcohol and water both by itself and by 2 replicates using micropipette the use of distilled water as a negative control sample incubated dishes at a temperature 37°C for a 18 to 24 hour and then measured the diameter of the inhibition zone .

RESULTS AND DISCUSSION

Isolation and Identification

Been isolated 25 isolation bacterial of tonsillitis and bacterial isolates were identified based on agricultural characteristics and microscopic characteristics and biochemical tests which included tests for the diagnosis of gram positive bacteria *S. aureus* and tests for the diagnosis of gram negative bacteria *E. coli* , *K. pneumonia* and the result showed that bacteria *Staph.aureus* is the commen cause of tonsillitis this result was consistent with the findings of the³ which isolated it from Tonsillitis (41.9%) and less that 14 which reported that the incidence of *S. aureus* in patients with tonsillitis (57.7%) rate is found naturally in the region and is responsible for many of the most important infections including recurrent tonsillitis and external ear infection and also involved in sinusitis it also isolated bacteria *K. pneumonia* and this result is consistent¹³

Table 1: Effect of the plant extract tea on the bacteria *S. aureus* by muller hinton agar.

Control	Water extract	Cohol extract	Concentrations (g/L)
A 0	C 0.35 ± 1.75	± 1.55 0.07 B	Concentration 5
A 0	B 0.45 ± 2.00	± 1.75 0.35 B	Concentration 7.5
A 0	2.55 ± 0.45 A	± 2.15 A 0.21	Concentration 10

*The similar letters indicate there are no significant differences ($p \leq 0.05$) among the group's vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among groups vertical comparison.

Table 2: Effect of the plant extract tea on the bacteria *S. aureus* by nutrient agar

Control	Water extract	Cohol extract	Concentrations (g/L)
A 0	B 0	0.21 ± 1.65 C	Concentration 5
A 0	B 0	1.95 ± 0.07 B	Concentration 7.5
A 0	± 1.65 0.21 A	± 2.4 0.14 A	Concentration 10

*The similar letters indicate there are no significant differences ($p \leq 0.05$) among they group vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among groups' vertical comparison.

Table 3: Effect of the plant extract tea on the bacteria *E. coli* by Muller Hinton agar

Control	Water extract	Cohol extract	Concentrations (g/L)
A 0	A 0	B 0	Concentration 5
A 0	A 0	B 0	Concentration 7.5
A 0	2.35 ± 0.40 A	2.00 0.35 ± A	Concentration 10

*The similar letters indicate there are no significant differences ($p \leq 0.05$) among the group vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among group's vertical comparison.

Table 4: Effect of the plant extract tea on the bacteria *E. coli* by nutrient agar

Control	Water extract	Cohol extract	Concentrations
A 0	A 0	B 0	Concentration 5
A 0	A 0	B 0	Concentration 7.5
A 0	A 0	1.35 ± 0.25 A	Concentration 10

*The similar letters indicate there are no significant differences ($p \leq 0.05$) among groups vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among groups vertical comparison.

Table 5: Effect of the plant extract tea on the bacteria *K. pneumoniae* by Muller Hinton agar

Control	Water extract	Cohol extract	Concentrations
A 0	A 0	1.00 ± 0.0 A	Concentration 5
A 0	A 0	1.15 ± A0.07	Concentration 7.5
A 0	A 0	1.55 0.07 ± A	Concentration 10

*The similar letters indicate there are no significant differences ($p \leq 0.05$) among groups vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among groups vertical comparison.

Table 6: Effect of the plant extract tea on the bacteria *K. pneumoniae* by nutrient agar

Control	Water extract	Cohol extract	Concentrations
A 0	A 0	B 0	Concentration 5
A 0	A 0	B 0	Concentration 7.5
A 0	A 0	1.45 ± 0.21 A	Concentration 10

*The similar letters indicate there are no significant differences ($0.05 \leq p$) among the groups vertical comparison).

*The different letters indicate there are significant differences ($p \geq 0.05$) among groups vertical comparison.

which recorded a ratio of isolation of these bacteria by (8.82%) it also isolated bacteria *E. coli* and that its presence in the area of pharynx is strongly related to the contamination of materials in the vicinity of the child and it does not settle the pharynx but moves to the original home is the small intestine through feoal contamination where contamination materials surrounding the child such as games and special purposes.

***S. aureus* Effect of Tea Extract on Bacterial Growth**

The results were shown when using muller hinton the effect of the cohol extract at the first concentration to add NaCl for media 1.5, 1.6 mL and the secondary concentration to add NaCl for media 1.5, 2 mmL when adding the third concentration for media 2, 2.3 mmL, and showed the water extract of tea at the first concentration to add NaCl for media 1.8, 1.7 mL and the

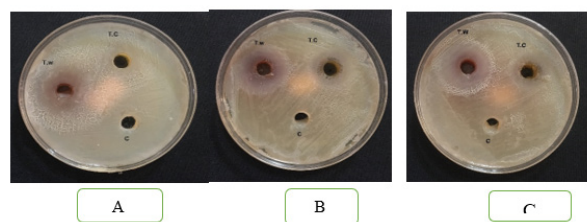


Figure 1: The effect of plant extracts on tea on bacteria *S. aureus* and using Muller Hinton agar A (Con First for NaCl) B (Con secondary for NaCl) C(Con third for NaCl)

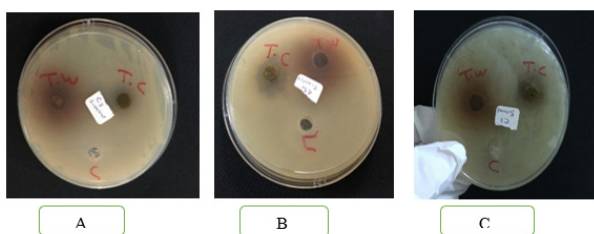


Figure 2: The effect of plant extracts on tea on bacteria *S.aureus* and using Nutrient agar A (Con First for NaCl) B (Con secondary for NaCl) C(Con third for NaCl)

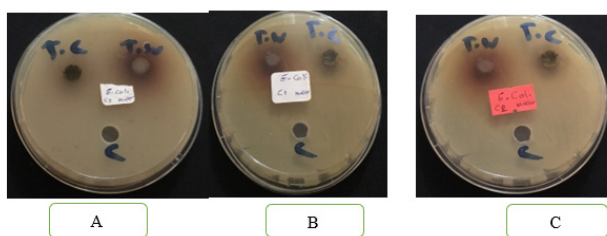


Figure 3: show the effect of plant extract on tea on bacteria *E. coli* and using Muller Hinton agar A (Con First for NaCl) B (Con secondary for NaCl) C(Con third for NaCl).

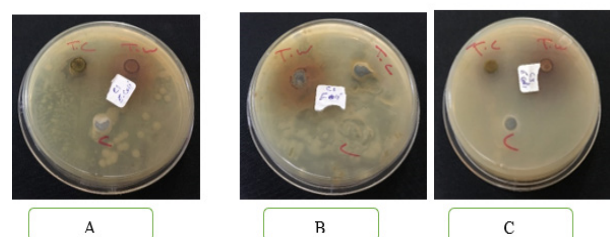


Figure 4: Effect of plant extract on tea on bacteria *E. coli* and using Nutrient agar A (Con First for NaCl) B (Con secondary for NaCl) C(Con third for NaCl).

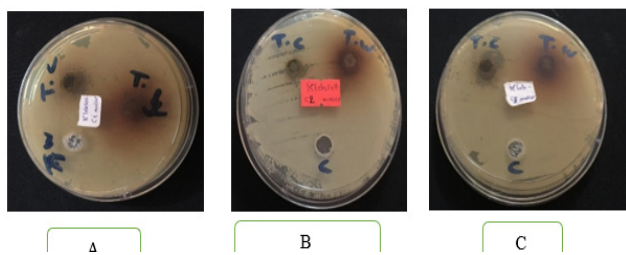


Figure 5: The effect of plant extract on tea on bacteria *K. pneumoniae* and using Muller Hinton agar A (Con First for NaCl) B (Con secondary for NaCl) C (Con third for NaCl).

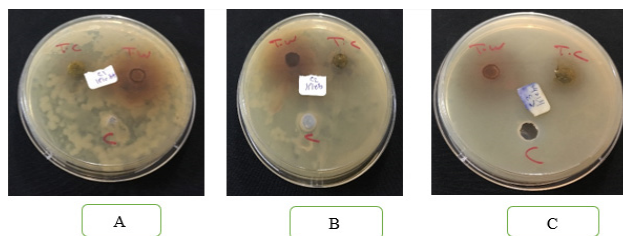


Figure 6: The effect of plant extract on tea on bacteria *K. pneumoniae* and using Nutrient agar A (Con First for NaCl) B (Con secondary for NaCl) C(Con third for NaCl).

secondary concentration to add NaCl for media 2, 2 mL when adding third concentration for media 2.6, 2.5 mL is shown in Table 1 and Figure 1.

When using the Nutrient agar, results showed the effect of alcohol extract at the first concentration to add NaCl for media 1.8, 1.5 mL, and the second focus is to add NaCl 1.9, 2 mL when adding the third focus was 2.5, 2.3 mL while adding the water extract showed on effect of bacteria when adding the first and secondary concentration for NaCl while the effect of the extract appeared to inhibit the growth of bacteria when adding the third concentration of NaCl 1.8, 1.5 mL as shown in Table 2 and Figure 2, It has been observed that inhibition values were greater in the gram-positive bacteria than in the gram-negative bacteria this is because the gram-positive bacteria have a wall that is more permeable than the wall of gram-negative bacteria as well as the active substance soluble in water high permeability of the cell wall of bacteria.¹⁵ NaCl was observed to inhibit growth of bacteria *S. aureus* and *E. coli* at 37°C.¹⁵

E. coli Effect of Tea Extract on Bacterial Growth

The results were shown when using Muller hinton agar, the bacteria did not affect the alcohol extract of the tea when adding the first and second concentrations of the bacteria; however, when the third concentration has added the effect of the bacteria was observed in the alcohol extract of the tea 2, 2 mL when adding the water extract of the tea did not show any effect on the bacteria in addition to the first and second focus of NaCl but when the third concentration was added, the results showed the effect of the bacteria 2.4, 2.3 as shown in Table 3 and Figure 3 the inhibitory effects of the water extract is because the active substances in the extract may be more active, water-soluble and more soluble in water¹⁶ pointed out that the active substances in the plant work differently among them in showing the inhibitory activity against the bacteria.

When the nutrient agar was used, the tea extract showed no effect on the bacteria by adding the first and second concentrations of NaCl, but when the third concentration of NaCl was added, the results showed the effect of the alcohol extract of the bacteria 1.2, 1.5 mL when the water extract was added there was no effect on the bacteria and all the concentrations used as shown in Table 4 and Figure 4. According to Ruwaiha (1983), although medicinal plants are widely used in traditional medicine, their effect may be optional based on the type of bacteria and the type of disease; NaCl

was observed to inhibit the growth of bacteria *S. aureus* and *E. coli* at 37°C.^{15,17}

***K. pneumoniae* Effect of Tea Extract on Bacterial Growth**

The results showed that when the Muller hinton agar was used the bacteria did not affect the alcohol extract of the tea when the first and second concentrations of NaCl, but when the third concentration of NaCl had added the effect of bacteria to the alcohol extract of tea 1.6, 1.5 mL when the water extract of tea was added, there was no effect on the bacteria and all the concentrations used in the study as shown in the Table 5 and Figure 5.

When the nutrient agar was used, the tea extract showed no effect on the bacteria by adding the first and second concentrations of NaCl, but when adding the third concentration, the results showed the effect of the alcohol extract on the bacteria (1.4, 1.5) mL when the water extract was added there was no effect on the bacteria and all the concentration used as shown in the Table 6 and Figure 6, this is due to the fact that the bacteria have virulence factor the ingredients of the extract were not able to be used with the saline concentration used to influence it not to the effectiveness of plant extracts used including green tea against bacteria *E. coli* and *K. pneumoniae*.¹⁸

CONCLUSION

- Bacteria cause tonsillitis, the most common of which are *S. aureus*, *E. coli*, and *K. pneumoniae*.
- Muller hinton agar was the best in the test for alcoholic and aqueous extracts of tea.
- *S. aureus* was effective with alcoholic and aqueous extracts at the three concentrations.
- *K. pneumoniae* and *E. coli* are affected by the alcoholic extract of tea at the third concentration on the muller hinton and nutrient agar.

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