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# The therapeutic role of Alcoholic Extract of Black Tea (*Camellia sinensis*) against Infection with *Staphylococcus aureus*

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**ABSTRACT**

The present study investigated the antibacterial activity of alcoholic extracts of black tea (*Camellia sinensis)* leaves on *Staphylococcus aureus* isolated from skin infection, The agar-well diffusion method was used in the concentrations 150, 250, 350 and 450 mg/ml respectively. That results shows the minimum inhibitory concentration (MIC) of black tea alcohol extract was 150mg/ml with inhibition zone of 19 mm, on the other hand MIC vancomycin against *S. aureus* was ≥ 64 µg/ml. The results obtained from the gene expression of Collagen -Binding Adhesion (CBA), showed there was significant induction of the expression in the groups treated with vancomycinin compared with black tea and the highest induction in expression of Collagen -Binding Adhesion (CBA) gene was at Sub MIC concentration of vancomicin (32 µg/ml) due to the activity of black tea as antibacterial has changed the resistance of *S. aureus.*

***Key words:*** *Black Tea, Vancomycin-resistant S. aureus, Collagen-binding adhesion*

## Introduction

Black tea have a variety of active compounds these including polyphenols, alkaloids, flavanoids, anthraquinoneglycosides, phenolic compounds, tannins, triterpenoids, saponins, carbohydrates, proteins and amino acids (Yue *et al.*, 2014).

*Staphylococcus aureus* bacterium that have ability to infected many hosts and causing disease that might be to the capability to created many virulence factors, for instance toxins and enzymes producing, polysaccharides and cell walls-associated proteins (Herbert, 2001). The virulence factors of *Staphylococcal* consist of membrane-active toxinscoaguelase, enterotoxins, proteases and staphylokinaes. Whereas the virulence factors related to cell wall these containing proteins A, fibronectins-binding adhesion (Arvidson, 2004) .

Staphylococci construct a family of adhesins that play a unique role in adhesion as a cell wall-anchored protein that mediate adherence to extracellular matrixs (ECM) proteins, in *S. aureus* collagen-binding adhesion has a major role in attaching to cartilage *in vitro* (Switalski *et al.* 1993), as well as that Collagen -Binding Adhesion (CBA) can act as an adhesin. in addition CBA contribute in pathogenesis of *S. aureus* that frequently originated to be the first or second major pathogen that cause arthritis, keratitis, endocarditis, mastitis, and osteomyelitis.

Using of black tea specially phenolic compound as antibacterial effect of various bacteria has been reported in many studies showed that black tea act as antibacterial activates to range of bacteria as *S. aureus , Vibrio cholera ,Campylobacter jejuni , Stapylococcus epidermidis* and *Vibrio mimicus*. Flayyih

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*et al.* (2013), showed that black tea had significant effect on virulence factors production by *P.areogenosa*.

In this studywe aimed to estimate the antibacterial activities of black tea and its effect on the collagen-binding adhesion (CBA) gene expression by *S. aureus* isolated from skin infection.

## Materials and Methods

### Bacteraial Isolation and Identification

The *Staphylococcus aureus* isolates were obtained from patients suffering from skin infection in Baghdad city . Diagnosis of all these isolates were according to the culturals and biochemicals examination, then the analysis was recognized with using API staph. Systemkit (Junkins, 2010).

### Black Tea (*Camellia sinensis*) Extraction

Al-gazallen black tea were bought from a local market in Baghdad city, then ground to a fine powder by an electrical grinder. Four serial dilution 150,250,350 and 450 mg /ml of *black tea* leaves were prepared by suspending 1.5 , 2.5 , 3.5 and 4.5 gm respectively in 10 ml of ethanol (95% ethyl alcohol) . Each concentration was mixed then filtered through whatman (No.1) ,and kept in sterile test tube at 4 **C**until used .

### Antibacterial Activity of black tea extract

The antibacterial activities of black tea extract were detection by agar-well diffusion technique ( *in vitro)* against *S.aureus* isolate according to Kavanagh *et al.* (1972): To achieve this purpose *S.aureus* pure colonies were selected. Five wells (6mm) in width were made in nutrient agar plate using a sterile cork borer , 200 Micro liters, of different concentrations of tea extract (150,250,350 and 450 mg /ml) were poured in each wells, other well were full with 0.1 ml of ethanol 95% as a control, the plates were incubated up down at the 37 **C** for 24hr.The diameter of inhibition zone was measured. The results and standard errors means principles were tabulate**.**

### Minimum Inhibitory Concentration Determination (MIC) for Vancomycina Ginst*S. aureus*

Minimum inhibitory concentration (MIC) of vancomicin to *S.aureus* was determined .This investigation was attained according to Morello *et al*. (2006).

Primers

The primer which used in this study include â-*actin* (530bp) :F (GGGTGGA GCCAAACGGGTC ) R: ( GGAGTTGCTGTTGAAGTCGCA) act as the housekeeping gene and the CBA gene ( 353bp) : F ( G G T A C C G G A T C C A CAGCTTCCGGTTTAATAGGTGTA), R: (CGAGGTACCAGAACTAAGAATAGCCTTATC) ,to determine mRNA expression of the Collagen Binding Adhesin from bacterial cell suspension through quantitative real-time PCR(Lehmann*et al* 2001).

### RNA extreaction

RNA was extracted from *S. aureus*isolates according to protocol of TRIzol™ Reagent. Cell grown in the suspension or pellet calls, 1.4 ml of cells culture was precipitated by centrifugation for 2 min at 13 $\times 10^3$rpm, supernatant then discarded and 0.75 milliliter ofTRIzol™ Reagent was added to pellet. The lysate was homogenized by pipetting up and downwards

### Quantitative reverse transcription Real-time PCR(RT-qPCR) master mix preparation

One strideReal Time-qPCR quantitative PCRmaster mix was organizedwith using kit that is dependent on relativeBRYT, elevoped by promega , discovery of gene amplification in Real Time- PCR system and incorporated the follow (Table 1). After that, these qPCR master mix component that mentioned above was pipeted in to smart tubes (cephied), next the tubes varied by centrifuge for three minutes, then located in Smart cycler RT-PCR organization. After that, the qPCR plate was laden in the following thermocycler protocol (Table 2).

### Reaction setup and thermal cycling protocol (One

**Table 1.** Master mix components for singlereaction

| QPCR master mix | Conc. | Volume |
|---|---|---|
| Master mix (10ng) | 2X | 10µl |
| Forward primer (10pmol) | 10µm | 1µl |
| Reverse primer (10pmol) | 10µm | 1µl |
| N.F water | | 10µl |
| RNA template | | 2µl |
| Total | 1-10ng | 20 µl |

**Step RT-PCR)**

PCR was carry out in optimization conditions (Table 2). The the logarithmic phase was evidenced by using the cycle number (Ct) at which signal cross a threshold set.

Expression level were quantify using relative quantitation. The differences in cycle threshold (ÄCt) and fold changes predictable between the treated groups and

calibrators of each gene. These values were normalized to CBAexpression as showed below:

**ÄCt**= Ct of tested gene-Ct of housekeeping gene

**ÄÄCt**=ÄCt (sample) – ÄCt (Calibrator)

**Fold changes** = $2^{-}$ÄÄCt

**Sample :**tested genes and housekeeping gene treatedwith antibiotics

**Calibration :**tested genes and house- keeping genewithout antibiotics.

**Effects of Green Tea on CBA gene expression**

1. Groups 1( G1) : the control negative) bacterial suspension)
2. Group 2( G2) : bacterial suspension with black tea 100 mg/ml .
3. Group 3 (G3): bacterial suspension with black tea 50 mg/ml
4. Group 4(G4): : bacterial suspension with vancomycin ( MIC) 64 µg/ml
5. Group 5(G5 ) : bacterial suspension with vancomycin (Sub- MIC) 32 µg/ml.

This experiment was done to conclude effect ofblack tea in virulence factor of*S.aureus*.

## Results and Discussion

**Identification of *S. aureus***

The *S. aureus* appeared as circular cells agreed in irregular grape-like cluster, Gram positive, the isolated grew on mannitol salt agar and fermented the mannitol, on Brain hart agar the colonies appear yellow to golden yellow in color, smooth, slightly raised and on blood agar gave beta hemolysis (Junkins, 2010). A number of biochemical tests were performed for identification that inveterate the isolates belonged to *S. aureus*, through using API Staph. System.

**Antibacterial effects of Black tea extract**

The results of agar-well diffusion method demonstrated that the inhibition zones of black tea against *S. aureus* were (19, 22, 23 and 25) mm, due to the four concentrations of alcoholic extract 150, 250, 350 and 450 mg/ml respectively as in Table (3) Various capital letteres indicate significant (P<0.05) results between diverse concontrations. The MIC of black tea against *S. aureus* was 150 mg/ml.

The result of the present study showed that the leaf extract of black tea produced zones of inhibition against *S.aureus*. Therapeutic value of plants might be the attendance of secondary metabolites (phytochemicals principles) which are create in all element of plant such as phenolic compound, polyphenols, flavonoids in addition tocatechin (Ayodele, 2003). The results showed the superiority of the concentration 450 mg /ml and this might be due to the solubility of high amount of active ingredient which inhibited the bacterial growth, these results come in agreement with that mentioned by Alfahmi, (2007), due to ability to bind with adhesions and to disturb the availability of inhibitory effects of ethanolic extracts of tea plant were found

**Table 3.** In-vitro antibacterial activity of different concentrations of black tea extract against *S. aureus.*

| Concentration mg/ml of Black *tea*. | *S.aureus* (inhibition zone-mm) (Mean ±SE) |
|---|---|
| 150 | 19 ± 34 |
| 250 | 22 ±48 |
| 350 | 23 ±53 |
| 450 | 25±69 |
| 95% ethanol | 0.00±0.00 D |

Values represent mean ±S.E

Different capital letters mean significant (P<0.05) results between different concentration.

**Table 2.** Thethermocycler protocol in Real-Time- PCR system

| QPCR step | Temperature | Time | Repeat cycle |
|---|---|---|---|
| Initial Denaturation | 95 °C | 5min | 1 |
| Denaturaetion | 95 °C | 20 sec | 40 |
| Annealing\Extention | 60 °C | 30 sec | |
| Detection(scan) | | | |
| Melting | 60-95°C | 0.5 sec | 1 |

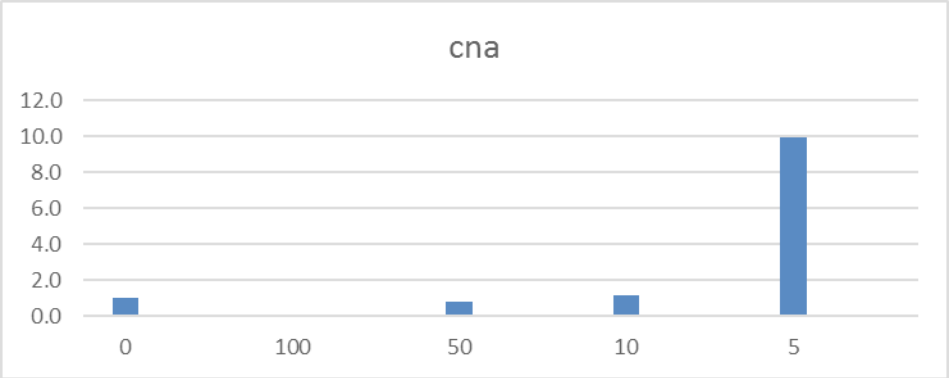


**Fig. 1.** The relative CBA gene expression treated group and non treated. Statistics are revealed as the fold alter in the mRNA stageon G1, G2, G3,G4 and G5by Q RT-PCR.

atconcentration 450 mg/ml.

Black tea extract has potent antibacterial activity which confirms its use against infection. That may be due to blacktea composites as cateichens and polyphenols that have been found to possess antibacterial action which are oxidation and polymerization products of simple isoflavanoids and main polyphenolic compounds in black tea (Mbata, 2008), these observation in agreement with Monagas (Monagas *et al*., 2010), who found that phenolic compound act as antibacterial may be related tothe significant oligoelement for heme-utilizing bacteriathat iron chelating possessions.

In the present study *S. aureus* strains isolated from skin infection, showed resistance to vancomycin antibiotics, the MIC value was 64 µg/ml. Infections associated with *S. aureus* were resistant to awide variety of antimicrobials might be due to a penicillin-binding protein (PBP2a) encoded for by gene *mec*A (Berger-Bachiand Rohrer, 2002). The vancomycin-resistants *S. aureus* lean to be multidrug resistants against a large amount of presently obtainable antimicrobial agents (De Lassence, 2006). These observation in agreement with Rao *et al.*, 2014, who found that infection associated with *S. aureus*are often complicated by the bactiral capability to develop into resistant to different module of antibiotics and to develop into virulence.

**Detection ofGene expression of CBA by using RT- qPCR**

The effect of black tea in compare with vancomycinon CBA gene expression by *S. aureus* was determined, the results (Fig. 1) shows that there was major induction ($P < 0.05$) of the expression in the groups treated with vancomycinin compare with black tea and the highest induction in the expressions was at 32 mg/ml of vancomycin.

The result of present study showed that black tea extract at 100 mg/ml concentration inhibit the expression of CBA mRNAcompletely,on the other hand black tea extract at 50 mg/mlconcentration showed significant lower fold change($P < 0.05$) in CBA mRNA gene expression as compared to the G1 (bacterial suspension ), these data may be due to the straight effects of the black tea on bacterial CBA, in addition to antbiactrial active of black tea which guide to reduce CBA ( Figure. 1).

G1: control negative, G2: bacterial suspension with Black tea 100 mg/ml, G3 : bacterial suspension with Black tea 50 mg/ml, G4: bacterial suspension with vancomycin64 mg/ml, G5: bacterial suspension with vancomycin 32 mg/ml.

collagen-binding protein CBA that which have significantaction in pathogcity of *S. aureus*. CBA has been established to be a virulence factor for *S. aureus* infection.The virulence of CBA in osteomyelitis, experimental arthritis as well as corneal infections[6, 20]. The target of antibodies against CBA which have the ability of *S. aureus* to reduce collagens adhesion (Visai, 200).

In this study black tea extract decreased the expressions of CBA these results shows that the antibacterial action of the tea polyphenols as well as the protectingresult of black tea extract against virulence factor against *S.* aureusby inhibiting the CBA expression ,This was in agreement with Yamabe*et al*[22] , who found the anti-inflammatory and antioxidant activity of tea polyphenolesby Sub—MIC be capable of support injury curative, that may provide in healing of blazeinjury and scar, in addition Klass *et al*[23] who showed that epicate

chingallate (ECG) has probable effects on injury reduction and curative .

The results of the CBA gene expression of G2 and G3 (black tea extract) demonstrated that which considerably ($P$ <0.05) lower fold change than G4 and G5 (bacterial suspension with vancomicin ), these result indicate the antibacterial effect of back tea (100 and 50 mg/ml), black tea actas antimicrobial by interferes with the pessimistically charge microbial cell surface, in addition to bactericidal effect against *S. aureus*, the occurrence of polyphenols which compensation bacterial cell membrane leading to decreased gene expression of CBA. ,eventually resulting in impairment of bacterial activities by declining virulence production of *S. aureus*, these product was concurrence with Bansal *et al* who found that the polyphenols composites act as antibacterial due to their capacity to damage to the bacterial cell membrane may be one of the detailed mechanisms with the antibacterial action.The antimicrobial possessions of tea have been recognized for its bacteriostatic and bactericidal effects due to catechinswhich have the capacityof bind to synthetic lipids bilayers (Yam, 1998). In addition to that black tea catechins act as effective antibacterial activity that polymerized and suppress the activity of *S. aureus* α-toxin. Holloway *et al.,* (2012), refered that the antibacterial actionof catechin against *S. aureus* strains attributed to enhanced the hydrogen peroxide construction aftermetal ions supplementations.

## Conclusion

This study has shown that black tea has antibacterial activity against *S. aureus* and that is superior than vancomycin in skin infection. As well as has effects on expression of virulence gene expression (CBA), suggesting it as alternatives to control *S. aureus*.

**Conflicts of interest:**The authors declare that they have no conflicts of interest.

**Ethical clearance** :This study approved by Department of Biology ethical committee

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