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Full Length Research

Evaluation the effects of black tea extract (*Comellia sinensis*) on *Staphylococcus aureus* at *invitro* condition

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Staphylococcus aureus **infections are an important cause of skin, soft tissue and invasive infections that are acquired in hospitals or community. Nowadays antibiotics play important role for treating bacterial infections, but due to the increasing resistance of bacteria and their side effects, the use of plant extracts as alternative treatment being used. Antibacterial affects different types of tea have been proven, synergistic effects of tea have also been reported with antibiotics. The aim of this study was to evaluate the inhibitory effect of black tea extract on *Staphylococcus aureus* compared with the standard antibiotic. In this study, black tea extract was prepared by using the percolation method. Bacteria were tested by antibiogram test with standard antibiotic discs containing ciprofloxacin, cefazolin and vancomycin. Data were analyzed using statistical tests and analysis of variance. The results were obtained from this study showed that black tea extract on the growth of *Staphylococcus aureus* inhibitory is dose-dependent 100 mg/ml has the maximum inhibitory effect.**

Key words: Black tea extract, *Staphylococcus aureus,* antibiogram, The inhibitory effect, invitro

INTRODUCTION

Comellia sinensis is the one of the most popular and consumed beverage in the world, Nataro (2006). Black tea use might be in China, Japan and some other countries in East Asia and India (Shoa hasani *et al*, 1998).Catkin is an antioxidant that accelerates the respiratory movements, blood circulation, energy, digestion, etc (Memarzadeh *et al.*, 2012). *Staphylococcus aureus*, is a Gram-positive bacterium which grow on the skin and Upper respiratory system (Grundmann *et al.*, 2006). It was leading pneumonia after viral infections. It also causes blood vessel inflammation, meningitis, urinary ducts infections, ect , Turkyilmaz and Kaya,(2006). It is one of the most important pathogens that make food poisoning (Adwan *et al*, 2005; El-Ghodban *et al.*, 2006). Nowadays antibiotics play important role for treating bacterial infections, but due to the increasing resistance of bacteria and their side effects, the use of plant extracts as alternative treatment being used. Khalaji and Neiestani (1996).

MATERIALS AND METHODS

Percolation method for the preparation of extracts, black tea transferred to a flask Meyer 2 L ethanol 70% was added. Incubation should result in confluent growth. Some *E. coli* isolates may not have sufficient growth after 24 h incubation. These are reincubated immediately and inhibition zones read after a total of 48 h incubation. Then the extract was filtered. Extract was concentrated with

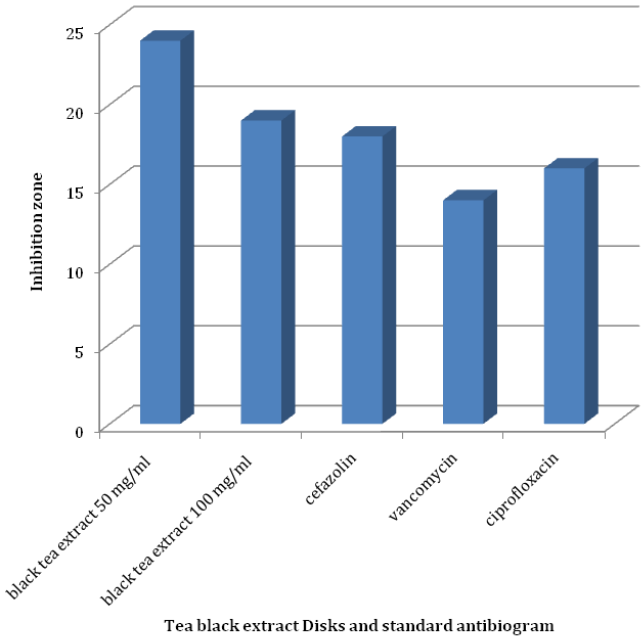

Figure 1: Evaluation inhibition zone of tea black extract disks and compare to standard antibiotic disks with Disk diffusion

extract evaporation tool and the volume titrated up to 20 mg/ml. The extract was dried with incubation at 50°C, 250mg/ml dimethyl sulfoxide (DMSO) was prepared respectively. Pure strains of *Staphylococcus aureus* ATCC: 29213 were used. Bacterial susceptibility testing was evaluated by two methods: *Disk diffusion* and *Pour plate*.

Disk diffusion method

50 and 100 mg in 25 ml of black tea extract were inoculated blank discs. Discs were incubated for one hour at 37 °C. Bacterial cultures were cultured with a sterile swab into Mueller Hinton agar medium. Black tea extract disks and antibiotics standard disks including vancomycin (5 mg), cefazolin (30 mg), and ciprofloxacin (5 mg) were placed on the Cultures individually. The plates were transferred into in an incubator at 37 °C. The results were read after 18 hours. Inhibition zone diameter around the disk containing tea extract and standard antibiotic discs were recorded with a ruler. SPSS was measured and analyzed. Statistical analysis was done by using SPSS software.

Pour plate method

In this method, black tea extract 50 and 100 mg/ ml were prepared in Thioglycolate 1ml bacterial suspension(equivalent to 0.5McFarland) was added to tea extracts which were incubated at 37 °C. One ml was transferred to sterile plates after 1, 2, 3, 5,7and 72 hours.10ml fused nutrient agar medium has been added at 45 °C. Bacteria are spread uniformly in the medium. Plates were incubated at 37 °C and the results of in vitro growth or no growth of bacteria was read after 24 hours.

RESULTS

Results shown black tea extract concentrations reduced the growth of *Staphylococcus aureus* bacteria in Pour plate method ($p>0.05$). Antibiogram test method was performed 8 times. Then the diameter of the growth inhibition were calculated and used for statistical analysis. The analysis of variance of mean inhibition zone diameter of standard antibiotic ciprofloxacin, cefazolin and vancomycin shows significant differences in two different concentrations of black tea extract.

Result indicated that black tea extract at a concentration of 100 mg/ ml had the greatest effect on *Staphylococcus aureus* compared to standard antibiotics. Comparison of the results showed that 50 mg/ml black tea extract were not significantly different with standard antibiotics ciprofloxacin, cefazolin and vancomycin ($p>0.05$) (Figure 1).

DICUSSION

Staphylococcus aureus has been introduced as a major cause of hospital infections. Future research is necessary in the organism due to the problems which make in the world and the second leading cause of wound infection after surgery.

So far, many reports on the antibacterial activity against a variety of microbes have been published (Bandyopadnyay *et al.*, 2005; Taguri and Tanakat, 2004). Synergy effects of tea have also been reported with antibiotics (Isogia *et al.*, 2001;Toda *et al.,*1989) demonstrated that tea extracts resulted in killing or inhibiting the growth of pathogenic bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Shigella dysentery,* and *Vibio* strains. Other studies have also shown that green tea polyphenols leaves have inhibitory effect on the growth of *E.coli*, streptococcus (Horiuchi *et al.,* 1992).

Deterrent effect of black tea extract on growth of *Staphylococcus aureus* has been found in *invitro* codition. 50 mg/ml black tea extract were not significantly different with standard antibiotics ciprofloxacin, cefazolin and vancomycin. Greatest effect on *Streptococcus mutans* was observed in a study on luteolin polyphenol anti-bacterial plant seeds of *Prila*. Inhibitory effects of apple polyphenols and some other plants have also been reported (Yanagida *et al.,* 2000). The strong antioxidant properties of black tea have been attributed to its chemical components of thearubigins, phenolic acids, catechins, and theaflavins. Theaflavins which impart color, brightness, and astringency to black tea infusion possess potent antioxidant properties(Luczaj *et al.,* 2005 ; Ngure *et al.,* 2009).

Plant polyphenols, tannins, have been suggested to exert their growth inhibitory effects through auto-oxidation and hydrogen peroxide production, but in certain circumstances some bacterial genes may be induced (like *OxyR* in *E.coli*) so that strengthening bacterial antioxidant defense mechanisms may overcome tannin inhibitory effects (Isogia *et al.*, 2001) The concentration of the polyphenolic compounds may have some role in this process. However, the inhibition of bacterial growth after 3–24 hours of incubation with tea extracts and also the attenuation of antibiotics by tea extracts at certain concentrations all strengthen the possibility of selective drug–tea interactions.

Considering the findings of this study and comparison with other studies in this field black tea extract can be controlled growth of *Staphylococcus aureus* in vitro condition. We suggest more research of black tea extract with different concentrations.

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