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Research Article

Determination of antibacterial activity of tea water concentrates against *E. coli* and *S. aureus*

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Abstract

Tea is one of the most common beverages consumed since centuries. It has antimicrobial activity against various pathogens. Current research study was designed to evaluate and compare the antibacterial activity of different commercially available tea (green and black) concentrates against pathogenic bacterial strains of gram-negative *Escherichia coli (E. coli)* and gram-positive *Staphylococcus aureus* (*S. aureus).* Modified broth micro dilution method was used for the evaluation and analysis of tea samples. For this purpose, stock solutions of green and black tea concentrates, mannitol broth and bacterial suspensions were prepared. *E. coli* and *S. aureus* 10 µl suspension was added according to labelled wells and incubated. Minimum inhibitory concentration was confirmed by well diffusion method. The results suggest that green tea water concentrate is effective against both strains of these pathogenic bacteria, inhibiting their growth at 6.2 mg/ml. The black tea water concentrate is found more efficient against *E. coli* than *S. aureus*, with the minimum inhibitory concentration at 6.2 mg/ml and 12.5 mg/ml respectively. The findings of the current study encourage and recommend the use of both green and black tea in normal routines as well as a traditional medicinal remedy for the treatment of various human ailments.

Keywords: Antibacterial activity; Growth inhibition; Micro dilution; Modified broth; Tea (*Camellia sinensis*)

Introduction

In beverages, tea (Camellia sinensis) is most commonly consumed in the world. Tea contains ingredients that refresh mind by stimulating and producing good feelings **[1]**. Tea has various types and flavours such as oolong tea, green tea and black tea. Green tea is most commonly used due to its antioxidant

and antimicrobial activities. In the modern world, green tea flavour is used in various items such as candies, soft drinks and ice creams [2]. Tea is well known as a therapeutic agent for different maladies [3, 4]. According to research studies, tea have antimicrobial, anti-inflammatory, antioxidant, anti-cancer and antibacterial activities against number of pathogens [5, 6]. In ancient India and China, human ailments were cured with tea as natural remedy [7].

Green tea is product of C. sinensis dried leaves, however black tea require fermentation process that produce thearubigins and theaflavins. These oligomeric polyphenolic compounds derived from flavanol tea units are biologically active components of tea [8]. Main flavonoids found in tea are tannins, catechins and theaflavins. Catechin is subdivided into epigallocatechin gallate (EGCG), epicatechin (EC), epigallocatechin (EGC) and epicatechin gallate (ECG) [9]. Polyphenols quantity in black and green tea is about 10 % and 40% respectively [10]. Green tea has antimicrobial activity against gram-positive and gram-negative bacteria [11]. EGCG have maximum radical scavenging activity and is the most abundant polyphenol, due to popularity of tea and absence to toxicity.

Tea and caffeine might contribute to and promote anticarcinogenic effects [12, 13]. Animal models and cell line studies have shown antiangiogenic, anticarcinogenic and antiproliferative effects of tea flavonoids [12, 13]. According to literature, in presence of tea, various pathogens (*Aeromonas sobria*, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* (*S. aureus*), *Pleisomonas shigelloides and Vibrio parahaemolyticus)*, fail to grow [13, 14]. The objective of current research study was based on determination of antimicrobial activity of green tea and black tea against gram-positive bacteria *(Staphylococcus aureus)* and gram-negative bacteria *(Escherichia coli).*

Materials and Methods

Tea concentrates preparation

Tea samples (20g) were collected in flasks (250ml), distilled water (150ml) was added and kept on shaking incubator (ES-20) at (26–30°C) for three days. After incubation, the samples solution was filtered with the help of Whatman filter paper. The filtrates were separated in two different screw capped flasks. For solvent evaporation Rota-vapor Buchi Rotavapor (R-200) equipment was used.

Mannitol broth preparation

Mannitol broth (50 ml) was prepared in (250 ml) flask and screw capped tubes. The broth was poured and stored at 5 °C. The test microorganisms (*E. coli and S. aureus*) were inoculated at 10^6 colony forming units into tubes and vortexed gently.

Determination of minimum inhibitory concentration (MIC)

Green tea and black tea stock solutions were prepared for each sample by adding 200 mg concentrate in 1 ml distilled water (200 mg/m1). To remove debris, the stock was centrifuged at 1000 rpm. Sterile micro-titer plate was used for determination of MIC. The plate first 4 rows were labelled, 1st row: s-g-w-1 to s-g-w-8, 2nd row: egw1 to e-g-w-8, 3rd row: s-b-w-1 to s-b-w-8 and 4th row: e-b-w-1 to e-b-w-8 respectively. Mannitol broth 40 µl was added through micropipette in to each well respectively. 50 µl mannitol broth was added to positive-control to 9th and 10th well and negative-control to 9th and 10th wells of microtiter plate (96U-MS-9096UZ) respectively.

For green tea, stock solution 40 µl was added to 1st row, well (s-g-w-1), the concentrates were properly dispensed than 40 µl solution was transferred to well (s-g-w-2). The process was repeated up to 8th well (s-g-w-8) respectively. Finally, 40 µl solution was discarded. Similar practice was repeated to

8th (s-g-w-8) well respectively and 40 ml solution was discarded from 8th (s-g-w-8) well. Similar steps was done for 2nd row (e-g-w-1 to e-g-w-8) wells. For dilutions of black tea, similar practice was done to 3rd row (s-b-w-1 to s-b-w-8) wells of plate and 4th row (e-b-w-1 to e-b-w-8). The concentrates of tea (green and black) were (0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 mg / ml) respectively.

Suspension of *Staphylococcus aureus* (*S. aureus)* 10 µl through micropipette was added to 1st and 3rd row of plate. 10 µl suspension of *E.coli* was added to 2nd and 4th row of microtiter plate and kept on incubation for one day at temperature (35 °C). Methyl red dye 15 µl solution was added to every well of microtiter plate respectively. With the help of plate-reader at (450 nm), optical density of wells was noted.

MIC (minimum inhibitory concentration)

Each concentrate stock solution (100 mg/ml) was prepared by adding 100 mg in 1 ml distilled water and centrifuged at 2000 rpm. Test microorganisms (*S. aureus and E. coli)*, suspensions (10^6 colony forming units) were inoculated on warmed mueller-hinton agar plates. Uniform size of wells was made on agar plates and (50 µl) tea concentrates was added to each well respectively and incubated at 37 °C for 24 hours.

Results

Green tea water concentrates antibacterial activity

The minimum inhibitory concentration value of green tea concentrate for both the test organism was found at the concentration of 6.2 mg/ml. The growth of test micro-organism was inhibited at 100, 50, 25, 12.5 and 6.25 mg/m1. Lower inhibition was found at 6.2 mg/m1 (Fig. 1). Well diffusion method and confirmatory test resulted that both test microorganisms are susceptible to green tea concentrations up to 10 mg/ml and resistant at 1 mg/ml (Table 1). *S. aureus* is susceptible up to 10 mg/ml concentrates of green tea with MIC 6.2 mg/ml, slightly resistant to concentrates of black tea with MIC 12.5 mg/ml.

Black tea concentrates antibacterial activity

By modified broth micro-dilution method, the MIC value of black tea concentrate was found at 12.5 and 6.25 mg/ml, for *S. aureus* and *E. coli*, respectively. Growth of *S. aureus* concentrates at 12.5 mg/ml at dilutions of 100, 50, 25 was inhibited whereas the growth of *E. coli* was inhibited at 6.25 mg/ml. At concentration of 100 mg/ml, maximum inhibition was observed and at concentrations of 6.25 and 12.5 mg/ml, *E. coli* and *S. aureus* was resistant respectively (Fig. 2). Well diffusion method indicated that *S. aureus* have shown resistance at 10.0 mg/ml concentration and *E. coli* have shown resistant at 1.0 mg/ml concentration as shown in (Table 1). The results show that *E. coli* is more susceptible to black and green tea concentrates having MIC 6.25 mg/ml.

Discussion

This research study conducted for antimicrobial activity of black tea and green tea at different concentrates resulted effects against pathogenic bacteria (*Staphylococcus aureus* (*S. aureus)* and *Escherichia coli (E. coli)*). Green tea concentrates resulted better antibacterial activity against *E. coli* and *S. aureus* that was confirmed by well diffusion method as indicated in (Fig. 1, 2 & Table 1). The effects of black tea concentrates as antibacterial agent is comparatively less pronounced than green tea **[15]**. The basic explanation behind this difference in antibacterial activity is due to presence of various bio-active secondary metabolite components **[16]**. The present analysis demonstrates that consumption of green tea and black tea is healthy in constrained

amount as it has potential to inhibit the growth of various pathogenic microorganisms.

According to literature, herbal plants tested for antimicrobial activity is widely accessible. Previous research studies have also reported that black tea and green tea solvents have potential of antioxidant properties and antimicrobial activity [16]. Black tea and green tea have strong antimicrobial activity against various pathogens i.e. *E. coli* and *S. aureus*. Tea (black and green) can also be used as alternate of antibiotics against bacterial infections [15]. Tea extracts are used as anti-adhesive agent to stop pathogenic bacteria adhesion to host cell membrane [17, 18]. It is confirmed that tea has been proven safe, less costly, and easily available compound [19].

Camellia sinensis leaves contain polyphenols such as alkaloids, tannins, catechin and different polyphenols that contain catechin as critical antibacterial compound [17]. Catechin pulverizes cell layer and decrease bacterial cell growth and development. Catechin is simplest compound, categorized in various components such as Epicatechin (EC), Epicatechin gallate (ECG), Epigallocatechin (EGC) and Epigallocatechin gallate (EGCG) that have strong anti-oxidant properties [20, 21]. Green tea and black tea polyphenols play important role by increase of hepatic AMPK (5′adenosylmonophosphate activated protein kinase) and induce weight loss to treat obesity [22]. It is suggested that green tea polyphenols increase production of AMPK with help of intestinal SCFA (short chain fatty-acids) production [23]. Another study reveals that green tea, black tea and oolong tea contain α-glucosidase inhibitors that have inhibitory potential to tea phenols that makes tea phenols capable to control postprandial hyperglycaemia [24].

Furthermore, green and black tea contains caffeine that has the capability to stop cell division and spore germination [25]. ECG and EGCG, break bacterial cell lipid bilayer by adhering on proteins that destroy morphology and biofilm relatively. Polyphenols (caffeine) is found in huge amount in black tea in oxidized form [19-26]. Therefore, green tea concentrates have potential antibacterial activity as compared with black tea concentrates [27]

Table 1. Effectiveness of tea (black and green) concentrations against gram-negative bacteria *(E. coli)* and gram-positive bacteria *(S. aureus)*

Microorganism	Inhibition activity					
	Black tea concentrates			Green tea concentrates		
Tea concentrates (mg/ml)	100.0	10.0	1.0	100.0	10.0	1.0
S. aureus	S	R	R	S	S	R
E. coli	S	S	R	S	S	R

R = Resistant, S = Susceptible

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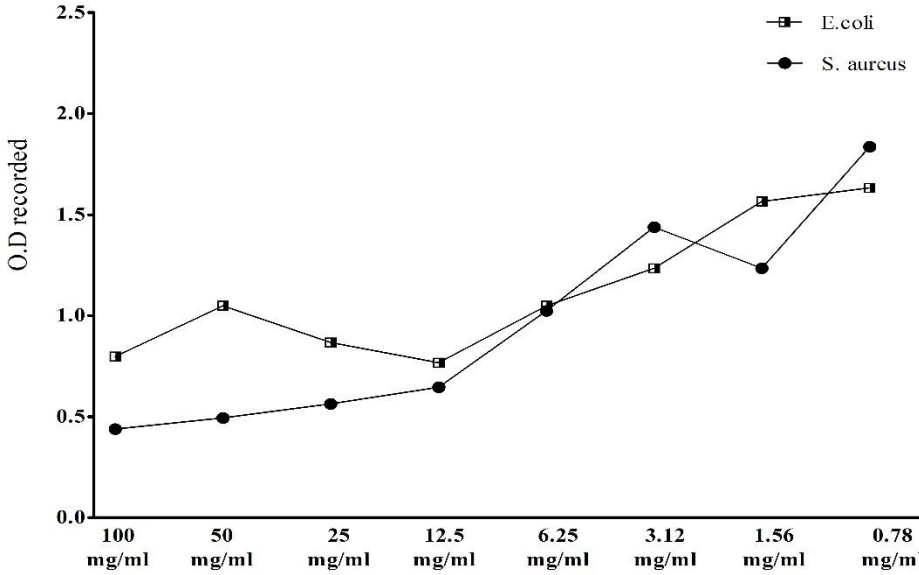

Figure 1. Green tea concentrates, antibacterial activity against gram-negative bacteria *(E. coli)* and gram-positive bacteria *(S. aureus)*

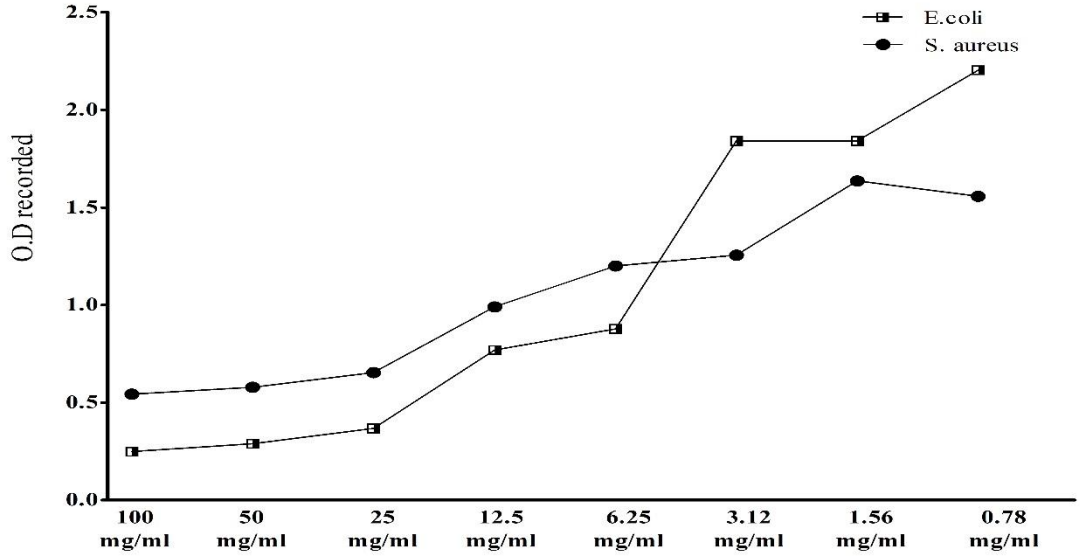

Figure 2. Black tea concentrates, antibacterial activity against gram-negative bacteria *(E. coli)* and gram-positive bacteria *(S. aureus)*

Conclusion

According to current research study, green tea has potential to inhibit growth of *E. coli* and *S. aureus*. However, *E. co1i* is relatively more susceptible to black tea than green tea concentrations. *S. aureus* indicates slight resistance to concentrates of black tea. Therefore, current research study supports the recommended use of tea (black and green tea) in folk medicines and beverages in satisfactory quantity.

Authors' contributions

Conceived and designed the experiments: A Ali, A Rohan, I Navid, WK Muhammad Performed the experiments: M Kiran, J Saqlain, R Abdur, Analyzed the data: U Hayat, Contributed materials/ analysis/ tools: A Ali, L Muhammad Wrote the paper: U Hayat.

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