Determination of antimicrobial activity of tea (Camellia sinensis) and coffee (Coffea arabica) extracts on common human pathogenic bacteria.

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ABSTRACT

Plant drive products specially tea and coffee as important sources of antioxidant and antimicrobial compounds which play a major role in reducing food pathogen. Tea and Coffee is the most preferred morning beverage throughout the world due to their pleasant flavor and stimulating properties, which may be due to caffeine and tannins. Their leaves produce organic compounds which act as defense against insect, bacteria, fungi and viruses. The aim of the present study was to determine the antimicrobial activity and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of tea and coffee extracts against some of common human pathogenic bacteria. Total four samples of tea and coffee extracts e.g. Raw tea leaves (RTL), Unpacked tea leaves (UTL), Commercial tea leaves (CTL) and Commercial coffee (CC) were used for antibacterial activity tested on six standard organisms Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae and Escherichia coli using agar well diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of tea and coffee extracts against tested bacteria were evaluated using micro-dilution plate technique. The antimicrobial activity of those extracts was also compared with commercial antibiotics. In the present study, all tea and coffee extracts showed strong antibacterial activity against standard strain of B. cereus and S. aureus but not against other four strains. On the other hand, only coffee extracts showed antibacterial activity against gram negative P. aeruginosa. From all extracts the lowest MIC was observed (3.125) mg/ml and MBC was 6.25 mg/ml and antimicrobial activity was much more effective than commercial antibiotics. Antibiotics have many side effects on human health and as such, we can use tea and coffee as natural anti-microbial agent, so that it is environment friendly. Thus, the present study reflects a hope for the development of novel chemotherapy agents.

Key words: Antimicrobial activity, extracts, tea, coffee.

Abbreviations: MIC, Minimal inhibitory concentration; MBC, minimal bactericidal concentration; RTL, raw tea leaves; UTL, unpacked tea leaves; CTL, commercial tea leaves; CC, commercial coffee.

INTRODUCTION

Tea is a beverage and is of infusion of variously processed leaves of one of the varieties of an evergreen shrub, Camellia sinensis (Tea plant). It is the most widely drunk beverage in the world (Vasudeo and Sonika, 2009). It is refreshing, mildly stimulating, and produces a feeling of well-being. These properties may be due to caffeine and...
tannins. These compounds are known to have stimulant and anti-soporific actions that elevate mood, decrease fatigue and increase capacity for work (Stagg, 1980).

The powerful antioxidant properties of the tea are generally attributed to its flavonoid components: the aflavins, bisflavanols and theaflavic acids (Rice-Evans, 1997). The effect of Black tea on stomach cancer has been studied. Out of 15 studies, five case-control studies showed a protective effect of tea on the risk of stomach cancer (Ji et al, 1997).

On the other hand, Coffee contains a number of useful nutrients, including riboflavin (vitamin B-2), niacin (vitamin B-3), magnesium, potassium, and various phenolic compounds, or antioxidants. In recent years, coffee has been associated with antimicrobial activity against a number of different bacteria and fungi. Researchers have looked at the benefits of drinking coffee for conditions such as diabetes, cardiovascular disease, inflammatory bowel disease, and liver disease. There is evidence to support some, but not all of these claims (Ukers, 1948). Research has shown that coffee possesses antibacterial activity against Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Listeria monocytogenes (Martínez-Tomé et al, 2011; Daglia et al, 1994).

The study aimed to assess antimicrobial activity and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of tea and coffee extracts against some common human pathogenic bacteria (vitamin B$\alpha$). The effect of Black tea on stomach cancer has been studied. Out of 15 studies, five case-control studies showed a protective effect of tea on the risk of stomach cancer (Ji et al, 1997).

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The study aimed to assess antimicrobial activity and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of tea and coffee extracts against some common human pathogenic bacteria using 96-Well micro-dilution plate technique. Comparison was done between antimicrobial activity of tea and coffee extracts with the inhibitory effects of some commercial drugs.

**MATERIALS AND METHODS**

**Selected test organism for experiment:**

Six standard bacteria Bacillus cereus (ATCC 14579), Staphylococcus aureus (MTCC 87), Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi (MTCC 531), Klebsiella pneumonia (ATCC 700721), Escherichia coli (O157:H7) were already available at the laboratory of microbiology, Department of Microbiology, Primeasia University, Banani, Dhaka, Bangladesh.

**Study design:** This study is an experimental research design.

**Study Location:** Laboratory of microbiology, Department of Microbiology, Primeasia University, Banani, Dhaka, Bangladesh.

**Study duration:** June 2019 to October 2019.

**Extraction preparation**

Fresh tea leaves were collected of different forms such as Raw tea leaves (RTL), Unpacked tea leaves (UTL), Commercial tea leaves (CTL) and Commercial coffee (CC). The tea and coffee leaves were washed with distilled water for eliminating the foreign particle attached to the surface of leaves and allow to air dry. Then dried by hot air oven and individually ground using sterile blender and then 10 grams of samples were soaked in 90 ml of 95% ethanol in sterile bottle and mixed well with constant agitation of 120 rpm at 37°C for 48 h by shaker incubator machine. After the incubation, the ethanol fraction was separated by sterile cheesecloth and filtered through Whatman filter paper no.1. Sample containers were then kept at 50°C in hot air oven for 5 to 6 days to allow evaporation. Finally, extracted samples were stored at 4°C for further use (Figure 1).

**Preparation of sample**

For the determination of antimicrobial activity, the extracted tea and coffee sample was concentrated into 0.1 mg/ml by the solvent methanol.

**Preparation and standardization of inoculums**

A standard stock of the bacteria isolates was prepared by suspending a loop full each microbial growth from the slants in about 4 ml of Nutrient broth. The test tubes containing the inoculated Nutrient broth was incubated at 37°C for 12 h.

The turbidity of bacterial suspension was measured and adjusted with the turbidity of 0.5 McFarland’s standard solution (0.5 ml of 1.75 % (w/v) barium chloride dihydrate (BaCl$_2$,2H$_2$O) giving a bacterial of about 1×10$^5$ CFU/ml.

**Agar well diffusion method**

After comparing the turbidity of inoculum with 0.5 McFarland solution and inoculated into Mueller Hinton Agar (MHA) plate. Agar wells were prepared with the help of sterilized cork borer of 8 mm diameter and different concentration of tea and coffee extracts were added to different agar wells in the plate, e.g 25, 50, 75 and 100 µl respectively and commercial antibiotic disk was used as a control. The plates were incubated in an upright position at 37°C overnight. Zone of inhibition was measured to the nearest millimeter using scale.

**Determination of MIC and MBC**

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight
incubation. MICs can be determined using agar or broth dilution methods. The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by sub-culturing to agar plates that do not contain the test agent (Figure 2).

**RESULTS**

Results in Table 1 and Figure 3 showed antimicrobial activity of all extracts against gram positive (*B. cereus, S. aureus*) bacteria and no antimicrobial activity shown against gram negative (*Salmonella typhi, K. pneumoniae, E. coli, P. aeruginosa*) bacteria. Only *P. aeruginosa* gram-
negative bacteria showed sensitivity against coffee extract.

The results in Figures 4, 5, 6 and 7 showed that different concentration of tea and coffee extracts were added on different agar wells in the plate where extracts concentration increased with increased inhibition zone as compared with the control (commercial antibiotic disk).

Results in Table 2 showed Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 4 tea and one coffee extracts against *B. cereus*, *S. aureus* and *P. aeruginosa* (only for coffee extracts).

**DISCUSSION**

Since ancient time to modern times, tea and coffee have contained medicinal value and possess less side effects as compared with commercial drugs.

In this study, CTL (Commercial Tae Leaf), RTL (Raw Tea Leaf), UTL (Unpacked Tea Leaf) and CC (Commercial Coffee) extract showed strong antimicrobial activity against *B. cereus* and *S. aureus*. In line with the present study, several other studies showed that different tea extracts had antimicrobial activity against *S. aureus* and *B. cereus*, but gram negative bacteria were resistance to the extracts (Wu et al., 2002; Zuo et al., 2008).

In a study by Chan et al. (2011) various green, black, and herbal tea extracts were evaluated against three Gram positive organisms, namely *M. luteus*, *S. aureus*, and *B. cereus*, where green teas inhibited all the three Gram positive bacteria, including *S. aureus*, and this result is similar to that obtained in the present study. They also evaluated the antimicrobial activity of three Gram negative bacteria such as *E. coli*, *Salmonella typhi*, and *P. aeruginosa*. It was found that none of the tea extracts showed any activity against. In addition, they found that black and herbal teas inhibited the growth of *M. luteus* and *B. cereus* but not *S. aureus* (Chan et al., 2011).

Contrary to the present study, Pruthviraj et al. (2011) demonstrated that the caffeine extracted from the leaves and leaf buds of *Camellia sinensis* (green tea), and beans of *Coffea Arabica* (coffee) inhibits the growth of gram-
Figure 5: Inhibitory effects of unpacked tea leaf on different concentration.

Figure 6: Inhibitory effects of raw tea leaf on different concentration.

Figure 7: Inhibitory effects of commercial coffee on different concentration.
negative bacteria such as *E. coli*, *P. mirabilis*, *K. pneumonia*, *P. aeruginosa*.

In the present study, we explored the least MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of RTL (Raw Tea Leaf) and CC (Commercial Coffee) and were found to be 3.125 mg/ml for *B. cereus* and 6.25 mg/ml for *S. aureus* and Only 12.5 mg/ml of CC (Commercial Coffee) observed for *P. aeruginosa*.

According to the study by Jazani et al. (2007) the average MICs and MBCs of the water soluble green tea extract against all strains of *P. aeruginosa* (of which 55.8% were MDR strains) was 2.06 ± 1.76 and 2.54 ± 2.22 mg/ml.

After the emergence of multi-drugs resistant pathogens, the research for new remedy alternatives has led to the recognition of the potential of medicinal plant extracts for treating the infections associated with these types of microorganisms. Moreover, there is a synergistic effect of antimicrobial plant extracts with commonly used antibiotics; this effect has become the foundation of multi targeted approach used against multi-drugs resistant bacteria (Zambare and Bhyote, 2009).

**Conclusion**

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.

From day to day, bacteria show resistance against antibiotics. Antibiotics also have numerous side effects on human health and as such, natural tea and coffee, which is environmentally friendly, can be used as an antimicrobial agent. However, further investigation is needed to determine the bioavailability of the active compounds and to determine the dose and toxicity before it can be used as therapeutic agents. Natural sources of treatment have long been sought by humans as a safe alternative to conventional, synthetic, medication and antibiotics in order to avoid their complication.

The present study further supports the use of these plants as potential antibacterial agents especially against nosocomial infections. Thus, the present study reflects a hope for the development of novel chemotherapeutical agents.

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**REFERENCES**


