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In vitro antibacterial activity of *Camellia sinensis* leaf extracts to some selective pathogenic bacterial strains

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Abstract

Plant and plant material are essential for animal and human health care and also important for microbial management program. Pakistan has a great wealth of *C. sinensis* which has valuable use as tea not only in Pakistan but all over the world. The present study was carried out *in vitro* to determine antibacterial activity of *C. sinensis* leaf extracts against Pathogenic bacterial strain by using leaf cold water extract. Antimicrobial activity of *C. sinensis* was evaluated by well diffusion methods. The zone of inhibition against the tested bacteria was found ranging from 20.00 to 24.31mm. The highest zone of inhibition produced by Cold water extract *S. typhi* was (24.21 mm), *E. coli* (20.31 mm) and *S. aureus* was (22 mm) respectively. The extracts of *C. sinensis* leaf were found to be valuable antibacterial agent. This study furnish the way for further consideration and investigate to find out the active compounds accountable for the plant biological activity with the necessary minimum inhibitory concentration (MIC). Further studies should carry out to find out the accurate mechanism of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development for safe health care services

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Introduction

Nowadays Antimicrobial activity of medicinal plant has turn out to be a worldwide concern. This problem is of great issue particularly in 3rd world countries because are one of the major causes of mortality in these countries is due to these infectious diseases. There is a continuous and urgent need to discover new antibacterial and anti fungal compounds for new infectious diseases. Therefore, researchers are increasingly revolving their concentration to traditional medicine and probing for new leads to develop better drugs against wide range microbial infections including bacterial and fungal (Parekh and Chanda, 2007). Although hundreds of plants species have been experienced for antimicrobial properties, the vast majority have not yet been adequately examined for there possible antimicrobial activity against varieties of infectious microorganism (Balandrin *et al.*, 1985).

Camellia sinensis is a perennial plant of the *Theaceae* family. It is cultivated in tropical and subtropical regions. Tea can be obtained from buds or first three tip leaves. The number of leaves from the harvested branches (two or three), as well as the yield season, have their own importance when shaping the tea's quality (Segneau *et al.*, 2012). Tea is the second most commonly drank liquid on earth after water. It is being consumed socially and habitually by people since 3000 BC. The pleasing astringent taste and stimulating boost it provides is so deep-pervasive that its potential health remuneration and medicinal properties are often overlooked. Ongoing scientific exploration points that the certain potential health benefits derived from tea have important implications on human health (Sharangi, 2012).

The most abundant components in tea are polyphenols, in particular flavonoids such as the catechins, catechingallates and proanthocyanidins. The fresh leaves contain caffeine (approximately 3.5% of the total dry weight, or about 50 mg/cup when brewed), theobromine (0.15–0.2%), theophylline (0.02–0.04%) and other methylxanthines, lignin

(6.5%), organic acids (1.5%), chlorophyll (0.5%) and free amino acids (1–5.5%) (Graham, 1992).

Extracts of *C. sinensis* leaves from the tea plant contain polyphenolic components with activity against a wide spectrum of microbes. Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins, in particular epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria species with moderate potency. Sub-inhibitory concentrations of EGCG and ECG can suppress the expression of bacterial virulence factors and can reverse the resistance of the opportunistic pathogen *Staphylococcus aureus* to β -lactam antibiotics (Peter *et al.*, 2005). Activity against various other microbial pathogens or factors involved in their virulence has been shown; these include viruses such as hepatitis, HIV (Fassina *et al.*, 2002), chlamydia, mycoplasmas (Chosa *et al.*, 1992), rotavirus, enterovirus and influenza virus (Song *et al.*, 2005), filamentous fungi (Okubo *et al.*, 1991), yeasts (Hirasawa and Takada, 2004), and parasites (Paveto *et al.*, 2004). The antimicrobial activity of tea, suggested for many years by anecdotal evidence, was first demonstrated almost 100 years ago in the laboratory by (McNaught, 1906), a major in the British Army Medical Corps. He recommended that the water bottles of troops be filled with tea in order to prevent outbreaks of infections because the black tea killed *Salmonella typhi* and *Brucella melitensis*. Other bacterial species against direct *in-vitro* antibacterial activity of tea extracts has been described include *Helicobacter pylori* and α -haemolytic streptococci.

A number of epidemiological surveys have indicated that green tea consumption is linked to lower incidences of various pathological conditions, including cardiovascular disease strokes, obesity and cancer. (Hertog *et al.*, 1993; Keli *et al.*, 1995; Bell and Goodrick, 2002). Recent clinical studies have exposed physiological responses to tea extracts including

promotion of health, as well as the treatment and prevention from these chronic diseases (Mckay and Blumberg, 2002).

The present study was conducted to investigate the antibacterial activity of *C. sinensis* leaf extracts against gram positive ATCC (American type cell Culture) bacteria *S.aerious* ATCC®6538, and gram negative bacteria *Escherichia coli* ATCC®25922, and *Salmonella typhimurium* ATCC®14028.

Materials and methods

This research work was conducted at the Microbiology research Laboratory, Department of Microbiology, Hazara University Mansehra, Pakistan.

Plant materials

Healthy, disease free, mature *C. sinensis* leaf was collected directly from local region of National Tea Research Institute (NTRI) Shinkyari District Mansehra, KPK and brought to Department of Microbiology, Hazara University, Mansehra Laboratory. The leaves were cleaned with tap water. After cutting the leaf into small pieces, they were air dried in room temperature for 7 days, and then dried leaves were crushed into a fine powder by blender machine.

Cold water used for leaf extraction

The Adebayo and Ishola (2009) method of extraction was used. Three grams powdered samples of leaf and root was soaked in 30 ml cold water in 250ml sterile flask and rotated on shaker at 150 rpm for 24 hours at room temperature. The extract was filtered through a muslin cloth and then centrifuged at 4400 rpm for 7 minutes. The supernatant were collected and the pellet was discarded. These steps were repeated three times. The coming supernatant was considered as 100% concentration of extract. The cold water extracts were evaporated to dryness using a rotary evaporator (Stuart, Barloworld and Model RE 300). Their crude extracts were evaporated in a water bath to give gummy solid residue. The obtained crude extracts were 0.54 g leaf and 0.72g root.

Media preparation

Nutrient agar

Nutrient Agar was enrichment medium for the growth of microorganisms. Medium was prepared by adding 13g of dehydrated powder using electrical balance into 1 liter of distilled water. PH was adjusted by electrical pH meter at 7.4 and was boiled to dissolve completely.

Media sterilization

All Media were sterilized by using automatic autoclave (SANYO) at 121°C for 15 minutes.

Media pouring and drying

Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

Test microorganisms

The in-vitro activity of the extracts was assayed against the bacterial strains. All the ATCC (MicroBioLogics) against gram positive bacteria *S.aerious* ATCC®6538, and gram negative bacteria *Escherichia coli* ATCC®25922, and *Salmonella typhimurium* ATCC®14028. which were kindly provided by Dr. Malik Mujaddad ur Rehman, Assistant Professor, and HOD Department of Microbiology, Hazara University, Mansehrastrains were maintained on Nutrient Agar Tubes at 4 °C. The antibiotic efficacy of the plant extracts was evaluated against given strains.

Standardization of inoculums

After the incubation time, single selective colony of each bacterium from their respective selective agar medium was inoculated into 5ml NB and incubated for 4-6 hours at 37°C in incubator (NAPCO). 1% v/v solution of sulphuric acid (H₂SO₄) was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water. 1.175% w/v solution of barium chloride was also prepared by dissolving 2.35 g of dehydrated barium chloride in 200 ml of distilled

water. McFarland Standard No.0.5 was prepared by mixing 0.1ml of 1.175% w/v of barium chloride with 9.9 ml of 1% v/v sulphuric acid. Nutrient agar plates were prepared and pure bacterial cultures were swabbed in these plates and incubated for 37°C for 24 hours. With the help of sterile wire loop, three to four well isolated colonies from each plate were transferred to test tubes containing fresh nutrient broth to make bacterial suspension. These test tubes were incubated at 37°C for six hours. Turbidity of these suspensions was adjusted by using nutrient broth to McFarland Standard No. 0.5 by visually comparing the turbidity of bacterial suspension with McFarland standard.

Inoculation of test organisms

100µl of 1McFarland bacterial suspensions were aseptically introduced and spread using pre-sterilized cotton swabs on surface of MHA plates.

Wells preparation by cork borer

Agar well diffusion techniques as described by Adeniyi *et al.*, (1996). Wells of 6mm diameter with sterile cork borer were aseptically punched in the 90mm MHA agar plates.

Evaluation of antimicrobial activity

Antimicrobial activity of *C. sinensis* leaf extract was tested using agar well diffusion method. With the help of sterile micropipette tips *C. sinensis* leaf extract (cold water) 100µl were poured into the wells. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the resulting zone of inhibition was measured with the help of Digital Vernier Caliper (Mitutoyo) and the average values were recorded. Each antimicrobial assay was performed three times. Mean values were reported in this report.

Data analysis

All data were measured average value of three replicates and standard error (\pm). Results were subjected to Microsoft excel 2007.

Results

In the present study, the antimicrobial activity of the Cold water extracts against two gram negative and one gram positive bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.

Table 1. Activity of Cold water extract of *C. sinensis* leaf against Gram negative bacterial strains.

Bacterial strains	Zone of inhibition on bacterial strain of <i>C. sinensis</i> extract
Well diffusion methods	
<i>E. coli</i>	20.31 mm
<i>S. typhimurium</i>	24.21 mm

In table 1 showed that Cold water extract of *C. sinensis* leaf make a zone of inhibition on gram negative bacterial strain including *E. coli* (20.31 mm) and *S. typhimurium* (24.21 mm) were determined.

Table 2. Activity of Cold water extract of *C. sinensis* leaf against Gram positive bacterial strains.

Bacterial strains	Zone of inhibition on bacterial strain of <i>C. sinensis</i> extract
Well diffusion methods	
<i>S. aureus</i>	22 mm

Antibacterial activity

The extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. Results of the antimicrobial activity obtained

using the well diffusion assay is summarized in Table 1 and 2 and Figure 1 and 2.

In Table 2 showed that Cold water extract of *C. sinensis* leaf make a zone of inhibition on gram positive *S. aureus* (22 mm) were determined.

Discussion

Our present research work was planned to carry out the study on in-vitro antibacterial activity of cold water leaf extracts of *Camellia sinensis* against some selective gram negative and gram positive pathogenic bacterial strains. The extracts of *C. sinensis* exhibited greater extend of antibacterial activities.

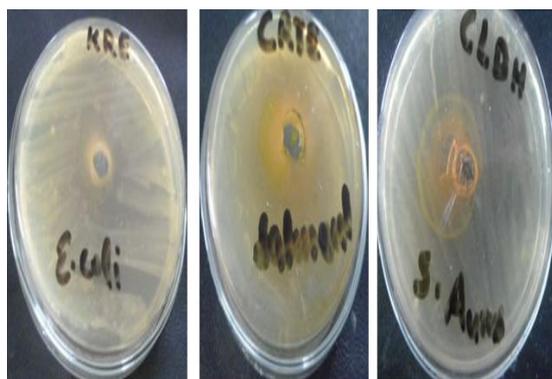


Fig. 1. Zone of inhibition of *C. sinensis* leaf extract on gram negative and positive bacterial strains. In table 2 showed that Cold water extract of *C. sinensis* leaf make a zone of inhibition on gram positive *S. aureus* (22 mm) were determined.

The antimicrobial activities of medicinal plants are qualified due to the presence of alkaloids, and flavonoids (Burapedjo and Bunchoo 1995; Fewell and Roddick, 1993). These reports and presence of flavonoids, alkaloids in different extract of *C. sinensis* confirm it's prospective against all selected pathogens bacterial strain.

The current study suggests that the Cold water leaf extract of *C. sinensis* have a board spectrum of antimicrobial activity, although the degree of vulnerability could different between different microorganisms. The antimicrobial activity found in this present conducted study may be ascribed to the presence of secondary metabolites either individually or in combination of various types of chemical composition present in the plant material.

The plant active substances were soluble in organic solvents so plant extracts obtained more activity than

commercial antibiotics (Boer *et al.*, 2005). Results of this study showed that the potential usefulness of *C. sinensis* in the treatment of various pathogenic diseases or infection as it may help in the innovation of new chemical classes of antibiotics or drugs that could serve as selective agents for the protection of human health and may provide life tools for the study of bacterial diseases or infection.

Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove expensive in case of prolonged treatment. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Therefore, there is needed to develop new antibacterial agents which can satisfy the present demand.

Conclusion

This study furnishes the way for further consideration and investigates to find out the active compounds accountable for the plant biological activity with the necessary minimum inhibitory concentration. Further studies should carry out to find out the accurate mechanism of action by which extracts exert their antimicrobial effect to recognize which can be used in drug development for safe health care services.

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Competing interests

The authors declare that they have no competing interests.

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