



INVITRO ANTIBACTERIAL ACTIVITY OF *CAMELLIA SINENSIS* AGAINST COMMON DENTAL PATHOGENS

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ABSTRACT

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases. Dental caries is one of the most common infectious microbial diseases. *Camellia sinensis* contains Qualitative and Quantitative Phytochemical analysis and Antioxidant activity. To evaluate the antimicrobial efficacy of *Camellia sinensis* extracts against some common oral pathogens like *Streptococcus mutans*, *Lactobacillus fermentum* and *Pseudomonas aeruginosa*. It has very important role in dental implications such as caries, halitosis and periodontal problems. The *in vitro* antibacterial activity was performed by agar well diffusion method. The most susceptible gram-positive bacteria were *B. subtilis*, *S. mutans* and *S. aureus*. The aqueous extract of *Camellia sinensis* was found to be effective against all the selected strains. Minimum inhibitory concentrations (MIC) of the extracts were also determined against the four selected microorganisms. This study was suggested that aqueous extracts of *Camellia sinensis* can be used as a potential source of novel antimicrobial agents used to cure dental caries.

KEYWORDS: Qualitative and Quantitative phytochemical analysis, Antioxidant activity, Antibacterial activity and Minimum Inhibitory concentration.

INTRODUCTION

Oral health has an impact on general health and well-being as the oral diseases cause pain and suffering, alter the eating pattern, speech, and affect the quality of life as a whole. Oral health also has an effect on other chronic diseases. Oral diseases are one among the most common chronic diseases affecting mankind. Dental caries is a very common problem that affects all age groups. It is a process in which the enamel and the dentine are demineralised by acids produced by bacterial fermentation of carbohydrates (De Soet and de Graaff, 1998). In real life, it is the most common infectious disease affecting human beings (Balakrishnan *et al.*, 2000). Dental caries and periodontal diseases are among the most important global oral health problems. Over 750 species of bacteria inhabit the oral cavity and a number of these are involved in oral diseases (Jenkinson *et al.*, 2005). Dental caries is a multifactorial human disease that has widely affected many populations all over the world. Current researches are showing that the properties of bacteria associated with a surface in a biofilm can be markedly different than those of the same cells growing in liquid broth (planktonic cells). Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest threat of disease (Scheie and Adv Dent Res, 1994). *Streptococcus mutans* (*S. mutans*) and

Streptococcus sobrinus are the major suspected pathogens in the initiation and progression of dental caries (Angius *et al.*, 2015). It is proved that cariogenic microorganisms, especially *Streptococcus mutans* plays an essential role in the pathogenesis of dental caries. It is involved in the initiation of almost all carious lesions in enamel (Samaranayake, 1996).

Green tea (*Camellia sinensis* L) is an important commercial plant that is produced in over 30 countries and has been consumed worldwide primarily as a beverage made from the processed leaf (Choi *et al.*, 2006). It has a variety of secondary metabolites such as catechin, caffeine, theanine and saponin that are important for human welfare (Yoon *et al.*, 2005). Green tea has many special antibacterial effects on oral cavity. They are includes: effect on dental caries, antiviral properties and abolition of halitosis. Investigation shows that tea consumption may decrease dental caries. *S. aureus* carriers are sources of (HAIs), which may also occur in dental healthcare settings (Petti and Polimeni, 2011). *S. aureus* transmission is promoted by the long-term survival, up to six months, on clinical contact surfaces (Petti *et al.*, 2012), that is, surfaces contaminated by patient materials generated during dental procedures (Kohn *et al.*, 2003), and by the passive

role of dental healthcare workers who may act as vectors in transmitting *S. aureus* from clinical contact surfaces to receptive patients (Dancer, 2008).

MATERIALS AND METHODS

Collection of plant material: The plant material *Camellia sinensis* (Leaves) was collected from Siddha shop. The spices were crushed and made in a fine powdered. This powdered was used for further studies.

Preparation of aqueous extracts (Mohammed et al., 2012): The plant material was powdered with the help of grinder. 10g of powder was taken and mixed with 50ml of Hot and Cold distilled water. This extract was kept at room temperature for 24hrs. After 24hrs the extracts were filtered through Whatman No.1 filter paper. This filtrate was used for this study.

Antibacterial Activity of Plant Extract

Agar well diffusion technique (Perez, 1990): The extract was tested for antibacterial activity by standard agar well-diffusion method against dental pathogenic bacteria *E.coli*, *Bacillus sp*, *Klebsiella sp*, *S.mutans* and *S.aureus*. The pure cultures of bacterial pathogens were sub cultured on nutrient broth 20ml of nutrient agar were poured into petriplates. Wells of 6mm diameter were made on nutrient agar using gel puncture. Culture was swabbed uniformly using sterile cotton swabs and then 50µl and 100µl of plant extract solution was loaded into the wells. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured.

Minimum Inhibitory Concentration (MIC) Micro dilution (Kumarasamy et al., 2002): Minimum inhibitory concentration was done by the lowest cost of the extract *Camellia sinensis* and was it can show the bactericidal and bacteriostatic effect. The test was performed in 96 well microtiterplate. Microtiter plate wells from each column in row 1 were marked and 100µl (500mg/ml) of stock (aqueous extract) was added. 50µl of sterilised distilled water was added to rows 2-12. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to 2, using a multichannel pipette. This was repeated down the row 2 to 12. 40µl of double strength nutrient broth and 10µl of bacterial culture was added to all the wells in separate column, so the final concentration of the inoculum in all the wells. To prevent dehydration, the plates were covered with a plastic cover and then incubated at 37°C for overnight. The bacterial growth was determined after addition of 40µl of 2, 3, 5. Tri phenyl Tetrazolium Chloride Red (0.02 mg/ml). The minimum inhibitory concentration (MIC) of the isolates was taken as the lowest concentration of the antibiotic of which the bacterial tested did not show visible growth.

RESULT AND DISCUSSION

Five different cultures were isolated from infected tooth. The isolated strains were identified by biochemical test

following the basis of Gram staining and biochemical test. The isolates were identified as *E.coli*, *Bacillus sp*, *Klebsiella sp*, *S.mutans* and *S.aureus*.

Table 1 showed the antibacterial activity of Cold and Hot water extract *Camellia sinensis*. A maximum zone was obtained for *Camellia sinensis* (23mm) against at *Klebsiella sp*. An et al., 2004 reported green tea extracts could enhance the antimicrobial properties against *S. aureus*, *S. epidermidis*, *E. coli* and *S. mutans*.

Figure 4 and 5 showed the Minimum inhibitory concentration of Cold and Hot water extract of medicinal plant. Cold water extracts of medicinal plant showed highest bactericidal effects than hot water. *Camellia sinensis* showed minimum inhibitory concentration at 6.25µl/ml against maximum strains. *Camellia sinensis* showed minimum inhibitory concentration at 100 µl/ml against minimum strains. Tzung et al., 2008 reported Minimum inhibitory concentration of green tea (water) extract against *S. mutans* was 0.8 mg/ml.

Table 2 showed the Qualitative phytochemical analysis in cold water and hot water extract of *Camellia. Sinensis*. Most of the compounds were present in the cold water while compared to hot water. Alkaloids, Flavonoids, Saponins, Tannins, Glycosides, Carbohydrates, Proteins Phenols, and Anthraquinone glycoside were present in all plants. Oil and Fat, Gum and mucilage were absent in both extracts. Bashir et al., 2014 studies screened the phytochemical constituents of *Camellia* supported the results of antibacterial assay. The leave extracts of *Camellia* varieties indicated the presence of active biological compounds such as flavonoids, proteins, phenols, alkaloids and glycosides which exhibited strong activity against selected bacterial strains.

Table 3 Quantitative estimation of Alkaloid, Flavonoid, Carbohydrate, Protein and Crude fiber tested in plant powders. Crude fiber content was rich in *Camellia sinensis* (0.31mg/g). Sarma et al., 2015 studied the quantitative phytochemical analysis of mature and immature leave of *Camellia sinensis* 63×10^{-5} of protein was present in mature leaves.

Figure 1, 2 and 3 showed the Antioxidant activity of *Camellia sinensis* plant extracts Cold and Hot water. Our results demonstrated good comparatively other. So it can chosen that plant. Reducing activity, Chelating activity and Scavenging activity of *Camellia sinensis*. It was observed that chelating activity followed by *Camellia sinensis* 366.63 - 1133.30 % for cold water extract. In Astley, 2003 work, 1gram of *Camellia sinensis* leaves extract carries 0.11g of reducing power.

Table 1: Antibacterial activity of Cold and Hot water extract of *Camellia sinensis*

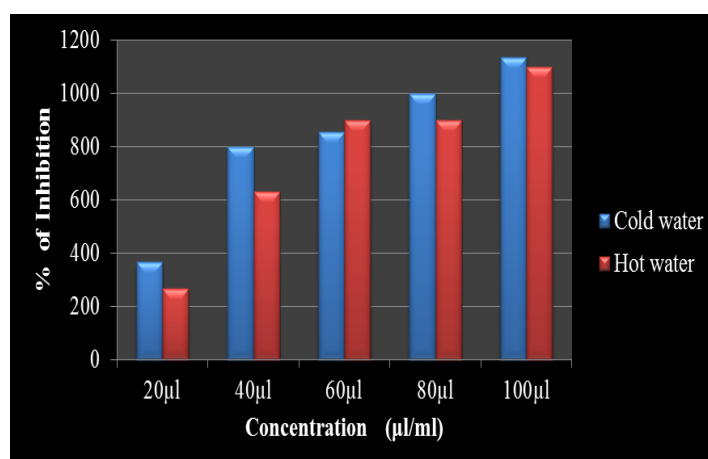
S.No	Isolates	Zone of inhibition (mm)	
		Cold water	Hot water
1	<i>E.coli</i>	17	10
2	<i>Bacillus sp</i>	14	18
3	<i>Klebsiella sp</i>	23	11
4	<i>S.mutans</i>	20	09
5	<i>S.aureus</i>	19	10

Table 2: Qualitative Phytochemical Analysis of *Camellia sinensis*

S. No	Parameter	<i>Camellia sinensis</i>	
		Cold water	Hot water
1	Alkaloid	Present	Present
2	Flavonoid	Present	Present
3	Saponins	Present	Present
4	Tannins	Present	Present
5	Glycosides	Present	Present
6	Carbohydrates	Present	Present
7	Protein	Present	Present
8	Terpenoids	Present	Present
9	Cardiac glycosides	Absent	Absent
10	Oil and fat	Absent	Absent
11	Phytosterols	Present	Absent
12	Gum and Mucilage	Absent	Absent
13	Phenols	Present	Present
14	Steroids	Present	Present
15	Anthraquinone glycosides	Present	Present

Table 3: Quantitative Phytochemical Analysis of *Camellia sinensis*

S.no	Compounds	<i>Camellia sinensis</i>
1	Alkaloids (mg/g)	1.34
2	Flavonoids(mg/g)	1.11
3	Carbohydrates (µg/ml)	120
4	Protein (µg/ml)	633.3
5	Crude fibre (mg/g)	0.31

Figure 1: Chelating Activity of *Camellia sinensis*

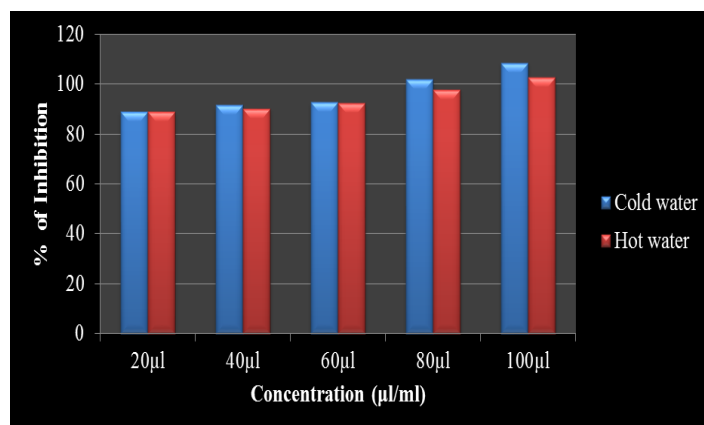


Figure 2: Scavenging Activity of *Camellia sinensis*

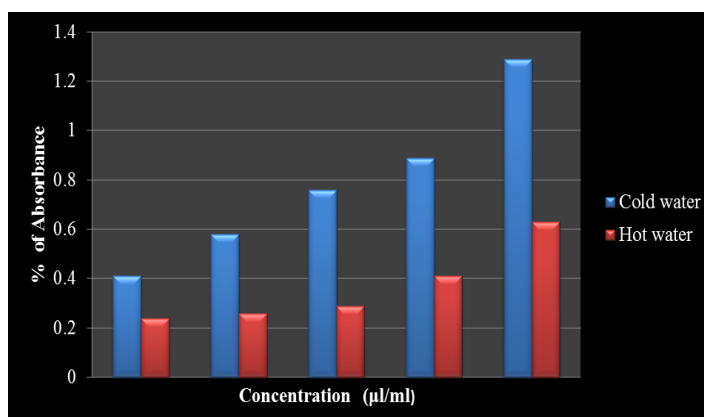


Figure 3: Reducing activity of Cold and Hot water extract of *Camellia sinensis*

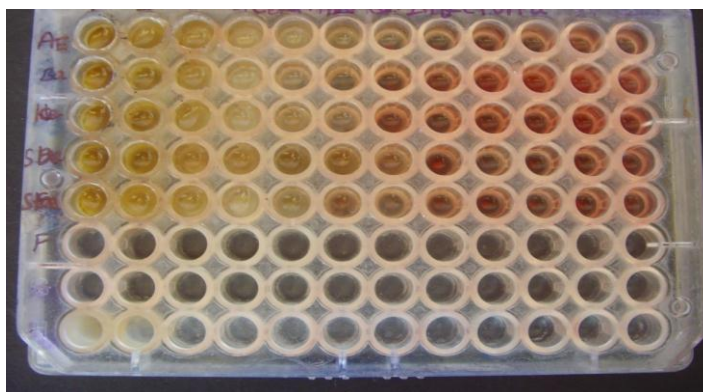


Figure 4 Minimum inhibitory concentration of Cold water extract of *Camellia sinensis*

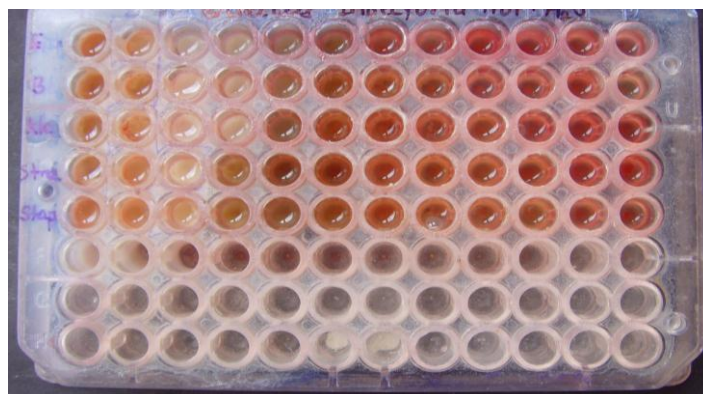


Figure 5 Minimum inhibitory concentration of Hot water extract of *Camellia sinensis*

CONCLUSION

❖ It can be concluded that *Camellia sinensis* was used for dental infection. These medicinal plants have potential antimicrobial agents against oral microorganisms. It may be used for mouthwash preparation.

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