



Research Article

Therapeutic Potential of Tea (*Camellia sinensis*) Against Oral and Intestinal Microbial Flora

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Keywords: Camelia sinensis; Anti-microbial plant extract; Oral pathogens; Tea

Abstract

The tea powder produced from plant *Camellia sinensis* (L), is popular as a daily beverage drink among the Indian population. The tea plant produces large number of metabolites which are of medicinal value. In the present study, the activity of tea leaf extract was studied by disk diffusion method against the bacterial strains, *Streptococcus mutans*, *Enterobacter fecalis*, *Escherchia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from the oral swab. From the disk diffusion assay, the tea extract has shown better activity against *S. mutans* and *P. aeruginosa* at the concentration, 25 mg/ml. It is effective against *E. fecalis* and *S. aureus* at higher concentration, 75 mg/ml and 100 mg/ml. Thus, the tea leaf can be considered as effective antibacterial agent against the oral pathogens.

Introduction

Plants produce an amazing variety of metabolites that are gaining importance for its therapeutic and biotechnological applications (Quiroga *et al.*, 2001). The application of herbs as the therapeutics is an ancient practice evolved along with human civilization. The vast majorities of people on this planet still relay on their indigenous system of medicine and use herbal drugs. According to the records, the medicine system of India and China are established around 3000 years. The medicinal plants' extract formulations and its

discovery is continued to discover the therapeutic molecules targeting the cancer, infectious diseases and all other health problems. The tea is discovered, and drinking practiced since long back. It was originating during the "Shen-Nong" era of ancient china, around 5000-6000 year ago (Chen, 1999). The probable center of origin of tea was in southwest china. The Chinese word "cha" is the first word coined for tea in the world which means tea (Yao, 1992). The first literary mention of tea all agree that its taste was bitter, at

the time it was also called bitter tea. The first ancient use of tea was as a “medicine” and during the course of time in late Zhou Dynasty (1124 B.C.- 222 B.C.) its being used as regular drink. Thus, during the time of Qin Dynasty (221-206 B.C.) tea drinking gained the popularity and practiced daily (Yao and Chen, 1995). In Tang Dynasty (618-906 A.D.) tea production including cultivation, harvesting and processing had developed rapidly and the population of tea drinkers reached to highest level.

The black tea or tea added with milk, is prominently preferred beverages in the world as compared to coffee, beer, wine and other carbonated soft drinks (Costa *et al.*, 2002; Rietveld and Wiseman, 2003). The tea is prepared

from cured leaves boiled in hot water and other ingredients also added; thus, it is also referred as aromatic beverage. Assam is one of the world’s single largest tea growing regions, situated on either side of the Brahmaputra River and bordering Bangladesh and Burma (Fig. 1 and Fig. 2). *C. sinensis* var. *assamica*, is attributed by the features such as briskness, malty flavour and strong bright colour and widely grown. The *C. sinensis* plant belongs to Theaceae family and it produces black, oolong and green tea from its leaves. Tea is oldest known medicine. It was taken in china 5000 years ago for its stimulating and detoxifying properties in the elimination of alcohol and toxin, to improve blood and urine flow, to relieve joint pains, and to improve resistance to diseases Balentine *et al.*, 1997).

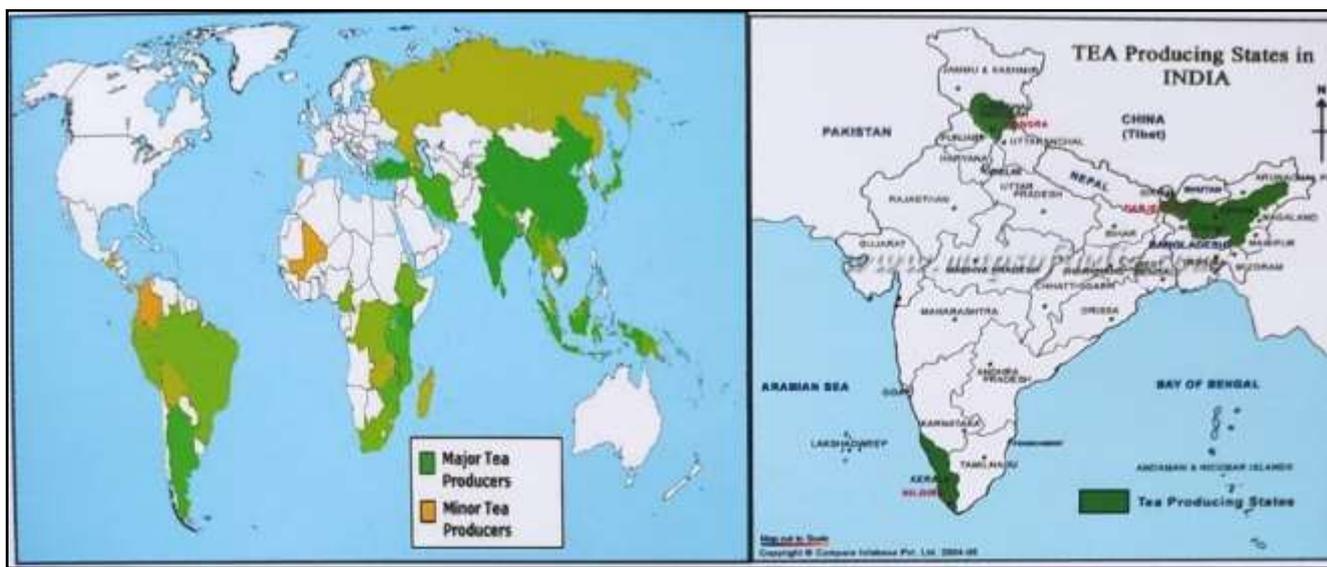


Fig. 1: Tea Producing States

(Source: (i) <http://sgritech.tnau.ac.in>; (ii) <http://in.pintereset.com/Pin/>



Fig. 2: Leaves of plant *Camellia sinensis*

Green tea catechins have demonstrated antibacterial activity against both gram positive and gram negative bacterial which can be harmful to humans. Dufresne and Farnworth (2000) reported that the tea extracts is capable of inhibiting the enteric pathogens, *Staphylococcus aureus*, *S. epidermis* and *Plasiomonas shigelloides*. It is well reported that the black and green tea extract is capable to disinfect the pathogenic bacteria, *Helicobacter pylori* responsible for gastric, peptic and duodenal ulcer diseases. Tea polyphenols can selectively inhibit the growth of clostridia and promote the growth of bifid bacteria in human large intestine. The bacterial balance in intestinal microflora may be important for the prevention of colon cancer (Diker and Hascelik, 1994). Several reviews addressed the local application of antimicrobial agents to the subgingival area for the treatment of periodontitis.

The use of medicinal plants for various therapeutic properties and the place of medicinal plants in modern day healthcare system. Medicinal plants commonly consumed worldwide contain different chemical substances that display a broad spectrum of biological activities, enabling the induction of positive effects in treatment of many human diseases. Antimicrobial of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants (Joshi *et al.*, 2009). Plants showing the anti-bacterial activity are rich in polyphenolic acids. The essential functions of microbial system might be damaged by the various secondary metabolites and essential oils of plant in adequate concentration (Samy and Gopalakrishnakone, 2010). Medical books written as early as in the Song Dynasty (960-1279 A.D.) in China mention that green tea in combination with ginger can effectively cure dysenteric disorders, including those so called red and white in appearance (Lin, 1992). Modern medical research has demonstrated that tea and tea products are active against a wide range of microorganism implying that tea may be potentially useful for treatment of some infectious illness. In the case of *Staphylococcus aureus*, black tea showed strong bactericidal activity than green tea and coffee (Toda, 1989). Tea polyphenols present in tea product are the major components responsible for the antibacterial activity. The active tea polyphenols include EC, ECG, EGC, EGCG and theaflavins and their MIC values were estimated in the range of 100-800 ppm (Hera *et al.*, 1989). In addition to the direct antibacterial activity, tea extracts can reverse the methicillin resistance in Methicillin Resistance *S. aureus* (MRSA) and, to some extent, the Penicillin resistance in beta-lactamase producing ones. There exists a synergy between beta lactam antibiotics and extracts (Yam *et al.*, 1998). Various tea extracts have shown bactericidal activity

against *Streptococci mutans*. Several catechins, the component from green tea, are active against cariogenic bacteria. The present study was carried out to study the antimicrobial activity of tea extract against the isolated oral pathogenic bacterial strains.

Scientific Classification

Kingdom: Plantae
Division: Angiosperms
Subdivision: Eudicots
Class: Asterids
Order: Ericales
Family: Theaceae
Genus: *Camellia*
Species: *C. sinensis*

(Source: Plant Database- <https://plants.sc.egov.usda.gov/>)

Material and Methods

Preparation of Plant Extract Disc

Various concentrations (100 - 500 µg) of hydro-alcoholic extract of plant *Camellia sinensis* were taken for the study. The 06 mm diameter Whatman filter paper no. 1 disc of different concentration of plant extract 75 mg/ml dilution (25%, 50%, 75% and 100%) were prepared for sensitivity test.

Oral Bacterial Strain Isolation

For antimicrobial activity important bacterial samples from different oral flora were isolated by using swab method and spread on specific media plates and then incubate at 37°C. After culturing the bacterial isolates stained by gram's staining and observed under compound microscope at 100X. According to morphometric characterization 5 different bacterial isolates, *Streptococcus mutans*, *Enterobacter fecalis*, *Escherchia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antimicrobial Activity of Extract *Camellia sinensis* Leaf Extract

The disc diffusion method (Kirby Bauer *et al.*, 1966) was used to test the antibacterial activity of plant extracts against 5 bacterial species. A zone of chemical components of the plant extract is formed around the impregnated filter paper disk on agar. The solubility of the chemicals and its molecular size determines the size of the area of chemical infiltration around the disc. The growth of susceptible microorganisms decreases in the area around the disc called as a "zone of inhibition". The culture plate were incubated for 24 hours. The diameter of zone of inhibition to nearest whole millimeter to the point, where there is 80% reduction in growth.

A loopful culture from the overnight grown culture in liquid broth having O.D at 660 nm up to (1×10^6) is spread on NAM agar medium. A 6 mm diameter Whatman filter paper disc of different concentration of plant extract 75 mg/ml dilution (25%, 50%, 75%, 100%) is placed at the center of plate each

respective plate. The plates were incubated at 37° C for 8 hours. The zone of inhibition in mm by zonal scale (Hi-Media) was measured adopting the methods of Doughari *et al.* (2008). The graphs were plotted using the Microsoft Excel software.

Results and Discussion

All over the world and in India, the collection of traditional herbal medicine knowledge and following the scientific confirmation through extensive research related to study of the optimization of plant extracts effectiveness and treatment conditions.

The present investigation is being aimed to explore the medicinal value of the leaves of plant, *Camellia sinensis* (tea), which is endemic in the whole India. The tea plant leaf extract was used to study its anti-microbial activity. The antimicrobial screening showed interesting results as it has shown activities at nearly all concentrations. Plant extracts are generally a crude mixture of number of active and inactive compounds and therefore the inhibition zone formed by the diffused plant extract must be interpreted accordingly.

Tea leaf extract showed maximum inhibition against selected oral isolates *Streptococcus mutans* i.e. 23 mm, 22 mm, and 20 mm respectively (Fig. 3, Fig. 4, Fig. 5, Fig. 6 and Fig. 7). The observations were comparable with that of other studies. The flavonoids and tannins present in the leaf are responsible for their antibacterial properties.

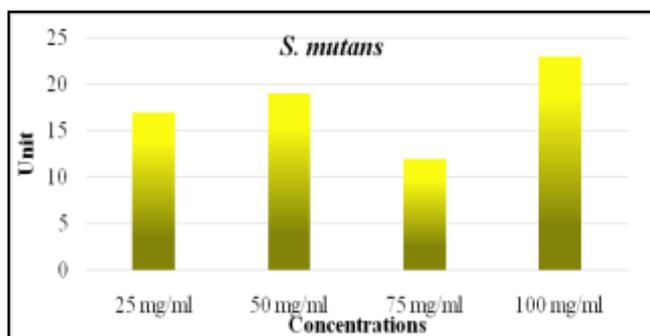


Fig 3: Antibacterial activity of Tea leaf extract against *S. mutans*

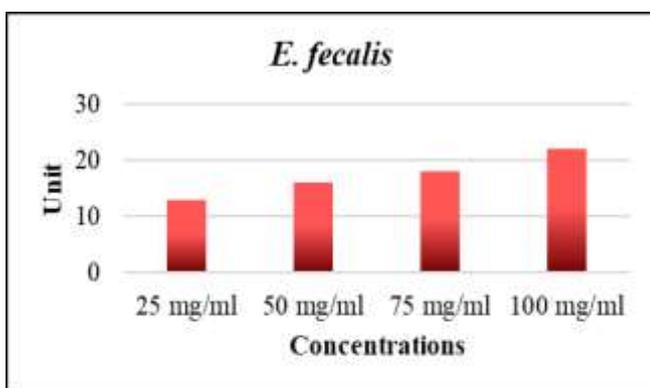


Fig. 4: Antibacterial activity of Tea leaf extract against *E. fecalis*

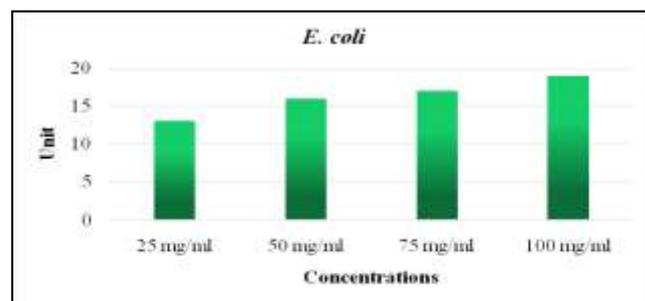


Fig 5: Antibacterial activity of Tea leaf extract against *E. coli*

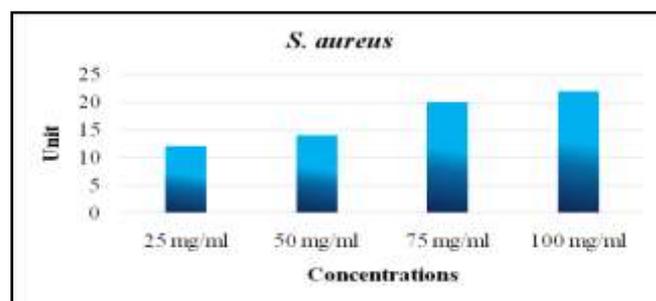


Fig 6: Antibacterial activity of Tea leaf extract against *S. aureus*

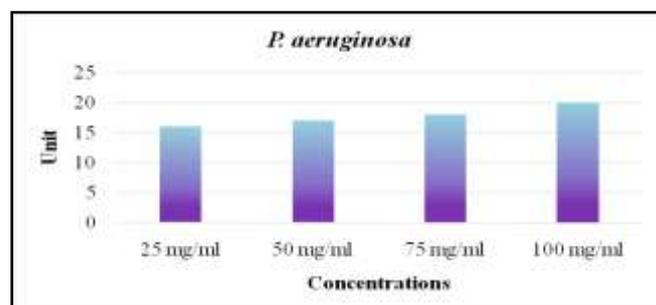


Fig 7: Antibacterial activity of Tea leaf extract against *P. aeruginosa*

Discussion

In this study we have noted that zone of inhibition less than 50 mg/ml should be interpreted as strong antibacterial potential. The zone of inhibition observed during this study are of standard that can be considered to be significant for plants and plants extracts. The antimicrobial activity of the plant extract against the gram-negative bacteria mainly depends on the bacterial strains, the plant parts used for extraction, its maturation state and nature of the extraction.

The antimicrobial activity of root extract of *Glycyrrhiza glabra* has shown in few studies but antibacterial effects of this plant against oral pathogens has not been studied extensively (Shapna *et al.*, 2010; Meghashri *et al.*, 2009). These studies propose that *G. glabra* can inhibit the growth of *Streptococcus mutans*, *Actinomyces viscosus*, *Streptococcus sanguis* and *Enterococcus faecalis*.

In the present study, the leaf extract of plant, *C. sinensis* showed antibacterial activity against *S. aureus* (fig 4);

similar to the results investigated on *G. glabra* root extract that showed antimicrobial activity against other oral microorganism *Actinomyces viscosus* and *Streptococcus sanguis* (Nirmala *et al.*, 2011). Glabridine, is one of the most important substances in *G. glabra* plant, had antibacterial activities and observed to be more active against gram positive strains than gram negative (Gupta *et al.*, 2008).

The flavonoids are known to be synthesized by plant leaves in response to microbial infection (Zhang *et al.*, 2017) hence leaf extracts possesses antimicrobial activity against a wide range of microorganisms. Considering the antibacterial activity, present study showed significant zone of inhibition

for crude plant extracts against all the oral pathogenic bacterial strains (Fig 8: a, b, c, d, e). The potent activity against *S. mutans* (fig 2) in particular is welcomed due to reduced bacterial frequency to the limit responsible to cause infections and major concern in clinical bacterial infections due to this strain. The equally strong activity against *S. aureus* opens the possibility to use the extracts either alone or in combination with other antibacterial drugs. The antibacterial activity is attributed to various phytochemical as investigated during the extracts screening that damage the pathogens cell membranes, resulting into leakage of cellular materials and ultimately the death of microorganisms.

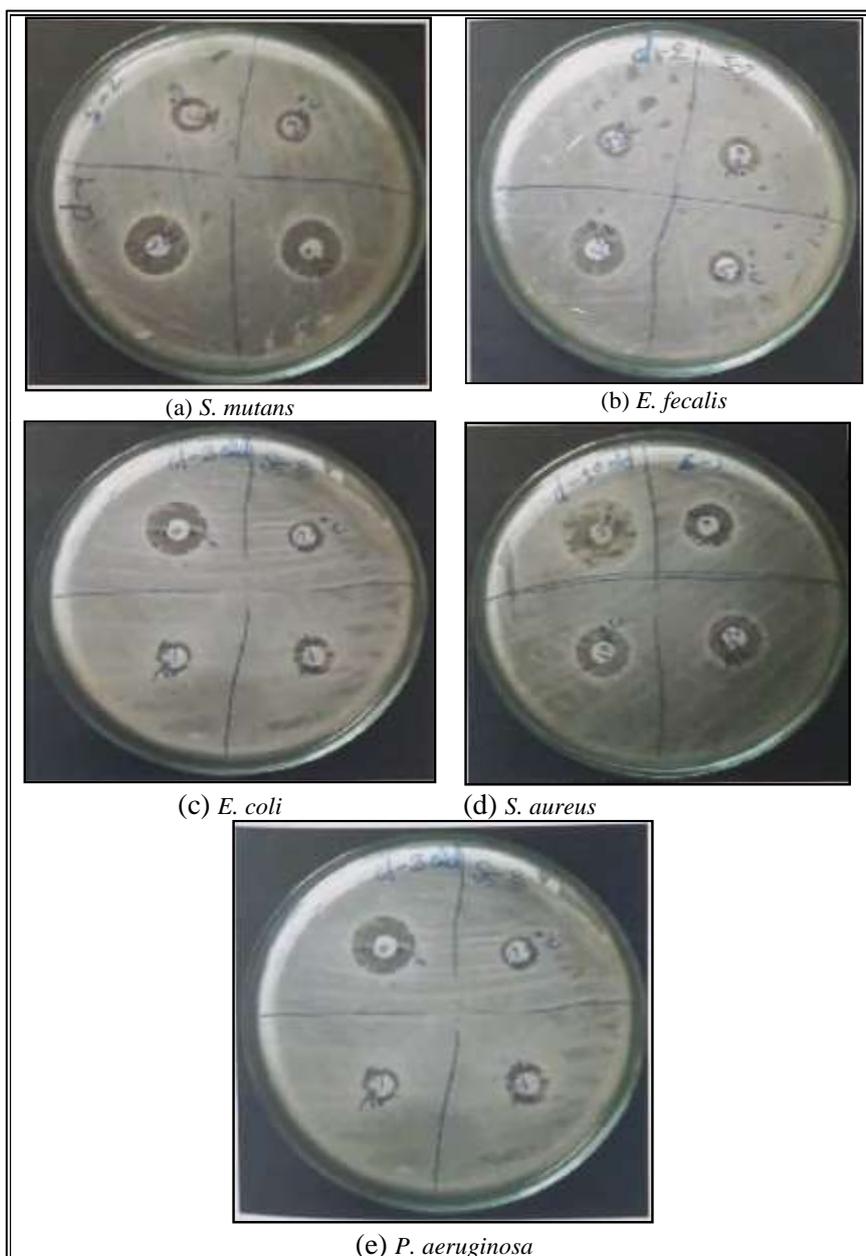


Fig. 8: Agar plate showing zone of inhibition against (a) *S. mutans*, (b) *E. fecalis*, (c) *E. coli*, (d) *S. aureus*, (e) *P. aeruginosa*.

Conclusion

The present study on antimicrobial activity of leaf extract of tea plant against the selected oral pathogens investigated to be significant by disk diffusion method. The studies further can be extended towards the isolation of active compounds from the Tea leaf extract. The biochemical analysis is required to confirm the molecular mechanism that is responsible for the antimicrobial activity of extract. The Tea leaf extract with its wide spectrum antibacterial properties can be considered an effective antimicrobial agent to counter act the oral infectious bacteria.

Authors' Contribution

Both authors contributed equally at all stages of research work as well as approval of the final form of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

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