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Research Article

Antibacterial activity of green tea (*Camellia sinensis*) Extract against dental caries and other pathogens

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Abstract

The present study has however, revealed that the herbal plant *Camellia sinensis* (green tea) possess antimicrobial properties. The isolated strains were confirmed by performing staining and biochemical techniques. Aqueous extract of green tea were taken and used for the study of inhibition effect against dental caries and other pathogens. The zone of inhibition was performed using agar well diffusion techniques different concentration of green tea extracts were studied for their antibacterial activity. The overall results showed that the microorganisms were susceptible to different concentration of aqueous extracts of *Camellia sinensis* which is a function of their antimicrobial properties. The effectiveness of active principle was studied and compared with the previous one. The nature of the chemicals present as active principle of the extract was studied using Paper chromatography and Thin layer chromatography. The chemicals involved in antimicrobial activity are commonly belonging to any one of the group such as flavanoids, alkaloids, saponins and polyphenols. It could be concluded that flavonoid in a potential natural, antimicrobial agent against dental caries and other pathogens.

Keywords: *Camellia sinensis*, antimicrobial properties, Paper chromatography and Thin layer chromatography

Introduction

Tea is drink derived from boiling the cured leaves of the *camellia sinensis* grown in many places around the world, especially India and China. There are four major types of tea that fall under the species of *C. Sinensis* such as white tea, green tea , black tea, and oolong tea. In the study of dental caries prevention, green tea, black tea, are involved. Tea is consumed by billions of people around the world along with water which is the most highly consumed beverage.

In traditional Chinese and Indian medicine, practitioners used green tea as a stimulant, diuretic (to control bleeding and help heal wounds), and to improve heart health. Other traditional uses of green tea include treating flatulene (gas), regulating body temperature,

blood sugar, promoting digestion, and improving mental processes.

Tea's antioxidant properties are derived from the catechins compounds found in tea leaves. Catechins are flavonoids found mostly in the leaves of tea, although they are also in wine, chocolate and fruits. Tea's antioxidant powers have a plethora of healthy effects, including tumour suppretion and cancer prevention. Epicatechin galate(ECG), is a catechin in Green tea. It can prevent the formation of carcinogens in animal studies .

Other flavonoids also derived from tea, they also have varied health benefits. They protect the heart against disease through their ability to prevent the oxidation of some lipoproteins in macrophages. Saponins extracted from Green tea have an anti-inflammatory effect, studied in rats because the saponins which decrease the ability of glucose and salt to be absorbed in the intestine.

Tea has been studied in experiments involving liver damage and HIV and are commonly used in household products such mouth wash and deodorant.

Green tea contains high concentration of Catechins such as (O)-epicatechin(EC),(O)-epigatec hingallate (ECG) and (O)-epicgallocatechin gallate (EGCg)-Isogai et al reported synergy between green tea extract and levofcoxacin against enteroahaemorrhagic *Escherichia coli.*

Green tea is made from unfermented leaves and reportedly contains the highest concentration of powerful antioxidants called poly phenols. It contains potent antiviral, antibacterial, antifungal and anti - tumour substances that can help treat diseases from the common cold to cancer. Green tea extracts also seem to be able to help prevent cold and flu illnesses from taking root in the first place.

Looking at the molecular process involved in teeth decay can help explain the nature of dental caries prevention. Dental caries is a gradual process that accumulation of bacteria in the mouth, usually caused by prolonged exposure of the teeth and mouth to sucrose. This process has three basic steps.

1. Bacteria, usually from a food source, attached to teeth.
2. Glycocalyx is formed when glucosyl transferase, a bacterial enzyme, react with sucrose.
3. Formation of bio film, as bacteria metabolize carbohydrates and produce acid that eventually decays the tooth.

Green, black, and oolong teas have specifically been studied in relation to teeth health. Green and black tea share many similarities, yet differ slightly in the structure of their catechins. Green tea has simple catechins with a lower molecular weight than black catechins. Many black catechins have been oxidized through fermentation and have a molecular weight, although some remain simple. Catechins can remain in the mouth for up to sixty minutes after tea has been consumed.

A cup of Green tea contain three times as many catechins than a brewed cup of black tea. Oolong tea is an intermediate between these two types, containing both simple and fermented catechins.

There have been many studies exploring the molecular methods behind the ability of tea to fight tooth decay. It has been discovered that these teas actually attack each of the three basic steps to dental caries. The first step is the adhesion of the bacteria to the tooth. Green and block tea extracts inhibits the ability of *Streptococcus mutans* to bind to the tooth surface. In addition , Oolong extracts have been found to have an affinity for proteins of the tooth, they prevent other bacteria from attaching there as well.

Once a bio film has been established on the surface of teeth, it becomes very hard to eradicate. Bio films are strong evolutionary communities that join bacteria together to provide structure and support, as well as other benefits including exchange of antibiotic resistance. It is much easier and more effective to break down growing bacteria before they reach this stage.

Lactobacillus acidophillus are also a gram positive bacteria. They are organotrophs that develop in a rod-like shape. They can sometimes grow in clusters, and are especially prominent in tooth bio film, were they thrive in the high sugar conditions. *Lactobacillus* main source of energy is found from metabolizing sugars into lactic acid, a process that makes them prime candidates for mouth dwellers.

Chemical constituents

The healthful properties of Green tea are largely attributed to polyphenols, chemicals with potent antioxidant properties. In fact, the antioxidant effect of poly phenols appear to be greater than vitamin C. The polyphenols in Green tea also give it somewhat bitter flavour.

Polyphenols contained in teas are classified as catechnis . Green tea contains six primary catechin compounds: catechin, gallaogatechin, epicatechin, epigallocatechin, epicatechin gallate, and apigallocatechin gallate (also known as EGCG). EGCG is the most studied poly phenol component in green tea and the most active.

Green tea also contains alkaloids including caffeine, theobromine, and theophlline. These alkaloids provide Green tea's stimulant effects. Tea leaves contain many compounds, such as polysaccharides, volatile oil, vitamins, minerals, purines , alkaloids eg:- catechins, flavonoids. Although all three tea types have antibacterial and free radical capturing (anti oxidising) activities, the efficacy decreases substantially the darker variety of tea. This is due to lower contents of antioxidising poly phenols remaining in the leaves. The polyphenols found in tea are more commonly known as flavanoids or catechins and comprise 30-40 % of the extractable solids of dried Green tea leaves. The main catechins in Green tea are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3- gallate (EGCG), with the latter being the highest in concentration.

Green tea polyphenols have demonstrated significant antioxidant, anti-carcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal and in vitro studies.

Causative agent of dental caries

Of the hundreds of different types in our mouth, only a handful are thought to cause gum diseases, but only *Streptococcus mutans* is implicated as the main cause of dental decay.

Streptococcus mutans was first isolated by J.K. CLARK in 1924. It is a gram positive bacterium that ranges in diameter from 0.5.to 0.75 micrometers. It is a facultative anaerobe and is most members of the *Streptococcus* family divided into four groups according to antibody they contain. *Streptococcus mutans*, however does not contain any of these antibodies.

Aim and objectives

The aim of present investigation is to utilize Green tea extracts and study their inhibitory action against dental caries causing microorganism like *Streptococcus mutans, Lactobacillus acidophillus* and other pathogenic bacteria such as *Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Bacillus subtilis.*

Materials and Methods

Sample collection

The sample (dental caries) was collected from the infected tooth with the help of sterile tooth pick or swab and other pathogens were enumerated from environmental source. The collected samples were used for isolation.

Isolation of microorganisms

The sample (dental caries) was inoculated in 1×PBS buffer. This was then diluted and streaked on blood agar and incubated anaerobically at 37°C for 2 days. The colonies from the agar plate was cultured in BHI (brain Heart Infusion broth) broth for 18 hours and streaked on MSB (Mitis - Salivaris Bacitracin) agar plates and synder's test agar plate incubated at 37° C for 2 days under anaerobic condition. The sample (Environmental source) was inoculated in Nutrient agar medium, Differential medium and Selective medium. Then the places were incubated an aerobically at 37°C for 24 hours. For further identification the organism has been maintained in nutrient broth.

Staining technique

Gram's staining
Motility test

Biochemical test

Indole test
Methyl red test
Voges proskauer test
Citrate utilisation test
Urease test
Catalase test
Oxidase test
Triple sugar iron test
Sugar utilisation test

Maintenance of culture

The selected colony was taken and maintained on nutrient agar slants and stored in refrigerator at 4°C.

Differential media

Blood agar

Overnight culture of the isolate from the nutrient broth was inoculated an aerobically at 37°C for 24 hours and observed the haemolytic properties.

Macconkey agar

Macconkey Agar plates were prepared, after sterilization at 121°C for 15 minutes. Hours were observed for lactose fermentation. A loopful of culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours. After incubation the plates were observed for Lactose fermentation.

Selective media

Synder's agar medium

Overnight broth culture of the isolate from the nutrient broth was inoculated on a sterile Synder's agar medium and incubated aerobically at 37°C for 2 days and observed the result.

Mitis salivarius bacitracin agar

Overnight broth culture of the isolate from the nutrient broth was inoculated on a sterile MSB medium and incubated anaerobically at 37°C for 2 days and observed the result.

MRS agar

MRS Agar plates were prepared, after sterilization at 121°C for 15 minutes. A loopful of culture was streaked on the surface of the agar plates and the plates were incubated anaerobically at 37°C for 2 days and observed the result.

Eosin methylene blue agar

Eosin Methylene blue agar plates were prepared, after sterilization at121°C for 15 minutes. A loopful of culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours.

Thiosulphate citrate bile salts sucrose agar

for 15 minutes. A loopful of culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours.

Cetrimide agar

Cetrimide agar plates were prepared, after sterilization at 121° C for 15 minutes. A loopful of culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours.

PLET agar

PLET agar plates were prepared, after sterilization at 121°C for 15 minutes. A loopful of culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours.

Nutrient agar

Nutrient agar plates were prepared, after sterilization at 121°C for 15 minutes. A loopful of each culture was streaked on the surface of the agar plate and the plates were incubated at 37°C for 24 hours.

Extraction of *Camellia sinensis*

10 g of green tea *(camellia sinensis)* were extracted by using 100ml of hot water and allow for 10 minutes and the extract is filtrated through a 0.2mm membrane filter and the filtrate were used for in vitro susceptibility testing.

Invitro study on antibacterial activity of green tea extract

The sterilized medium was used into a petridish in a uniform thickness and kept a side for solidification. Using sterilised swabs even distribution of lawn culture was prepared using derived bacteria such as Dental caries (*Streptococcus mutans, Lactobacillus acidophillus)* and other pathogenic bacteria *(Escherichia coli ,Pseudomonas aeruginosa, Vibrio cholerae, Bacillus subtilis, Klebsiella pneumonia)* in Muller Hinton Agar (MHA) plates.

All culture plates were allowed to dry for about 5 minutes.

Using the well cutter 5 wells were made on the agar plates.

Using sterilised micropipette green tea extracts were introduced into each of the wells in different volumes such as 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml. After diffusion the plates were incubate at 37°C for 24 hours.

After incubation, the inhibition of growth was analysed and results were observed.

Isolation of active principle

Paper chromatography

Paper chromatography has been one of the most popular chromatographic producers as it is simplest one. Paper chromatography is a form of partition chromatography. Where a mixture of solvents is separated one sheet of paper two types of partition involve in the separation of compounds in paper chromatography viz. Liquid - liquid partition.

Procedure

Whatman filter paper no: 1(or) 3 is used for paper chromatography. Whatman no.1 for smaller quantities and whatman no.3 for larger quantities are used (But the resolution is better in whatman no.1 paper). A line with pencil is drawn about 2 cm from the bottom. The samples are applied as small spot using a capillary tube on the pencil and dried.

A beaker was taken and filled with 10ml of solvent (n- butanol: acetic acid: water 4:1:5). The sheet of paper was inserted into the beaker supported on the sides of beaker with the edge of in contact with solvent. The beaker was covered with a watch glass and left for 10-15

minutes when the solvent reached 80% of the paper, then it was taken out of the beaker and dried.

Thin Layer Chromatography

Preparation of TLC plates

In thin layer chromatography the stationary phase was spread uniformly as a layer (0.25-0.50mm) over the surface of a glass plate.

The stationary phase was prepared as sherry with water at 1:2 and slurry was placed in the applicator and applied evenly to a required thickness using a metal scale.

Activation of adsorbent

After making thin layers on plates, the next step is to remove as completely as possible liquid associated with the thin layer. During the thin layer plate, for 30 minutes, did this. This during made the adsorbent layer active.

Sample application

Capillary tubes were used for transferring the sample solution to the thin layer for qualitative and quantitative work. Solvents used for sample solutions were volatile and nonpolar as possible.

Development tank

The development tank used in paper chromatography was used in TLC for this study. Ascending chromatography is the most common technique in TLC, the plate was placed in a development chamber at an angle of 45°C. The bottom of the chamber is covered up to nearly 1mm by the solvent. Three sides of the tank are lined with solvent impregnated paper while top was covered tightly with the lid. The TLC that development chamber was perfectly saturated with solvent vapour by closing it by a lid.

Solvent system

The plates were developed in a solvent mixture of n-butanol: acetic acid: water. (4:1:5)

Development methods

Ascending technique was used in which the solvents were allowed to rise to the height of about 15-40 minutes. At the end of this time, the plate was marked and the plate was finally allowed to dry.

Detection of components

By spraying the plates with 9:1 ratio of Ethonolic Ferricchloride, presence of flavonoid can be detected.

Flavonoid compounds - Bluish grey colour.
No flavonoid compounds -No such colour.

Results

In the present study samples were collected from dental caries the bacterial isolates were found to be predominant.

Isolation and identification of pathogenic organisms

By inoculating the samples on various selective media, characteristics growth pattern observed in Nutrient Agar, Macconkey Agar, Blood Agar, Synder's agar medium, Mitis Salivarius Bacitracin Agar, MRS Agar, Eosin methylene Blue Agar, Thiosulphate Citrate Bile Salt sucrose agar, Citrimide agar and PLET Agar medium.

On inoculating the organisms in bio-chemical set-ups, the result obtained were tabulated. The results obtained from the various bio - chemical tests confirms that the bacteria we identified to be.

(1)Streptococcus mutans
(2)Lactobacillus acidophilus
(3)Escherichia coli.
(4) Vibrio cholera
(5) Klebsiella pneumonia
(6)Pseudomonas aeuruginosa
(7) Bacillus subtilis

TABLE - I Characterization of *Streptococcus mutans*

S.NO	TEST FOR IDENTIFICATION	RESULTS
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram positive cocci
	Motility	Non-motile
II	**BIOCHEMICAL TESTS**	
	IMVIC	++++
	Catalase	Negative
	Oxidase	Positive
	Urease	Negative
	TSI	Acid butt &alkaline slant
III	**SELECTIVEMEDIUM**	
	Mitis salivarius bacitracin medium	Raised, convex, opaque, pale blue, granular froasted glass appearence
	Synder's agar	Greyish yellow colour colonies
IV	**DIFFERENTIAL MEDIUM**	
	Blood agar medium	Beta haemolysis
	MacConkey Agar	Lactose fermenting pink colour colonies

TABLE -II Characterization of *Lactobacillus acidophillus*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram positive
	Motility	Non-motile
II	**BIOCHEMICAL TEST**	
	IMVIC	- + - +
	Catalase	Negative
	Oxidase	Positive
	Urease	Positive
	TSI	Acid butt and Alkaline slant with gas production.
III	**SELECTIVE MEDIA**	
	MRS (De man Rogosa and sharpe) Agar.	Colony appears as creamy white transparent and smooth round in shape.
IV	**DIFFERENTIAL MEDIA**	
	Blood agar medium	Haemolytic colonies.
	MacConkey Agar	Lactose fermenting pink colour colonies.

TABLE -III Characterization of *Escherichia coli*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram Negative straight rod
	Motility	Motile
II	**BIOCHEMICAL TEST**	
	IMVIC	+ + - -
	Catalase	Positive
	Oxidase	Negative
	Urease	Negative
	TSI	Acid butt only glucose, Alkaline slant with gas production.
III	**GROWTH MEDIA**	
	Nutrient Agar	Large, thick, greyish white, moist, smooth opaque colonies.
IV	**SELECTIVE MEDIA**	
	Eosin Methylene Blue Agar.	Metallic sheen colour colonies.
V	**DIFFERENTIAL MEDIA**	
	Blood agar medium	Heamolytic colonies.
	MacConkey Agar	Lactose fermenting bright pink colour colonies.

TABLE -IV Characterization of *Vibrio cholerae*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram Negative rod
	Motility	Motile
II	**BIOCHEMICAL TEST**	
	IMVIC	+ + + -
	Catalase	Positive
	Oxidase	Negative
	Urease	Negative
	TSI	Acid butt and Alkaline slant but not gas production.
	GROWTH MEDIA	
	Nutrient Agar	Moist, round discs
III	**SELECTIVE MEDIA**	
	TCBS (Thiosulphate citrate bile salt sucrose) agar	Large yellow colonies becomes green on continued incubation.
IV	**DIFFERENTIAL MEDIA**	
	Blood Agar	α - Heamolysis- Green colour on prolonged incubation clear zone is found.
	MacConkey Agar	Non - Lactose fermenting colony

TABLE: V Characterization of *Klebsiella pneumoniae*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram Negative rod shape
	Capsular staining	Positive
	Motility	Non – Motile
II	**BIOCHEMICAL TEST**	
	IMVIC	- - + +
	Catalase	Positive
	Oxidase	Negative
	Urease	Positive
	TSI	Acid butt and Acid slant with gas production and H2S
III	**GROWTH MEDIA**	
	Nutrient Agar	Large mucoid colonies.
IV	**DIFFERENTIAL MEDIUM**	
	Blood Agar	Non haemolytic colonies
	MacConkey Agar	Pink colour mucoid colonies

TABLE: VI Characterization of *Pseudomonas aeuruginosa*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram negative
	Motility	Motile
II	**BIOCHEMICAL TEST**	
	IMVIC	- + - +
	Catalase	Positive
	Oxidase	Positive
	Urease	Positive
	TSI	Acid butt only glucose, Alkaline slant.
III	**ORDENERY MEDIA**	
	Nutrient Agar	Large, opaque, irregular colonies. Iridesent patches with earthy smell.
IV	**SELECTIVE MEDIA**	
	MRS (De man Rogosa and sharpe)	Colony appears as creamy white transparent and smooth round in shape.
V	**DIFFERENTIAL MEDIA**	
	Blood agar medium	Heamolytic colonies.
	MacConkey Agar	Non- Lactose fermenting colonies.

TABLE: VII Characterization of *Bacillus subtilis*

S.NO	TEST FOR IDENTIFICATION	RESULT
I	**STAINING TECHNIQUES**	
	Gram's staining test	Gram Positive
	Motility	Motile having lateral flagella
II	**BIOCHEMICAL TEST**	
	IMVIC	- - + +
	Catalase	Positive
	Oxidase	Negative
	Urease	Negative
	TSI	Acid butt and Acid slant with glucose.
III	**ORDENERY MEDIA**	
	Nutrient Agar	Greyish yellow, large colonies.
VI	**SELECTIVE MEDIA**	
	PLET Agar	Irregular round, dull, opaque, greyish white colour colonies.
V	**DIFFERENTIAL MEDIA**	
	Blood agar medium	Heamolytic colonies.
	MacConkey Agar	Non- Lactose fermenting colonies.

TABLE: VIII ANTI BACTERIAL ACTIVITY OF CAMELLIA SINENSIS

S.NO	TEST ORGANISMS	ZONE OF INHIBITION(in mm)				
		0.2µl	**0.4µl**	**0.6µl**	**0.8µl**	**1µl**
1	*Streptococcus mutans*	3mm	4mm	8mm	9mm	11mm
2	*Lactobacillus acidophilus*	2mm	3mm	5mm	6mm	7mm
3	*Escherichia coli*	2mm	3mm	5mm	5mm	7mm
4	*Vibrio cholerae*	2mm	4mm	6mm	8mm	10mm
5	*Klebsiella pneumoniae*	3mm	5mm	7mm	8mm	10mm
6	*Pseudomonas aeuruginosa*	2mm	4mm	6mm	7mm	9mm
7	*Bacillus subtilis*	2mm	4mm	6mm	7mm	8mm

Determination of antibacterial activity

The results of antibacterial activity of green tea extracts studied by the agar well diffusion method revealed the efficiency of the herb against a wide range of bacteria like *Streptococcus mutans, Lactobacillus acidophillus, Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae, Pseudomonas aeuruginosae* and *Bacillus subtilis.*

The zone of inhibition were tabulated in table (I, II, III, IV, V, VI, VII, VIII, IX respectively).

Discussion

Diseases are very common and increasing day by day, which makes the man to search for new and high effective medicines as therapeutic agents.

In this study, the invitro activity of the green tea (*Camellia sinensis) extract against Streptococcus mutans, Lactobacillus acidophilus , Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae, Pseudomonas aeuruginosa* and *Bacillus subtilis.*

Their activity was studied using agar well diffusion method in Muller Hinton Agar.

Although the differences was observed for that zone of inhibition against all the isolates.

Trial was made to separate the active principle using paper and Thin layer chromatography (TLC). The effectiveness of active principle was also studied and compared with the previous one.

It can be concluded that green tea (*Camellia sinensis*) leaves extract can be used as complementary medicine in trealing can be used as complementary medicine in trealing disease caused by multi drug resistant strains of *Streptococcus mutans* and other pathogens.

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.

Conclusion

Dental caries in a bacterial disease that usually can be successfully prevented or controlled . Green tea were chosen to combat the disease. Intrestingly, the flavanoids in green tea may actually promote dental health by helping to decrease plaque formation.

Thus from our findings , it was concluded that the active principles responsible for an antimicrobial activity against the tested microorganisms should be isolated and identified to develop a new lead of therapeutic interest.

From this we conclude that the Flavanoids present in this green tea contribute the antimicrobial activity. So instead of using synthetic drugs, it is better to consume of green tea against dental caries and other pathogens.

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