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Antibacterial activity of green tea (Camellia sinensis) extracts against various bacteria isolated from environmental sources

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Abstract
Tea is cultivated in many countries of the world. India is largest tea (black tea) producer in world followed by Japan (green tea) and China. In the present study *Camellia assamica* (Green tea) leaves extracts were tested for antibacterial activity against various bacteria isolated from environmental sources. Different bacteria were isolated from sewage samples collected from different places at Solan Himachal Pradesh. Isolated bacteria were identified by Gram staining and biochemical tests. A total of six bacteria were identified at Department of Microbiology at SILB Solan (H.P) Green tea leaves extracts were tested for antibacterial activity. Tea leaves were collected from Palampur, Himachal Pradesh. Three different extracts were prepared by using standardized protocols. All the extracts were tested for antibacterial activity by disc diffusion method. Antibacterial assay was performed at 10µl, 20µl, and 30µl concentrations. Significant antibacterial activity was reported for all extracts with results. Aqueous extracts has shown little antibacterial activity against six bacteria isolated. Maximum antibacterial activity was found in methanolic extracts. Our study reflects the chemotherapeutic use of green tea.

Keywords: Tea, Antibacterial activity, Extracts

INTRODUCTION

Tea is produced from leaves and non-developed buds of a tea shrub having two botanical varieties: *Camellia assamica* (L) and *Camellia sinensis* (L). India is tea (black tea) producer in world followed by Japan (green tea) and China (different sorts of tea). Depending on tea manufacturing method tea is divided mainly into: green and the black one [1].Microorganism like *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the common pathogens of human infection. *Staphylococcus aureus* is an opportunistic pathogen of human skin. *Pseudomonas aeruginosa* is a pathogen associated with pyogenic infection and urinary tract infection. These microorganisms are highly pathogenic and rate of infection caused by these microorganisms are considerably increasing in recent years. Hence the use of plant products has been increasing worldwide, to minimize side effects [2]. Green tea is generally safe, non toxic and having no side effects after use. However over consumption may cause in treatment infection, human disease because to associated to lower side effects [3]. The present study is designed to check antibacterial activity of tea leaves extracts against various bacteria isolated from environmental samples. This will helps us to see the antibacterial activity of tea against pathogenic bacteria and to design chemotherapy against the disease cause by them.

MATERIALS AND METHODS

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Collection of samples

Samples were collected from different places at Solan District of Himachal Pradesh. Moist soil was taken in sterile polythene bags and processed immediately in the laboratory for bacterial contamination

Isolation of bacteria from soil

Isolation of microorganism from soil was done by serial dilution agar plate method as described by Dubey RC.*et al*., 2006. A known amount (1g) of soil was suspended into 9ml of sterile distilled water in a sterile test tube. 1ml suspension was transferred to other test tubes containing 9ml sterile distilled water. All tubes get serially diluted with soil suspension at concentration of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8}. Aliquot of 1ml suspension was poured on the surface of plate containing nutrient agar [4].

Isolation of bacteria from water by MPN method

An estimation of the coliform bacilli in water supply was done as per methodology described by Dubey RC.*et al*., 2006. Added varying quantities of water sample (0.1ml-10ml) in test tubes containing MacConkey broth with Durham's tubes. All the test tubes were incubated at 37°C for 24 hrs. Observed test tubes for production of acid and gas. This test stated the smallest quantity of water containing coliform bacilli expressed the degree of contamination with these organisms. Double strength and single strength MacConkey broth containing inverted Durham's tube were used for indication of gas production.10ml volume of water were inoculated to each to 10ml double strength MacConkey broth. 1ml of water were inoculated to each sample to 5ml single strength MacConkey broth. 0.1ml of water were inoculated to each sample

5ml single strength MacConkey broth. The inoculated tubes were incubated at 37°C for 48hrs. The presumptive coliform count per 100ml of sample was determined from tubes showing acid and gas production using standard McCardy table [4].

Isolation of bacteria from air sample

Isolation of bacteria from air was done by settle plate method as per methodology described by Dubey RC *et al*., 2006. Petri dishes containing nutrient agar medium were kept in open air outside the microbiology laboratory at SILB, Solan (H.P). All the plates were exposed in removing lids for 10 min. The plates are then incubated at 37°C for 48hrs. Plates were examined for appearance of microbial colonies [4].

Identification of bacteria

Isolated bacterial colonies were identified by using appropriate microscopic and macroscopic as per methodology described by Aneja K R., 2003. The colony morphology and biochemical characteristics of the bacterial isolates were studied carefully. Gram staining was performed by preparing a thin homogenous bacterial smear on a clean glass slide from the bacterial culture grown on nutrient agar, air-dried and heat-fixed. The smear was stained with crystal violet for 1 min, washed with distilled water and flooded with Gram's iodine solution for 1 min. The slide was again washed with water and decolorized with absolute alcohol until no violet colour came off. The smear was counter stained with safranine for 30 sec., washed with water, blot-dried and observed under Microscope using oil immersion objective [5].

Biochemical tests

Isolated bacteria were further identified by various biochemical tests. Catalase test was performed by taking a drop of 3% hydrogen peroxide was added to 48 hr old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The appearance of air bubble indicated catalase activity. Coagulase test was performed by placing a drop of normal saline on a clear glass slide. Gently added the pure bacterial colonies. Mix and adding one drop of plasma. Further mixed by tilting the slide. Observed for immediate formation of granular clumps. For Oxidase test old bacterial culture (48 hrs.) was rubbed to oxidase disc soaked with sterilized water placed over a clean slide. The appearance of dark purple colour within 10 to 20 second indicated oxidase activity**.** Indole test was done by inoculating Peptone broth with 48 hr old bacterial cultures grown on N.A and incubated at 37°C for 24 hr. To the test tubes were added 0.5 ml Kovac's reagent and shaken gently. Indole production was indicated by development of deep red colour on the top of tubes. Methyl red test was performed by inoculating MR-VP medium in duplicates with 48 h old bacterial cultures grown on N.A and incubated for 24 h at 37°C.Sterile test tubes were added with 3-4 drops of methyl-red reagent and incubated for 24 h at 37 °C. Positive test was indicated by a change in the medium colour from yellow to red. Voges proskauer test was performed by inoculating test tubes with culture and added 3 ml of 5% α-Naphthol in absolute ethanol and 1 ml of 40% KOH. Shaken well to ensure aeration. Positive test was indicated by the appearance of strong red colour which changed to crimson in about 30 min. Citrate utilization test was done with Simmon's citrate medium by inoculating with 48 h old bacterial cultures grown on N.A. and incubated at 37°C for 24-48 h. Citrate utilization was indicated by a change in green colour of the medium to blue For nitrate reduction test nitrate broth was inoculated with 48 hr old bacterial cultures was incubated at 37°C for 48 h. To the tubes were added 6-8 drops of sulphanilic acid and 6-8 drops of α-naphthylamine. Positive test was indicated by the appearance of pink to red colour. Urease test was performed by inoculating Christensen's medium with 48 h old bacterial cultures was incubated at 37°C for 24-48 h. Positive test was indicated by a change in the medium colour from orange to pink. Carbohydrate fermentation test was done by taking pure bacterial cultures and inoculating into a broth containing the test sugar and incubated at 37°C for 24-48 hr. A bright yellow color indicated the production of enough acidic fermentation products to drop the medium pH to 6.9 or less. Gas production was determined with Durham tube, a small inverted vial filled with the carbohydrate fermentation broth. If gas was produced, it was trapped at the top of the Durham tube and appears as a bubble. Motility test was checked using semisolid medium inoculated with 48 hr old bacterial cultures with the help of straight wire at about 8-10mm, deep once only and incubated overnight at 37°C. Positive test was indicated diffuse growth or swarm extends as zone turbidity from the stab line [4, 5].

Collection of green tea leaves

The green tea leaves were collected from Palampur, Himachal Pradesh. Various extracts of leaves were prepared in different solvents and tested for antibacterial activity against bacteria isolated from environmental samples.

Preparation of Plant Extracts

Fresh plant leaves were washed under running tap water and ethanol (30-40%). The leaves were cut into pieces and grind into powdery form using pestle and mortar and shade dried. The powder was stored in air tight bottle [6]. Aqueous extract was prepared by mixing 15.0gm of dry powder of plant leaves with 100ml. of sterile distilled water in a round bottom flask with occasional shaking. The extract was then filtered through a muslin cloth for coarse residue and finally filtered through Whatman No.1 filter paper and stored in an airtight container at 4^{o}C until use [7]. Ethanolic extract was prepared by mixing 15.0gm of dry powder of plant leaves with 100ml. of 95% ethanol and kept at room temperature for 5 days in a round bottom flask with occasional shaking. After a five days period, the extract was filtered through a muslin cloth for coarse residue and finally filtration was done through whatman No.1 filter paper and stored in airtight bottle at 4^{o}C until use [8]. The air-dried and powdered plant material (12gm of each) was extracted with 50 ml methanol and kept on a rotary shaker for 24hr. filtered and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and stored in bottle at 4°C until use [9].

Preparation of Inoculum

Inoculum was prepared by using isolated bacterial colonies. The bacterial colonies were inoculated in nutrient broth. All bacterial cultures were maintained by weekly transferring into nutrient broth and storing in sterile test tubes at low temperature [10]

Antibacterial Susceptibility testing

Antibacterial Susceptibility testing was done by using Disc diffusion method. To check antibacterial activity of tea leaves extracts, sterile Muller Hinton agar plates were used. Muller Hinton agar was prepared and autoclaving at 121°C for 15 minutes. Poured the medium in sterile Petri plates under aseptic conditions. Then allowed the media to solidify at room temperature and stored at 4°C until use. After solidification, 0.2 ml of inoculum suspension was inoculated with micropipette and

spread uniformly with sterile glass spreader over agar surface, the inoculum was allowed to dry for 5 minutes. Different concentrations of tea leaves extracts (10, 20, 30µl) were loaded on sterile individual discs. The loaded discs were placed on the surface of medium and the extract was allowed to diffuse at least for 5 minutes. The plates were kept for incubation at 37°C for 24-48 hours. Methanol, Ethanol and distilled water were used as negative control. Plates were observed after 24-48 hrs incubation for appearance of zones of inhibition around the discs. Antibacterial activity was evaluated by measuring diameter of zones of inhibition (in millimeters) of bacterial growth. [11, 2, 8].

RESULTS AND DISCUSSION

Six bacteria were isolated during the study. All bacterial isolates were identified by Gram staining and biochemical testing. Six different bacteria identified were *Staphylococcus, Streptococcus, Pseudomonas, E.coli, Bacillus,* and *Proteus*. Antibacterial activities of extracts were checked by disc diffusion method. The concentrations of green leaves aqueous, ethanolic and methanolic extract used were 10, 20, 30µl. In methanolic extracts greater zone of inhibition was found than ethanolic extracts. Maximum activity of methanolic extracts were found against *Staphylococcus, Streptococci* and *Bacillus,* however, all the extracts were least active against *Pseudomonas* and *Proteus.* Tea is known to possess antibacterial activity against a number of bacteria. Antimicrobial property in tea is due to presence of polyphenols. Specific antioxidant polyphenols, called catechins, play an important role in green tea's inhibition of bacterial growth. Several significant catechins include: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) [12]. Antimicrobial activities of tea extracts are very selective. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on the bacterial species so that they may be either growth inhibitory or stimulatory [13]. Green tea leaves extracts tested in current study have also shown varying activities against environmental bacteria. The active substance found in tea is supposed to reduce growth and development of microorganisms. The highest antimicrobial activity of tea is due to presence of catechins and polyphones which damages bacterial cell membrane. The green sorts of tea have shown higher antimicrobial activity than the black ones. This difference in results is probably due to presence of different contents of active substance in these tea sorts.[14]. The daily consumption of green tea can kill Gram positive *staphylococcus aureus* including many other harmful bacteria. Tea constituents also possess antibacterial, antiviral action, anticarcinogenic and anti mutagenic properties. [15]. However, the antimicrobial activity of plant extracts also depends upon presence of different secondary metabolite like hydroxyl group on the active constituents. The biologically active compounds of plants extracts are considered as antimicrobial

Table 1.Cultural characteristics and Gram's staining results of various bacteria isolated from environmental sources.

Sample	Isolate no.	Cultural characteristics				Gram's staining
		Nutrient agar	MacConkey Agar	Blood agar	Mannitol salt agar	
Soil	I	Colonies were large, circular convex, smooth, shiny, and opaque.	Colonies were small, pink due lactose fermentation.	Colonies were 2-4mm in diameter, circular, smooth, shiny and hemolytic.	Yellow coloured colonies	Gram positive cocci
	II	Colonies were smooth , translucent , large 2-4 diameter	Non lactose fermenting	Colonies 2-3mm, large, opaque, hemolytic.	No growth	Gram negative bacilli
	III	Colonies spread or swarm over the surface of the medium	Non lactose fermenting	No growth	No growth	Gram negative bacilli
	IV	Colonies were Circular, thin, even	Lactose fermenting	Small 0.5-1.0mm diameter, circular, convex colonies, β-hemolytic.	No growth	Gram positive Cocci
Water	I	Colonies were round 2-3 mm in diameter, greyish white.	Colonies were Bright pink due to lactose fermentation	Colonies were 2-3mm in diameter Hemolytic	No growth	Gram negative bacilli
Air	I	Colonies were round 2-3 mm in diameter, greyish white	Colonies were Bright pink due to lactose fermentation	Colonies were Non-hemolytic.	No growth	Gram positive bacilli

agents, because of their ability to bind with adhesions and to disturb the availability of inhibitory effects of aqueous, methanolic and ethanolic extracts of tea plant were found at concentration of 10, 20, 30µl. extracts Minimum antibacterial activities of the aqueous were reported against most of bacteria isolated in the study. Methanolic and ethanolic extracts have shown significant zones of inhibition against bacterial were isolated in the study from various environmental samples. The soil is composed of five major components such as inorganic matter, organic matter, soil air soil water and soil They cause many clinical manifestations such as urinary infection, diarrhea, abdominal pain, pyogenic infection. septicemia, sepsis. Green tea possesses antimicrobial activity against a variety of pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia, and skin infection [17]. The results noticed in the study showed that the extrac

Table 2. Biochemical test for identification of bacteria isolated from environmental sources.

Biochemical tests	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Catalase	+	+	+	+	+	-
Oxidase	-	-	+	-	-	-
Indole	-	+	-	-	-	-
M R	-	+	-	-	+	-
VP	+	-	-	-	-	-
Citrate	+	-	+	+	-	-
Lactose	-	AG	-	-	A	A
Glucose	A	AG	A	A	A	A
Sucrose	A	AG	-	A	A	A
Mannitol	A	AG	-	A	A	-
Urease	+	-	+	-	-	-
Motility	+	+	+	+	-	-
Nitrate reduction	+	+	+	+	+	-
Coagulase	-	-	-	-	+	-
Results	*Proteus sp*	*E. coli*	*P. aeruginosa*	*Bacillus sp*	*S. aureus*	*Streptococcus*

Table 3. Zone of inhibition in (mm) for ethanolic extracts of *Camellia sinesis* against various bacteria isolated from environmental sources.

Bacteria	Size of zones of inhibition (mm)			Control diameter (mm)
	10 µl	20 µl	30 µl	
Staphylococcus aureus	8	9	12	-
Streptococcus	9	10	13	-
Pseudomonas aeruginosa	7	9	10	-
Bacillus	10	11	12	-
E.coli	10	12	13	-
Proteus	8	9	11	-

obtained from tea plants had shown strong antibacterial activity and can be serve as a very good source for the invention of new therapeutic agents to kill pathogenic bacteria isolated from environmental samples.

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REFERENCES

[1] Islam G.M.R, Iqbal M, Quddus K.G. and Ali M. Y. 2005. Present status and future needs of tea industry in Bangladesh. *Proc. Pakistan Acad. Sci.* 42(4); 305-315

[2] Padmini E, Valarmathi A. and Rani Usha M. 2011. Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinesis. Asian J. Exp. Biol. Sci*. 4. pp 772-78.

[3] Der M. The Review of Natural Products.1999; 20:14-15.

[4] Dubey R.C. The Practical book of Microbiology. 2th ed. 2007: 37-39.

[5] Aneja K.R. Experiments In Microbiology, Biotechnology and Plant pathology 4ed. 2003. *NAI.*

[6] Mohan M.C.H., Rao S.M. and Kumari P. 2009. Antimicrobial Activity of Selected Indian Medicinal Plants. *Jr. of Microbiol. Biotech. Env. Sc*, 11 (2). pp 355-360.

[7] Nayak A, Nayak R.N, G Soumya B, Bhatt K. and Kudalkar M. 2011. Evaluation of antibacterial and anticandidal efficacy of aqueous and alcoholic extracts of Neem (Azadirachia India) and *in vitro* study. *IJPAP.*1; 230-235.

[8] Yildirim A, Mavi A, Oktay M, Kara A.A, Algur OF, Bilaloglu V.2000. Comparison of antioxidant and antimicrobial activities of Tilia (Tilia Argentea Desf Ex DC), Sage (Salvia Triloba L.), and Black Tea (Camellia Sinensis) Extracts. *J Agric Food Chem.* 48:5030–503.

[9] Parekh, J. and Chanda S.2008. In vitro antifungal activity of methanol extracts of some Indian medicinal against pathogenic yeasts ant molds. *Afr. J. of Biotech.*7 (23):4349-4353.

[10] Shihabudeen M.S., D. Hans Priscilla and Thirumurugan K. 2010. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. *International Journal of Pharma Sciences and Research*.10. pp 430-434.

[11] Opara A.A. and Ansa M.A. 1993. The antibacterial activity of tea and coffee on selected organisms. *J. Med. Lab. Sci.* 3: 45-48.

[12] Isogai E, Isogai, H, Hirose K, Hayashi S and Oguma K. 2001. *In vivo* synergy between green tea extract and levofloxacin against enterohemorrhagic *Escherichia coli* 0157 infection. *Curr Microbiol. 42:* 248-51.

[13] Tiwari, R.P, Bharti S.K., Kaur H.D., Dikshit R.P. and Hoondal G.S.2005. Synergistic antimicrobial activity of tea and antibiotics. *J. Med. Res*. 122: 80-84.

[14] Wang, Z.Y., M.T. Huang, R. Chang, W. Ma and Ferraro T. 1992. Inhibitory effect of green tea on the growth of established skin papillomas in mice. *Cancer Res*. 52: 6657-6665.

[15] Toda M, Okubo S, Ikigai H, Suzuki T and Shimamura T. 1991. The productive activity of tea against infection by *Vibro cholerae*. J. Appl. Bacterial. 70: 109-112.

[16] Branter A., Males Z. and Antolic A. 1996. Antimicrobial activity of paliurus spina-christi Mill. (christis thorn). 52. pp119-122.

[17] You S. 1999. Study on feasibility of Chinese green tea polyphones for preventing dental caries. 28:197-199.