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ANTIBACTERIAL POTENTIAL OF GREEN TEA (*CAMELLIA SINENSIS)* AGAINST URINARY TRACT INFECTION

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ABSTRACT

Urinary tract infection is the most common disease in females and males, which is a big threat of kidney failure. In the present study *Camellia sinensis* (Green tea) leaves extracts were tested against various bacteria isolated from urine samples collected from various hospital in Thanjavur. Isolated bacteria were identified by gram staining and biochemical tests. Tea leaves were collected from Ooty. The natural and commercial green tea extracts were prepared by using standardized protocols. The antibacterial activity of two extracts were tested by disc diffusion method. Significant zone of inhibition was reported in all bacterial isolates of UTI infection. All bacterial pathogens isolated from UTI samples showed maximum growth suppression in natural green tea extracts than the commercial green tea extracts. The methanolic extracts of *Camellia sinensis* showed the antibacterial activity against the bacteria *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis,* and *Proteus mirabilis* which cause UTI. The highest susceptibility was observed in Proteus mirabilis and Staphylococcus aureus in both natural and commercial green tea extracts.

KEYWORDS: Bacterial isolates of UTI infection, Green tea extract (*Camellia sinensis)*, antibacterial activity.

INTRODUCTION

Urinary tract infection (UTI) are the most common type of infection world wide can have resulted in billions of dollars in medical care costs.[1,2] UTI that affects part of the urinary tract. When it affects the lower urinary tract it is known as bladder infection and when it affects the upper urinary tract it is known as kidney infection. The urinary tract infection is caused by several bacteria the most important cause of 80-90% of all UTI is due to *Staphylococcus aures, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcos facalis, Proteus mirabilis and Escherichia coli.* A numbers of bacterial isolates have been collected from urine samples of patients with UTI that are resistant to antimicrobial agents commonly uses to treat UTI.[3,4] The challenges have been receiving growing interest to find alternative antimicrobial agents from plant extracts that need to be developed and use to control multidrug resistant bacteria.[5,6] The Green tea is obtained from the tea plant *Camellia sinensis* belongs to the family Theaceae. It is one of the most popular beverages in the world and has been reported to have antimicrobial effects against various pathogenic bacteria.[7] Green tea is generally safe, non toxic and having no side effects after use. However over consumption may cause in treatment infection. This plant leaves are commonly used traditionally as a natural antimicrobial agents for various drug resistant microorganism for thousands of years by many cultures of peoples in the past decade.[9] Tea constituents also possess antibacterial, antiviral action, anticarcinogenic and anti mutagenic properties.[17] Green tea possesses antimicrobial activity against a variety of pathogenic bacteria that cause cystitis, diarrhea, dental caries, skin infection.[18] The present study is designed to check antibacterial activity of natural tea leaves extracts and commercial extracts against various bacterial isolates of urinary tract infection urine samples. This will helps us to see the antibacterial activity of tea against urinary tract infection and to design a chemotherthapy against the disease caused by them.

MATERIALS AND METHODS

Collection of Urine Samples

The Urine samples of UTI patients were collected from various hospital in Thanjavur. The urine samples were collected in sterile, dry, wide, necked, leak proof container. About 25 ml of sample was taken for analysis. Clean catch method is used to collect mid-stream urine. The collected urine samples was stored in refrigerated at 4°C.

Isolation of Bacteria from UTI Affected Urine Samples

Bacteria were isolated from the collected urine samples using Nutrient agar by streak plate method. After the streaking the bacterial plates were incubated at 37˚C for 24-48 hrs. After incubation cultural characteristics and colony morphology were observed. These colonies were sub cultured and stored in refrigerator for further study.

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.[10]

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 αnaphthylamine. 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.[11]

Collection of test plant (*Camellia sinensis)*

The fresh leaves of *Camellia sinensis* (leaves) were collected from the dense tea state garden, Ooty, Tamilnadu. South india The plants were identified based on the morphological plant characteristics. Commercial lipton tea packets were purchased from local market at Thanjavur.

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.[12] 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˚ C until use.[13] 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 4oC until use.[14] 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.[15]

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.[12]

Analysis of Antibacterial activity

The antibacterial activity of the tea extract was assessed by Kirby Bauer disc diffusion technique (Bauer *et al.*, 1966). The sterile Muller-Hinten agar plates were prepared. The isolated UTI bacterial strains like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Proteus mirabilis* were spreaded over the Muller-Hinten agar plates by using separate sterile cotton buds. After the microbial lawn preparation, the extracts of fermented tea disc were placed on the organism inoculated plates with equal distance control and the natural and commercial green tea (*Camellia sinensis.)* All bacterial plates were incubated at 37˚C for 24 hrs. The plates were observed for inhibitory zones by measure of Antibiotic zone scale.

RESULT AND DISSCUSION

Camellia sinensis is the species of plant leaves and leaf buds are used to produce Chinese tea.[19] The presence of phytochemical namely alkaloids, Flavonoids, Steroids, gallic tannins, catecholic tannins plays the vital role in the plant defence mechanisms. The active substance found in tea is supposed to reduce growth and development of microorganisms.[20] The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damage bacterial cell membrane.[21,22] They also serve in plant defence mechanisms to counteract reactive oxygen species in order to survive and prevent molecular damage and caused by microorganisms, insects, and herbivors.[23] In the present study Six different bacterial isolates such as Escherichia coli, Klebsiella pneumoniae, Pseudomonasaeruginosa, Staphylococcs aureus, Enterococcus faecalis and Proteus mirabilis. Were identified from the urine sample of UTI patients. Antibacterial activities of Camellia sinensis extracts were checked by disc diffusion method. The concentration of natural and commercial green tea leaves were noted. In this work different concentration of leaf extracts were used against six different pathogenic bacteria causing UTI infection. Among the six.

Table 1: Biochemical Test for Bacterial Identification.

S. No.	Biochemial test	UTIB 1	UTIB 2	UTIB 3	UTIB4	UTIB 5	UTIB 6
1	Gram staining	-	-	-	+	-	-
2	Motility	+	-	+	-	+	+
3	Shape	Rod	Rod	Rod	Cocci	Rod	Rod
4	Indole	+	-	-	-	-	-
5	Methyl red	+	-	-	+	-	-
6	Vp	-	+	-	+	-	-
7	Citrate	-	+	+	-	+	+
8	Urease	-	+	-	-	-	-
9	TSI	**A**	**A/A**	**A/K**	**K/A**	-	-
10	Catalase	+	+	-	-	+	+
11	Oxidese	-	-	+	-	+	+
12	Lactose	**AG**	**AG**	**AG**	**A**	-	-
13	Dextrose	**A**	**AG**	**AG**	**A**	**AG**	**AG**
14	Sucrose	**A**	**AG**	**AG**	**A**	-	-
	Result	*E.coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococccus aureus*	*Enterococcus faecalis*	*Proteus mirabilis*

UTIB- Urinary Tract Infection Bacteria, (+) Poitive, (-) Negtive, A/A – Acid slant and acid butt
K/K – Alkaline slant and alkaline butt, K/A – Alkaline slant Acid butt

Table 2: Antibacterial activity of *Camellia sinensis* against bacterial isolates of UTI.

S. No.	Bacteria	Zone of Inhibition (mm in diameter)	
		Natural Extract(Leaves)	Commercial Extract(tea powder)
1	Escherichia coli	24±1.4	22±1.0
2	Klebsiella pneumoniae	22±0.8	21±1.5
3	Pseudomonas aeruginosa	21±1.5	20±1.2
4	Staphylococcus aureus	25±1.0	23±1.8
5	Enterococcus faecalis	23±1.0	22±1.0
6	Proteus mirabilis	25±1.5	24±1.0

Values are expressed in Mean ± Standard deviation; n=3

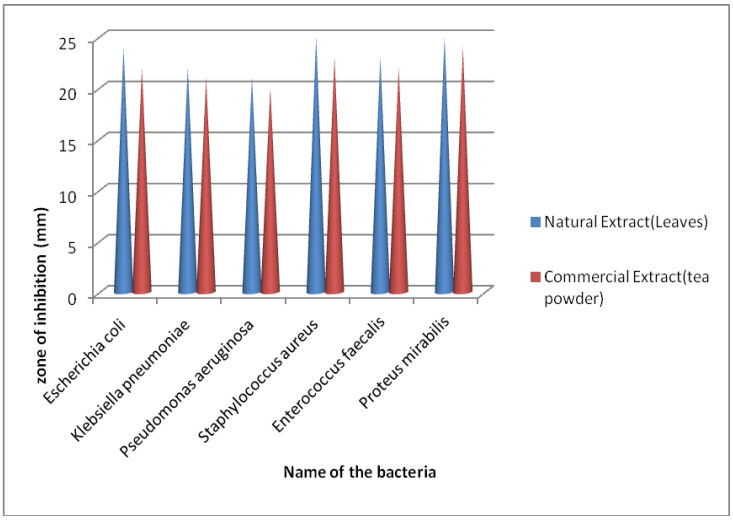

Fig 1: Antibacterial activity of *Camellia sinensis* against bacterial isolates of UTI.

Bacterial species, highest zone of inhibition was observed against *Proteus mirabilis* followed by *Staphylococcus aureus* and *E.coli*. maximum zone of inhibition was observed in highest concentration of both natural and commercial tea extracts. However least zone of inhibition was observed against Pseudomonas in both natural and commercial tea extracts. Antimicrobial activities of tea extracts are very selective. The result showed in Table-2. 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.[17]

Green tea leaves extracts tested in current study have also shown varying activities against UTI 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 specific antioxidant catechins and polyphenols which damages bacterial cell membrane.[12] 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.[18] The daily consumption green tea can kill Gram positive *staphylococcus aureus* including many other harmful bacteria. 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 agents, because of their ability to bind with adhesions and to disturb the availability of inhibitory of extracts of tea plant were found at the concentration. The results noticed in the study showed that the extract 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 urinary tract infection.

CONCLUSION

Camellia Sinensis leaves are having the duel benefits as medicinal values and food value. In this study we found that leaf extracts were found to be potential antibacterial agents against the various bacteria causing UTI. Thus *camellia sinensis* leaves can be used an alternative medicine against the bacterial infection.

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