IJPSR (2016), Vol. 7, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 March, 2016; received in revised form, 12 May, 2016; accepted, 30 June, 2016; published 01 August, 2016

EFFECT OF *CAMELLIA SINENSIS* EXTRACTS ON GROWTH OF VIRULENT GRAM NEGATIVE UROPATHOGENS

Vishaka Labar, Rinzi Bhutia and Y.V.S. Annapurna*

Department of Microbiology, St. Francis College for Women, Begumpet, Hyderabad, Telangana India

Keywords:

Green tea, Black tea, *E.coli, Klebsiella spp,* Urinary tract infections

Correspondence to Author:
Y. V. S. Annapurna

Department of Microbiology,
St. Francis College for Women,
Begumpet, Hyderabad, Telangana India

E-mail: annapurna2103@yahoo.com

ABSTRACT: Urinary tract infections causing bacteria play an important role in expression and spread of drug resistance among bacteria in the hospitals and in community. Because of rapid increase in antibiotic resistance, alternative antimicrobial agents are needed to be developed and employed to control multi-drug resistant bacteria. The present study focuses on antibacterial activity of Green and Black tea (*Camellia sinensis*) extracts against the virulent uropathogens. Twelve bacterial isolates from urinary tract infections were studied for their ability to produce virulence phenotypes like hemolysin, biofilms, gelatinases, hydrophobic nature and penicillinases. Antibacterial susceptibility testing was done. Out of the total isolates tested 67% of *E.coli* and 60% of *Klebsiella spp* were multi drug resistant Penicillinase production as tested by filter paper iodometric method and biofilm production on Congo red plates were the virulence features exhibited by maximum number of *E.coli* and *Klebsiella Spp.* Among the aqueous, methanol and ethanol extracts tested for antibacterial activity, methanol extracts showed maximum dimeter of zone of inhibition. Minimum inhibitory concentration for ethanol and aqueous extracts was 0.8mg /ml whereas that for methanol extracts was 0.4 mg/ml with both Green and black tea crude extracts.

INTRODUCTION: First cultivated in China nearly 5,000 years ago, tea is consumed in greater quantity worldwide than any other beverage except water. The beverage is made from the leaves of the plant *Camellia sinensis*, (family Theaceae), which is native to India and perhaps parts of China and Japan. Black, green and oolong teas are all made from this plant but differ in their methods of preparation.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(8).3373-78

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (8).3373-78

All tea leaves are withered, rolled and heated, but black teas go through an oxidative process known as fermentation before the final heating process. Oolong teas are partially fermented [1]. The secondary metabolites found in the tea plant and the unique combinations of these secondary metabolites are responsible for the popularity of this crop as source of consumed soft beverage. Components of the tea such as epigallocatechin gallate, epicatechin gallate, epicatechin act as effective scavengers of free radicals [2].

It has been shown that natural antioxidants in tea possess stronger antioxidative activity than synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and dl-

α-tocopherol interestingly, tea polyphenols are much less toxic than that of butylated hydroxyanisole, butylated hydroxytoluene and dl-α-tocopherol [2]. India is a major tea (black tea) producer in world followed by Japan (green tea) and China [3] (different sorts of tea). Green tea is generally safe, non toxic and having no side effects after use. Catechins from tea extracts have been observed to be cytotoxic to microbial pathogens and therefore may be useful as antibacterial agents.

There is growing evidence that indicates that the catechin components of green tea are responsible for the observed antibacterial activity, and that Epigallocatechin, Epigallocatechin gallate and Epicatechin gallate constitute the most important antibacterial agents. Black tea which is a major source of phenolics, including theaflavins and thearubigin [4] has also been shown to have antibacterial properties both *in vivo* and *in vitro* [5].

The development of antibiotic resistance in bacteria is a major issue in the prevention of infectious diseases. Currently the spread of multi-drug resistant bacteria is not only through nosocomial infections, but also occur in the community. Predominant multi-resistant bacteria that causes the infection such as *P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) have been found in several hospitals [5]. As the bacteria that cause the infection was resistant to first-line antibiotics, treatment options are usually replaced with a second or third choice of antibiotics, which are generally much more expensive.

Therefore, alternative antimicrobial agents are needed to be developed and employed to control multi-drug resistant bacteria. To face this challenge, there has been growing interests to find antimicrobial compounds from medicinal plant extracts as an alternative approach to discover new antimicrobial compounds. The antimicrobial activities of some herbal medicines against different pathogens have been reported from different countries. *Camellia sinensis* (*C. sinensis*), has been reported to have antimicrobial activities against various pathogenic bacteria [6]. Urinary tract infections play a significant role in transmission of drug resistance, as these infections present in asymptomatic form and are, caused by opportunistic pathogens of intestinal tract. The present study was designed to check antibacterial activity of tea extracts against bacteria isolated from urine samples of patients suffering with UTI. The isolates were also checked for expression of virulence features. This will helps us to see the antibacterial activity of tea against pathogenic bacteria and to design chemotherapy against the disease caused by them.

MATERIAL AND METHODS:

Extract preparation:

The samples, Green tea and Black tea, for the present study were collected from Makaibari tea garden and Matelli, Fulbari tea garden located in Darjeeling district, West Bengal. Both the tea samples were washed and dried in the sun. 10g of each tea sample were then mixed with 30 ml of distilled water in a round bottom flask and boiled. It was then filtered through Whatman No.1 filter paper and filtrate was stored at 4^oC in screw capped bottle until use as crude aqueous extract.

For alcoholic extract ethanol and methanol, 3g of green tea and black tea were mixed with 15ml of 95% ethanol and 95% methanol separately. They were allowed to stand for two days and filtrate was evaporated at room temperature (37^o C). It was reconstituted in DMSO (10mg in 100ml) stored at 4^oC to be used as alcoholic extracts. Qualitative phytochemical analyses [7] were performed on tea extracts to detect various phytochemical constituents such as saponins, alkaloid, phenolics, flavonoid, terpenoid, tannins and proteins.

Phytochemical analysis:

About 2g of the dried extract was weighed and dissolved in distilled water. The solution obtained was then subjected to various phytochemical screening to identify the components present in the extract using qualitative chemical tests for saponins, alkaloid, phenolics, flavonoid, terpenoid, tannins and proteins.

Isolation and identification of Uropathogens:

Twelve microorganisms used in this study were isolated from patients suffering with Urinary Tract Infections, obtained from the Central Railway

Hospital, Mettuguda, Hyderabad. The organisms were inoculated on Nutrient Agar (simple media), Mac Conkey Agar (differential media), Eosin-methylene blue medium and cultured at 37^{o}C for 24 hrs. Microorganisms were initially identified using the colony characters on the above medias and Gram staining, Stock cultures were maintained at 4^{o}C on nutrient agar. The bacterial strains were identified by performing IMViC tests [8], and extended biochemical reactions like catalase, oxidase, H_2S production, urease test [9].

Evaluation of virulence phenotypes:

The ability of these organisms to produce virulence phenotypes like hemolysin, biofilms, gelatinase, hydrophobic nature and penicillinase were studied. Hemolysin production among the isolates was detected by growing on Blood agar plates and observing for hemolysis [10]. Ability of the organisms to produce biofilms was detected by growing the organisms on congo red medium[11]. Congo red agar (CRA) medium was prepared with brain heart infusion broth, sucrose, agar and congo red indicator [11]. CRA plates were inoculated with test organism and incubated at 37^{o}C for 24 hrs. Black colonies with dry consistency indicate strong biofilm formation. Brownish or reddish growth was considered as negative biofilm formation. The cell surface hydrophobicity of isolates was determined by using Salt Aggregation Test (SAT) [12].

Gelatinase production was tested using gelatin agar[9]. Production of beta lactamases was determined by iodometric method by Sng et al, 1980 [13]. The mechanism of the test involves a preliminary positive starch test as evidenced by the filter paper stripimprignated with starch (1%) becomes purple when iodine solution is added.

The test area of the strip turns white if penicilloic acid is produced by the action of penicillinase which converts the iodine to iodide, which is then no longer available to form the purple starch-iodine complex. Susceptibility of isolates to antibiotics were tested using the disk diffusion Bauer-Kirby method, against the following commonly used antibiotics. As per Clinical Laboratory Standards Institute inhibition zones sizes were interpreted in accordance to Performance Standards for Antimicrobial Disk Susceptibility Test [14].

Antibacterial activity of extracts:

Agar well diffusion assay [15] was the key process used to evaluate the antibacterial potential of plant extracts. Petri dishes (100mm) containing 18ml of Mueller Hinton Agar were seeded with 100 µl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10 CFU/ml with 0.5 Mac Farlands standards). Media was allowed to solidify. Wells of 8 mm diameter were cut into solidified agar media using a sterilized cup-borer. 100 µl of each extract was poured in the respective well and the plates were incubated at 37°C overnight. The zone of clearance around each well after the incubation period confirms the antimicrobial activity of each spice extract. The experiment was performed in triplicate and antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition in mm, produced by each extract at the end of incubation period. Organic solvents used in preparation of extracts and DMSO were also used as negative controls during the study.

Minimum inhibitory concentration:

Tea liquors that presented inhibitory properties *in vitro* in the screening activity were evaluated for their MIC using the Agar well diffusion method. Different concentrations of Green and Black tea extracts (0.2, 0.4, 0.6, 0.8 and 1mg/ml) were loaded in individual wells. 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. The MIC was determined as the lowest drug concentration that inhibited growth, as recommended by the National Committee of Clinical and Laboratory Standards (NCCLS, 2011)[5].

RESULTS AND DISCUSSION:

Table 1 shows the results of qualitative phytochemical analysis. The present study shows presence of alkaloids, flavanoids, saponins, tannins, phenols and carbohydrates. Proteins could not be detected by Ninhydrin method. Tariq et al[16] showed the presence of phytochemicals namely alkaloids, flavonoids, steroids, gallic tannins and catecholic tannin by changing the colour of the solution to yellow, white, green bluish, blue, green black

respectively. They indicated the absence of terpenoid, saponins, and glycosides as there was no colour change in the solution with respect to them. The differences in the results obtained and that reported in previous studies may be due to environmental factors that prevail in production areas, cultivars used to produce seeds and also due to the different methods used to prepare these local spices. Generally the presence of phytochemical namely alkaloids, flavonoids, steroids, gallic tannins, catecholic tannin plays the vital role in the plant defense mechanisms [16, 17].

TABLE 1: PHYTOCHEMICAL ANALYSIS OF GREEN TEA AND BLACK TEA

Phytochemicals tested	Green tea	Black tea
Alkaloids	+	+
Flavanoids	+	+
Saponins	+	+
Tanins	+	+
Phenols	+	+
Carbohydrates	+	+
Proteins	-	-

+: Presence of phytochemical; -: Absence of phytochemical

TABLE 2: BIOCHEMICAL REACTIONS PERFORMED FOR THE ISOLATES

Biochemical tests	Organisms		
	E. coli	***Klebsiella spp***	***Pseudomonas spp***
Indole	Positive	Negative	Negative
Methyl red	Positive	Negative	Negative
Voges proskaur	Negative	Positive	Negative
Citrate	Negative	Positive	Positive
Catalase	Positive	Positive	Positive
H_2S	Negative	Negative	Positive
Oxidase	Negative	Negative	Positive
Urease	Negative	Positive	Negative

TABLE 3: EXPRESSION OF VIRULENCE PHENOTYPES AMONG THE ISOLATES

	Virulence Characters				
Isolates tested	**Gelatinase**	**Biofilm**	**Hydrophobicity**	**Penicillinase**	**Haemolysin**
E.coli (n=6)	2 (33%)	4 (47%)	2 (33%)	4 (67%)	1 (17%)
Klebsiella spp (n=5)	0 (0%)	3 (60%)	2 (40%)	4 (80%)	2 (40%)
Pseudomonas spp (n=1)	1 (100%)	1(100%)	0 (0%)	1 (100%)	1 (100%)

Table 2 gives a list of biochemical tests performed to identify *Klebsiella spp* and *E.coli*. **Table 3** represents the virulence phenotypes presented by the different bacterial isolates. Penicillinase production as detected by filter paper iodometric method and biofilm production on Congo red medium are seen in nearly 50% or above among the isolates. Penicillinase production confers resistance to beta lactam antibiotics. Biofilms play an important role in colonization of the bacteria in the urinary tract[18]. They also play an important role in colonization of catheters, thus increasing the incidence of urinary tract infections in patients undergoing surgical interventions [19]. In the present study as shown in **Table 4** all the isolates were sensitive to three aminoglycoside tested i.e Amikacin, Netilimicin and Gentamicin. With both *E.coli* and *Klebsiella spp* maximum resistance was observed with Cephalosporin- Cefuroxime and Quinolone-Norfloxacin and Nalidixic acid. Out of the total isolates tested 67% of *E.coli* and 60% of *Klebsiella spp* were Multi drug resistant[20]. Three isolates which were MDR ie resistant to 3 or more antibiotics and in addition showed more than three virulence features were selected for antibacterial activity against the Green and black tea extracts. **Table 5** depicts the antibacterial activity of the extracts. The results obtained from this study revealed that different tea products exerted significant antimicrobial activity against antibiotic

resistant clinical isolates of bacteria. Methanol extracts of Green tea and Black tea extracts showed maximum antibacterial activity against all three isolates tested viz *E.coli*, *Klebsiella spp* and *Pseudomonas spp.*

TABLE 4: ANTIBIOTIC RESISTANCE PATTERN OF DIFFERENT ISOLATES

Antibiotic	Concentration (mcg)	*E.coli* (n=6)	%	*Klebsiella spp* (n=5)	%	*Pseudomonas spp* (n=1)	%
Amikacin	30	0		0		0	
Netilmicin	10	0		0		0	
Gentamicin	10	0		0		0	
Norfloxacin	5	(5)83.33		(4)80		(1)100	
Ciprofloxacin	5	(3)50		(3)60		0	
Ofloxacin	10	(2)33.33		0		0	
Nalidixic acid	30	(5)83.33		(4)80		(1)100	
Ceftriazone	30	(5)83.33		(2)40		0	
Cefaclor	30	(4)66.66		(4)80		(1)100	
Cefadroxil	30	(5)83.33		(2)40		(1)100	
Cefuroxime	75	(5)83.33		(4)80		(1)100	
Cefoperozone	30	(2)33.33		(1)20		0	

TABLE 5: ANTIBACTERIAL ACTIVITY OF GREEN AND BLACK TEA EXTRACTS AGAINST THE UTI ISOLATES

Isolates tested	Green tea extracts (Diameter of zone of inhibition in mm)			Black tea extracts (Diameter of zone of inhibition in mm)		
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous
E.coli	18	22	18	19	20	19
Klebsiella spp	20	23	17	16	22	14
Pseudomonas spp	15	20	10	14	18	10

TABLE 6: MINIMUM INHIBITORY CONCENTRATION AMONG THE ISOLATES

	MIC in mg/ml					
	Green tea			Black tea		
Organism	Aqueous	Ethanolic	Methanolic	Aqueous	Ethanolic	Methanolic
E.coli	0.8	0.8	0.4	0.8	0.8	0.4
Klebsiella spp	0.8	0.8	0.4	0.8	0.8	0.4
Pseudomonas spp	0.8	0.8	0.4	0.8	0.8	0.4

As per the results presented in **Table 6** variations in the MIC of Green and Black tea extracts was observed. Aqueous and ethanolic Green and Black tea extracts showed MIC of 0.8 mg/ml against *E.coli, Klebsiella spp* and *Pseudomonas spp* whereas methanolic extract inhibited the cultures at 0.4mg/ml. Maksum Radji et al in their study found that the MIC of green tea extract against MRSA was 400 µg/mL, while the MIC for MDR-*Pseudomonas spp* was 800 µg/ml.

Conclusions- Based upon our data and these earlier findings, the daily intake of green tea might be expected to alter the growth and composition of the microbial community and to modulate the genesis of potentially harmful products such as carcinogenic N-nitroso compounds or aromatic steroids within the intestinal tract, thus protecting from a variety of diseases and helping to maintain optimal human health. Further work need to be aimed to identify the biologically active materials of green tea and black tea. Studies on *in vivo* research using human volunteers are in progress.

ACKNOWLEDGEMENT: The Authors would like to thank UGC, Delhi for the financial support.

REFERENCES

1. Lagasse P. 2000. Columbia Encyclopedia, Sixth Edition.
2. Taheri M, Giahi M, Shahmohamadi R, Ghafoori H, Aghamaali M R and Sariri R. 2011. Screening Antioxidant Activity of Extracts from Different Tea Samples. Deparment of Biology, University if Guilan, Rasht, Iran. Department of Chemistry, Islamic Azad University Lahijan Branch, Lahijan, Iran Review Pharmacologyonline 3: 442-448
3. Kumar A, Kuma A, Thaku P, Patil S, Payal C, Kumar A and Sharma P. 2012. Antibacterial activity of green tea (*Camellia sinensis*) extracts against various bacteria

isolated from environmental sources. Recent Research in Science and Technology, 4(1): 19-23 ISSN: 2076-5061

4. Cameron A R, Anton S, Melville L, Houston N P, Dayal S, McDougall G J, Stewart D, Rena G. (2008). Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell* **7** (1): 69–77.
5. Koech K R, Wachira F N, Ngure R M, Wanyoko J K, Bii C. and Karori S M. 2013. Antibacterial and Synergistic Activity of Different Tea Crude Extracts against Antibiotic Resistant *S. Aureus*, *E. coli* and a Clinical Isolate of *S. Typhi*. Science Journal o f Microbiology ISSN:2276-626X
6. Radji M, Agustama R A, Elya B and Tjampakasari C R. 2006. Antimicrobial activity of green tea extract against isolates of methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa*. J Am Coll Nutr. 25(2):79-99.
7. Obwoge J O, Kinyua J K, Kariuki D W and Magoma G N. 2012. Phytochemical screening and antimicrobial studies of green, orthodox and black kenyan tea. Departmentof Biochemistry, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya. 200-207
8. Cappunccino J C and Sherman N.2001. Microbiology laboratory manual.6thedition, Pearson education.
9. Chatterjee B, Kulathinal S, Bhargava A, Jain Y, Kataria R. Anti - microbial resistance stratified by risk factor among Escherichia coli strains isolated from the urinary tract at a rural clinic in Central India. Indian J Med Microbiol [serial online] 2009; 27:329-34.
10. Siddiqui A A, Ali M. 1997. Practical Pharmaceutical Chemistry. 1st edition, CBS Publishers and Distributors, New Delhi.126-131.
11. Freeman D J, Falkner F R, Keane C T. 1989 New method for detecting slime production by coagulase negative Staphylococci. J clin pathol, 42; 872-874
12. Jadhav S, Hussain A, Kumar A, Parveen S, Gandham N, Wielar L H, Ewers C and Ahmed N.2011.Virulence characteristics and Genetic affinities of Multiple Drug Resisant Uropathogenic Escherichia coli from a semi urban locality in India. PLoS ONE 6 (3) e18063
13. Y.V.S. Annapurna and Rashmi Savant: Multi Drug Resistance and MAR Index Among Bacteria Associated With Fruits and Vegetables; 2nd May 2014; Int.J.Curr.Microbiol.App.Sci (2014) 3(6): 222-229
14. Wayne, P A.2005. National committee for clinical laboratory standards. Performance standards for antimicrobial susceptibility testings; 15th informational supplement 9M100-S15. National committee for clinical laboratory standards.
15. B. Rajendar et al.,: Comparative Antibacterial activities of the Combined crude Leaf extract of Eucalyptus Globulus, *Azadirachta Indica* and *Ocimum Scantum*, J. Pharm. Res., 2015; 4(4): 164-166.
16. Tariq A L and Reyaz A L. 2012. Phytochemical analysis of Camellia sinensis Leaves. Int. J. Drug Dev. & Res., 4(4): 311-316.
17. Artacho R, Cabrera C, Giménez R. 2006. Beneficial effects of green tea: a review. J Am Coll Nutr, 25:79-99
18. Koare C R, Chakraborty S, Khopade A N and Mahadik K R. 2009. Indian Journal of Biotechnology.159-168
19. Tambyah P A. 2004. Catheter associated urinary tract infections: diagnosis and prophylaxis. International Journal of Antimicrobial Agents, 24.44-48
20. E.O. Adeleke and B.O. Omafuvbe, 2011. Antibiotic Resistance of Aerobic Mesophilic Bacteria Isolated from Poultry Faeces. Research Journal of Microbiology, 6: 356-365.

How to cite this article:
Labar V, Bhutia R and Annapurna YVS: Effect of *Camellia Sinensis* Extracts on Growth of Virulent Gram Negative Uropathogens. Int J Pharm Sci Res 2016; 7(8): 3373-78.doi: 10.13040/IJPSR.0975-8232.7(8).3373-78.