Antimicrobial activity of Green Tea: A comparative study with different Green Tea extract

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ABSTRACT

Objective: The aim of this present study is to evaluate the activity of green tea extracted in different solvents on different microorganism such as on Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Salmonella abony NCTC 6017, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 10231, Esteritia coli mutant NCIM 2567, Bacillus subtilis ATCC 6633 &Lactobacillus lichmani ATCC 7830.

Method: Zone of inhibition of aqueous, methanolic and ethanolic extracts were measured and compared by using cup plate method. 5µg, 10µg, 15µg & 20µg concentration of the extract were used. MIC (Minimum inhibitory concentration)& MBC (Minimum bactericidal concentration) were observed with the concentrations of 100µg, 75µg, 50µg & 25µg .

Result: Result of zone of inhibition shows that aquous extract at the concentration of 20µg/ml is most effective to inhibit Escherichia coli growth compare to standard.Also minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC) result suggest that aquous extract is more effective than ethanolic and methanolic extract to inhibit the growth of both Escherichia coli &Escherichia coli mutant.

Conclusion: Significant antimicrobial activity has been shown by all extracts against Escherichia coli but other microorganism inhibition is not so significant compare to Escherichia coli . Methanolic and ethanolic extract has shown little antimicrobial activity against all microorganisms as compared to the aqueous extract.

Keyword: Antimicrobial activity, Extracts, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Zone of Inhibition

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern (Westh H et.al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow JE et.al, 2003). Among them many infectious diseases have been known to be treated with tea extract. Tea is produced from leaves and non-developed buds of a teashrub 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 (Islam G.M.R, et.al, 2005)(Sang Hee Kim, et.al, 2008).The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechins and polyphenols (Sofowara A, 1984). Recent reports however indicate the tea's antibacterial and bactericidal properties on various bacterial strains isolated from patients with infected root canal (Horiba N, et.al, 1991). A Recent study also showed that moderate daily consumption of green tea killed Staphylococcus aureusand other harmful bacteria (Toda M, et.al, 1989a). Subsequently, several studies on the antimicrobial properties of Japanese tea have been reported (Toda M, et.al. 1989b). Candida albicans, Escherichia coli mutant, Bacillus subtilis, Lactobasilluslichmani these are the harmful microorganism causing various harmful diseases like opportunistic oral & genital infection, Colitis, Food poisoning, Gastro intestinal infection etc. 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.

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Green tea is generally safe, nontoxic and having no side effects after use. However over several research works were done on green tea extract and its activity but the form of tea which we are using after processing regularly in our daily life left behind. So in this study we are trying to evaluate the effect of processed green tea on some microorganisms.

MATERIALS & METHODS

Source of Tea:

All experiment was done with rosted CTC green tea. Tea sample was purchase from The Santoshpur Tea centre, Kolkata, India. The voucher specimen have been deposited at the Tea Reasearch Association (TRA), Kolkata, India

Bacterial strains & media:

Microbial stains (Salmonella abony NCTC 6017, Staphylococcus aureus ATCC 6538,Esteritia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027,Candida albicans ATCC 10231, Esteritia coli mutant NCIM 2567, Bacillus subtilis ATCC 6633, Lactobacillus lichmani ATCC 7830) & Antibiotic assay media no. 1 containing peptone 6%,yeast extract 3%,casein enzyme hydrolysate 4%,beef extract 1.5%,dextrose 1%,agar 15% with final pH 6.6 ± 0.2 (at 25º C) supplied by Holy Cross Research Laboratories, Baruipur Industrial Estate, Kolkata, India. Peptone, beef extract is purchase from HiMedia laboratories, India. All other chemicals used were of analytical grade purity.

Preparation of different extracts of tea:

Water extracts:

15 gm of dry powder of fresh processed green tea leaves was taken in a round bottom flask and then 100ml of distilled water was added to it. Then it was gently heated over the flame for 2hrs. After that the mixture was filtered with muslin cloth. Then the filtrate was again filtered by the help of whattman filter paper no.1. Then the filtrate was centrifuged at 5000 rpm for 30 mins. The supernatant liquid was collected and then further centrifuged at 5000 rpm for 30 mins. Then the liquid obtained was poured in a petri dish and heated gently over the water bath. When the liquid gets evaporated cool the content and scratch it, powder like substance was obtained which was then covered properly by aluminium foil and stored at 4^{o}C.

Ethanolic extract:

15gm of dry powder of fresh processed green tea leaves was taken in a round bottom flask and then 100ml of ethanol (95% pure) was added into it, and then kept for 5 days at a normal temperature. After 5days the mixture was filtered 1st by muslin cloth, then the filtrate was again filtered by whattman filter paper no.1, then the filtrate was centrifuged at 5000rpm for 30mins. The supernatant was collected then again centrifuged at 5000rpm for 30mins. Then poured the in a petridish and heated gently over the water bath. When the liquid gets evaporated cool the content and scratched it, powder like substance was obtained which was covered properly by aluminium foil and stored at 4^{o}C.

Methanolic extract:

12gm of dry of dry powder of fresh processed green tea leaves was taken in a round bottom flask and then 50ml of methanol was added to it and then kept for 3days. After 3days the mixture was filtered 1st by muslin cloth, then the filtrate was again filtered by whattman filter paper no.1, then the filtrate was centrifuged at 5000rpm for 30mins. The supernatant which was collected centrifuged at 5000rpm for 30mins. Then poured the liquid in a petridish and heated gently over the water bath. When the liquid gets evaporated it was cooled and the remnant portion was scratched. Powder like substance was obtained which was then covered properly by aluminum foil kept and stored at 4^{o}C.

Preparation of Nutrient Broth:

Peptone 2grm,beef extract 2grm & sodium chloride 1grm used for preparation of nutrient broth.All the ingredients were taken and weighed properly and kept in a conical flask. To this mixture 200ml of distilled water was added with continuous stirring. When all the ingredients gets dissolved, plugged the mouth of the conical flask with cotton, covered it with brown paper. After that, the plugged conical flasks were autoclaved at 121^{o}C at 15lb pressure. After autoclaving, the broth was poured in four cleaned & sterilized test tube equally. The test tubes were marked accordingly for the different microorganisms. Then the broth was inoculated with the microorganism according to the respective marked test tube for the different microorganism

under laminar airflow chamber. Then it was incubated in the incubator at 37°C for 24hr.

Preparation of media:

Antibiotic assay media no. 1 was taken and 14.64gm was weighed properly and taken in a conical flask and 480ml of distilled water was added to it (so that it can be equally divided into 20ml for each 24petridish). Then it was slightly heated over the flame to dissolve it. When it gets fully dissolved the mouth of the conical flask was plugged with cotton then covered with brown paper and tied properly. Then it was autoclaved at 121°C at 15 lb pressure.

Testing of antibacterial susceptibility:

The antibacterial activity of different green tea extract was found by cup-plate method (Sarvananan et al,2010). About 15 ml 0f sterile Muller Hilton agar (Himedia) in petri dish was seeded with 1.0 ml of standard broth culture of the bacteria (1.0×107 CFU/ml) and spread gently to ensure uniform distribution of microorganisms and then allowed to solidify on a flat surface. Four wells were made in the plate(about 0.5 mm diameter) using a sterile cork borer and with 5 µl,10µl,15µl,20µl of a solution of aqueous extracts of green tea were transferred into well using micropipette. 0.1 ml of Ciprofloxacin at a concentration of (0.65µg/ml, 0.8µg/ml, 1.0µg/ml, 1.2µg/ml) was taken as a standard reference. The pates were allowed to stand for one hour for pre diffusion to the extract to occur and were incubated at 37ºC for 24 hours. After incubation for 24 hours the zone of inhibition was calculated by measuring the diameter of zones of growth inhibition by using colony counter.

Determination of Minimum Inhibitory Concentration (MIC):

At first four different concentration of the test samples were made (100µg/ml, 75µg/ml, 50µg/ml & 25µg/ml) with three types of extract.Then 32 cleaned and sterilized test tube was taken and marked them for 4 different concentrations and for 8 microorganisms respectively and kept in a cleaned test tube stand. (4 test tube for each microorganism) Followed by the nutrient broth preparation. (as above mentioned). Then poured 2ml of the nutrient broth and 2ml of the aqueous extract of different concentration in different test tube respectively. After that inoculated the test tubes as per the respective microorganism. Then the mouth of the test tubes was plugged properly with cotton and subjected them in the incubator for 24hrs at 37.5°C. Then, after 24hrs the test tubes were taken out from the incubator and the observation has been noted. The same procedure had been repeated for both ethanolic and methanolic extract

Determination of Minimum Bactericidal Concentration (MBC):

MBC was determined by subculturing the 5 µl of test dilution from each well on to a nutrient agar (Himedia,India) plates and incubating further at 37 °C for 24 h. The complete absence of growth at applied concentration was considered as the minimum bactericidal concentration

RESULT & DISCUSSION

Zone of inhibition. Table 1:Inhibition zone of aqueous extract:

Sample type (µg/ml)	Conc	Microorganisms								Ctrl .b
		P.aerogenosa	E.coli	S. abony	S. aureus	C. albicans	E.coli mutant	B. subtilis	L. lichmani	
Test	5	1.7cm	2.0cm	1.9cm	1.8cm	1.4cm	1.9cm	1.6cm	1.3cm	__
	10	1.9cm	2.2cm	2.0cm	1.9cm	1.4cm	2.0cm	1.7cm	1.4cm	__
	15	2.0cm	2.2cm	2.2cm	2.0cm	1.6cm	2.0cm	1.9cm	1.5cm	__
	20	2.3cm	2.4cm	2.3cm	2.2cm	1.7cm	2.1cm	1.9cm	1.7cm	__
Std.a	0.65	2.1cm	2.1cm	2.1cm	2.0cm	2.0cm	1.9cm	2.0cm	2.1cm	__
	0.8	2.1cm	2.2cm	2.2cm	2.0cm	2.1cm	2.0cm	2.1cm	2.2cm	__
	1	2.3cm	2.3cm	2.2cm	2.2cm	2.4cm	2.2cm	2.2cm	2.2cm	__
	1.25	2.4cm	2.4cm	2.3cm	2.4cm	2.5cm	2.3cm	2.4cm	2.5cm	__

[a.Standard, b. control]

Table 2: Inhibition zone of ethanolic extract:

Sample type (µg/ml)	Conc	Microorganisms								Ctrl. b
		P. aerogenosa	E.coli	S. abony	S. aureus	C. albicans	E.coli mutant	B. subtilis	L. lichmani	
Test	5	1.4cm	1.8cm	1.6cm	1.3cm	1.5cm	1.8cm	1.4cm	1.7cm	__
	10	1.5cm	1.9cm	1.8cm	1.4cm	1.6cm	1.8cm	1.5cm	1.8cm	__
	15	1.6cm	1.9cm	1.9cm	1.5cm	1.6cm	2.0cm	1.7cm	1.8cm	__
	20	1.8cm	2.1cm	1.9cm	1.7cm	1.8cm	2.1cm	1.9cm	2.0cm	__
Std.	0.65	2.0cm	1.9cm	2.0cm	2.1cm	2.1cm	2.1cm	2.1cm	2.0cm	__
	0.8	2.1cm	2.0cm	2.1cm	2.2cm	2.1cm	2.2cm	2.2cm	2.0cm	__
	1	2.4cm	2.2cm	2.1cm	2.2cm	2.3cm	2.3cm	2.2cm	2.0cm	__
	1.25	2.5cm	2.3cm	2.4cm	2.5cm	2.4cm	2.4cm	2.3cm	2.4cm	__

Table 3: Inhibition zone of methanolic extract:

Sample type (µg/ml)	Conc	Microorganisms								Ctrl
		P. aerogenosa	E.coli	S. abony	S. aureus	C. albicans	E.coli mutant	B. subtilis	L. lichmani	
Test	5	1.0cm	1.1cm	0.9cm	0.6cm	0.8cm	1.0cm	0.7cm	0.8cm	__
	10	1.1cm	1.3cm	0.9cm	0.8cm	1.0cm	1.2cm	0.9cm	0.8cm	__
	15	1.1cm	1.4cm	1.0cm	1.0cm	1.1cm	1.3cm	1.0cm	1.0cm	__
	20	1.3cm	1.4cm	1.1cm	1.1cm	1.3cm	1.4cm	1.1cm	1.2cm	__
Std.	0.65	2.1cm	2.0cm	2.1cm	2.2cm	2.0cm	1.9cm	1.8cm	2.1cm	__
	0.8	2.2cm	2.2cm	2.2cm	2.2cm	2.0cm	2.2cm	2.0cm	2.1cm	__
	1	2.2cm	2.3cm	2.3cm	2.2cm	2.2cm	2.3cm	2.0cm	2.2cm	__
	1.25	2.3cm	2.4cm	2.4cm	2.4cm	2.3cm	2.4cm	2.1cm	2.3cm	__

Minimum Inhibitory Concentration. Table 4: MIC in aqueous extract:

Microorganisms	Test tubes				MIC (µg/ml)
	A	B	C	D	
P.aerogenosa	_	+	++	+++	50
E.coli	_	+	++	+++	50
S.abony	_	+	++	+++	50
S.aureus	+	++	+++	++++	75
C.albicans	_	+	++	+++	75
E.coli mutant	_	+	++	+++	50
B.subtilis	_	+	++	+++	50
L.lichmani	+	++	+++	++++	75

Table 5: MIC in ethanolic extract:

Microorganisms	Test tubes				MIC (µg/ml)
	A	B	C	D	
P.aerogenosa	_	+	++	+++	50
E.coli	+	++	+++	++++	75
S.abony	_	+	++	+++	50
S.aureus	_	+	++	+++	50
C.albicans	_	+	++	+++	50
E.coli mutant	+	++	+++	++++	75
B.subtilis	_	+	++	+++	75
L.lichmani	_	+	++	+++	50

Table 6: MIC in methanolic extract:

Microorganisms	Test tubes				MIC (µg/ml)
	A	B	C	D	
P.aerogenosa	+	++	+++	++++	50
E.coli	+	++	+++	++++	75
S.abony	_	+	++	+++	50
S.aureus	_	+	++	+++	75
C.albicans	+	++	+++	++++	50
E.coli mutant	+	++	+++	++++	50
B.subtilis	_	+	++	+++	50
L.lichmani	_	+	++	+++	75

[(-) = clear solution, (+) = very less turbidity, (++) = less turbidity, (+++) = quite turbid, (++++) = very turbid.]
[Test tube A - 100µg/ml, Test tube B - 75µg/ml, Test tube C - 50µg/ml, Test tube D - 25µg/ml}]

Minimum Bacterial Concentration.

Table 7: MBC for aqueous extract:

Microorganism	Concentration(µg/ml)	Zone of inhibition	Control
P. aeruginosa	50	2.7cm	__
E.coli	50	2.8cm	__
S. abony	50	2.4cm	__
S.aureus	75	2.5cm	__
C.albicans	75	2.7cm	__
E.coli mutant	50	2.9cm	__
B.subtilis	50	2.5cm	__
L.lichmani	75	2.5cm	__

Table 8: MBC for ethanolic extract:

Microorganism	Concentration (µg/ml)	Zone of inhibition	Control
P. aeruginosa	50	2.3cm	__
E.coli	75	2.4cm	__
S. abony	50	2.2cm	__
S.aureus	50	2.0cm	__
C.albicans	50	2.2cm	__
E.coli mutant	75	2.4cm	__
B.subtilis	75	2.3cm	__
L.lichmani	50	2.0cm	__

Table 9: MBC for methanolic extract:

Microorganism	Concentration (µg/ml)	Zone of inhibition	Control
P. aeruginosa	50	2.0cm	__
E.coli	75	2.3cm	__
S. abony	50	1.9cm	__
S.aureus	75	2.0cm	__
C.albicans	75	2.1cm	__
E.coli mutant	75	2.2cm	__
B.subtilis	50	1.9cm	__
L.lichmani	50	1.8cm	__

It has been observe from all of this data, that most significant result has been seen on E.coli and E.coli mutant. The observation of the zone of inhibition study has been tabulated in table 1, 2&3 and it has been found that both aqueous extract and methanolic extract at concentration of 20µg/ml inhibit E.coli significantly as compare to standard drug Ciprofloxacin. From this data it is observed that both methanolic and aqueous extract of green tea is effective against E.coli infection in concentration dependent manner. But more significant inhibition of E.coli has been seen for aqueous extract only.

The result of MIC shows that concentration required for inhibit E.coli is less for aqueous extract as compares to ethanolic and methanolic extraction. For aqueous extract the concentration is 50µg/ml (Table 6 & 7).

The result of MBC indicate that the highest range of inhibition is seen at concentration of 50µg/ml and in this concentration both E.coli and E.coli mutant is inhibited significantly(Table 7).For ethanolic and methanolic extract at the concentration of 75µg/ml inhibit both E.coli & E.coli mutant, but this inhibition is less in compares to aqueous extract. From this data we also came to know that at aqueous extract less concentration (Table.7) is required for inhibition of E.coli&E.coli mutant as compare to ethanolic and methanolic extract of green tea.

CONCLUSION

This experimental work showing, that all the active ingredients present in roasted green tea is better soluble in water than the other organic solvents like ethanol and methanol. When the comparison of the result was done among the microorganisms, then it was observed that the roasted green tea is more active against the microorganism Escherichia coli mutant and also it can be concluded that all the extract having broad spectrum activity. At last it can be concluded that the aqueous extract of green tea which is available in the market as a roasted form having better inhibitory action on E.coli. However, further extensive study is required to explore all this activity.

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