COMPARATIVE ANTIBACTERIAL ACTIVITY OF GREEN TEA AND LEMON GRASS EXTRACTS AGAINST *STREPTOCOCCUS MUTANS* AND ITS SYNERGISM WITH ANTIBIOTICS

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ABSTRACT

Tea is the most common beverage after it is extracted from the leaves of *Camellia sinenesis*. It is classified in to fermented tea (black tea), non fermented tea (Green tea). The decoction of *Cymbopogon citrates* (Lemon grass) also used as Qawa. Extract of tea samples in methanol and ethanol which was further diluted in methanol and water tested for antibacterial activity against *Streptococcus mutans*. Methanol was found to be best antimicrobial solvent. MIC of methanolic extract of green tea diluted in methanol was determined and found to be bactericidal in concentration of 0.1g/ml against *Streptococcus mutans*. MIC values of methanolic extracts of green tea diluted in ethanol was 0.1 mg/ml. Synergistic results of antibacterial activity of methanolic extracts (Diluted in ethanol) was poor. Synergistic activity of tea samples with antibiotics (Chloroamphenicol, Tetracycline, Levofolxacin and Gentamycin) showed best response against the bacteria (0.1mg/ml).

Key words: Antibacterial activity, Green Tea, Lemon grass, *Streptococcus mutans*, synergistic activity ,Antibiotics

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INTRODUCTION:

Tea is an infusion made by steeping processed leaves, buds or twigs of tea bush, *camellia sinensis*, in hot water for several minutes after, which it is drunk. The term herbal tea usually refers to infusion or tisane of fruit or herbs that contain no *Camellia sinensis*. Tea came into English language from Chinese word of tea which is pronounced in "te" in the Min Nan spoken variant .The British English slang word "char " for tea arose from its mandarin " Chinese pronunciation "cha " with its spelling affected by British English erotic dialect pronunciation (Macfarlane). (Chow.K and Kramer.L .,1990)

Tea is one of the most consumed beverages of the world. Presently, it is cultivated in at least 30countries around the world. Approximately 20-22% of tea produced and consumed is green tea and less than 2% is oolong tea. While green tea in drink in China, Japan Korea and Morocco (Wu C.D, Wei GX .2002)(Zuo et.al., 2008). The powerful antioxidant properties of the tea are generally attributed to its flavonoid components theaflavins, bisflavanols and theaflavic acids. (Rice.E. C. 1999). The effect of tea on stomach cancer has been the most extensively studied. Out of 15 studies, five case-control studies showed a protective effect of tea on the risk of stomach cancer (Ji B.T, *et.al*., in 1997). Green tea polyphenols have chemo preventive effect against cancers in smokers. The anti-carcinogenic activities of tea Polyphenol are generally believed to be related to their antioxidative properties. Tea may affect the metabolism of carcinogens by induction or inhibition of various cytochrome P450s, but the practical importance of this mechanism is not known (Katiyar S,et.al., 1992).

lemon grass include lemon grass barbed wires grass ,Andropogon , silky grass citronella grass ,fever grass ,hierba luisa.(Shadab *et. al.* , 1992). Main constituents of lemon grass are citral, myrcene, dipentene, linalool, geraniol, nerol, citronellol, farneol and borneol . Its oil in nontoxic in nature it is yellow amber or reddish brown in color. Lemon grass also includes nutritious calcium, iron, magnesia, phosphorous, potassium, selenium and zinc (Wilkinson .J.*et.al.*, 2003). Citral has been used for centuries to treat a variety of ailments, however, the discovery of its effectiveness against s relatively a new development citral helps to combats depression and bad mood. Used to treat fever cold and cough. A great remedy for stomach ache relaxes the stomach and intestine (Ming.L., *et al* 1996).

Streptococcus mutans is a non motile, non spore forming coccoid shaped, gram positive facultative anaerobic is a part normal bacteria flora of a mouth it is an alpha hemolytic streptococci . Bacterial cells appear in a form of chains due to cellular division in one division and incomplete cytokinesis following mitosis. It can thrive in temperature 18-40ºC and pH below 5.5. (Smith, T. J., Blackman, S. A. & S. J Foster 2000).

REVIEW OF LITERATURE

Sakanaka *et .al,* (2000) reported that the inhibitory action of tea polyphenols towards the development and growth of bacterial spores of *Bacillus* bacteria, tea polyphenols showed antibacterial effects towards *Bacillus stearothermophilus,* which is a thermophilic spore-forming bacterium. The heat resistance of *B. stearother-mophilus* spores was reduced by the addition of tea polyphenols. *Clostridium thermoaceticum*, an anaerobic spore-forming bacterium, also exhibited reduced heat resistance of its spores in the presence of tea polyphenols. *Epigallocatechin gallate*, which is the main component of tea polyphenols, showed strong activity against both *B. stearothermophilus* and *C. thermoaceticum.* The heat resistance of these bacterial spores was more rapidly decreased by the addition of tea polyphenols at high temperatures.

Hosseini Jazani *et.al.,* (2007) studied that the antibacterial activity of water soluble green tea extracts on 43 hospital isolates of *Pseudomonas aeruginosa.* Antibacterial activity of water soluble green tea extract was measured by Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) 35.6% of isolated strains showed resistance to 5 antibiotics or more and 55.8% of all strains were Multi-Drug Resistant (MDR) strains. The average MICs and MBCs of the extract against all strains of *Pseudomonas auroginosa* were 2.06+/-1.76 and 2.54+/-2.22 mg/ml, respectively. It was proved that green tea has significant activity with bactericidal action on multi-drug resistant strains of *Pseudomonas aeruginosa.*

Tiwari *et.al.,* (2005) studied that the synergistic antimicrobial activity of tea and antibiotics against enteropathogens. Antimicrobial activity of boiled water tea extract and organic solvent extract

were studied against *Salmonella typhimurium* 1402/84, *S. typhi*, *S. typhi Ty2a*, *Shigella dysenteriae*, *Yersinia enterocolitica C770*, and *Escherichia coli* determining minimum inhibitory concentration, minimum bactericidal concentration and death rate kinetics at MBC of tea extract in presence of sub inhibitory concentration of antibiotic. Green tea extracts effectively inhibited the growth of *S. typhimurium 1402/84, S. typhi, S. typhi Ty2a, S. dysenteriae, Y. enterocolitica C770, and E.coli.* Antimicrobial activity of green tea, methanol and water extract tea was better as compared to boiled water tea extract of green tea. Based on death rate kinetics results, *S.typhi Ty2a* appeared to be highly sensitive and *Y. enterocolitica* C770 the most resistant. Chloramphenicol and tea extract in combination inhibited the growth of *S.dysenteriae* at 2.5 µg/ml chloramphenicol (MIC 5 µg/ml). Tea extract showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against test strains.

MATERIAL AND METHODS

All chemicals solvents were analytical grade (Merck). Mueller-Hinton agar was used. Microbial strain *Streptococcus mutans* ATCC 20572 (*S. mutans*) was obtained from Dental plaque centre Railway Hospital Lahore growing on MSB agar.

Tea samples:

Tea samples used in the present study are green tea and lemon grass was collected from different departmental store of Lahore.-Pakistan. All the material was in the branded form.

Extraction:

The purchased tea samples(*green tea and Lemon grass)* were grinded to obtain fine powder .The sample powder were triturated with redistilled methanol for 4-6 days by Soxhlet apparatus and process was repeated three times. The solvent was removed in the rotary evaporator to yield the crud methanolic extracts stored at 4ºC. The methanolic extract of all tea samples were diluted with solvent (Ethanol and Methanol) and with combinations of different antibiotic (Chloramphenicol, Tetracyline, Levofloxacin and Gentamycin) with Concentration (0.1,0.2,0.3, 0.4,0.5,0.6,0.7,0.8,0.9 and 1.0mg/ml) was used.

Table-1: Detail of Tea Samples

Tea samples	Botanical Name	Family	Local Name	Part of Plant
1	*Cymbopogon citratus*	Poaceae	Lemon grass	Stem &leaves
2	*Camellia sinensis Linn*	Theaceae	GreeTea	Leaves (Fresh dried)

Bioassay:

Bactericidal activity was determined by agar well diffusion method Norrel and Messley,(1997). This test was performed in triplicate by spreading 18-24 hour old pathogenic bacterial cultures containing approximately 10^6 -10^{10} colony forming units (CFU/ml) on the surface of MSB agar medium (**Mitis-Salivarius Bacitiracin Agar**) plates. Wells (4 mm) were dug in the media with the help of sterile metallic borer. Test samples of different concentrations prepared in different solvents (Merck) were added (50 µl) in their respective wells. Pure methanol was used as negative control (3 mm).

Other wells were supplemented with reference compound i. e. Chloramphenicol, Tetracyline, Levofloxacin and Gentamycin serving as positive control.

Synergistic activity against bacterial strains was determined by taking equal amounts (0.1mg/ml) of antibiotics(Chloramphenicol, Tetracyline, Levofloxacin and Gentamycin)

RESULT AND DISCUSSION

From the results obtained it could be observed that methanol was the best solvent for extracting antimicrobial substances from tea samples based on the number of organisms inhibited and the diameter of inhibitory zones produced. It could also be seen that different extracts were different in their antimicrobial effectiveness depending on the extractive solvent used. This result agrees favorably with the suggestion of Oloke and Kolawole (Oloke and Kolawole, 1988) that bioactive components of any medicinal plant may differ in their solubility depending on the extractive solvents used. The water extract was least bactericidal as compared to other solvent extracts and showed medium activity against *S.mutans*.

The crude methanolic extract of Green tea diluted in methanol showed highest activity against *S.mutans* at the concentration of 0.3 mg/ml (Zone of inhibition 39 mm)while it showed good bactericidal activity at other concentrations. The Lemon grass was not soluble in methanol.

Table 2. Antibacterial Activity of Methanol Extract of Tea samples (Dilution in Methanol)

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Lemon Grass	Green Tea
1-	0.01	*Lemon grass not soluble in methanol.	25
2	0.2		30
3-	0.3		39
4-	0.4		19
5-	0.5		22
6-	0.6		21
7-	0.7		25
8-	0.8		22
9-	0.9		20
10-	1.0		21

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Green Tea	Lemon Grass
1		21	-
2-	0.2	11	15
3-	0.3	13	30
4-	0.4	17	15
5-	0.5	10	25
6-	0.6	12	22
7-	0.7	_	21
8-	0.8	_	22
9-	0.9	_	20
10-	1.0	_	22

The crude methanolic extract of Green tea diluted in Ethanol showed moderate activity against *S.mutans* at the concentrations from 0.1-0.6 mg/ml while the concentrations between 0.7-1.0mg/ml inhibit the growth of *S.mutans*. Lemon grass showed good bactericidal activity nearly at all concentrations (Table 2)

Table 3. Antibacterial activity of crude methanol extract of Tea Samples (dilutions In Ethanol)

		Zone of inhibition (mm)		
Serial No	Concentration mg/ml	Green Tea +Lemon Grass	Tea +Lemon Grass	Green Tea + Tea
		-	-	_
2	0.2	-	-	_
3	0.3l	-	-	_
4	0.4	-	-	_
5	0.5	-	-	15
6	0.6	-	-	14
7	0.7	-	10	21
8	0.8	-	-	21
9	0.9	-	-	20
10	1	-	-	22

Synergistic activity:

The crude methanolic extract of Green tea mixed with lemon grass in same ratio(1:1) diluted in Ethanol showed poor activity against *S.mutans* at all concentrations. The mixed crude methanolic extract of Green tea (diluted in Ethanol) showed the good result at the concentrations of 0.5-1.0 mg/ml (Table -3).

Table 3. Synergistic Antibacterial activity of crude methanol Extract Dilutions in Methanol

		Zone of inhibition (mm)	
Serial No	Concentration mg/ml	Lemon Grass	Green Tea
		20	25
2	0.2	21	26
3	0.3	20	20
4	0.4	25	20
5	0.5	21	25
6	0.6	23	22
7	0.7	24	25
8	0.8	25	20
9	0.9	20	20
10	1.0l	28	20

Synergistic activity with different antibiotics:

The crude methanolic extract of Green tea mixed with chloroamphenicol in same ratio(1:1) diluted in Ethanol showed good activity against *S.mutans* at all concentrations. The crude methanolic extract of Lemon grass with same antibiotic (diluted in Ethanol) showed the good result at the

concentrations. Mixing of extracts with chloroamphenicol enhance the activities of all tea samples. (Table 4)

Table 5. Synergistic Antibacterial activity of crude methanol extract of Tea samples with *chloroamphenicol* (Antibiotic)

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Lemon Grass	Green Tea
1	0.1	12	15
2	0.2	13	20
3	0.3	14	18
4	0.4	25	15
5	0.5	10	15
6	0.6	18	18
7	0.7	17	18
8	0.8	1 8	16
9	0.9	30	15
10	1.0	20	16

The crude methanolic extract of Green tea and tetracycline diluted in ethanol mixed in same ratio (1:1) showed good bactericidal activity against *S.mutans* at all concentrations. The synergistic combination of green tea with tetracycline showed good result at all the concentrations. (Graph-11, 12 & 13)

Table 6. Synergistic Antibacterial activity of crude methanol extract of Tea samples with *Tetracycline* (Antibiotic)

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Lemon Grass	Green Tea
1	0.1	30	22
2	0.2	31	22
3	0.3	28	30
4	0.4	25	24
5	0.5	24	27
6	0.6	20	22
7	0.7	26	20
8	0.8	21	25
9	0.9	20	20
10	1.0	20	21

The synergistic activity of crude methanolic extract of Green tea and Lemon grass with Levofloxcin diluted in ethanol showed best activity against *S.mutans* at all concentrations. (Table 7)

Table 7. Synergistic Antibacterial activity of crude methanol extract of Tea samples with *Levofloxacin* (Antibiotic)

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Lemon Grass	Green Tea
		30	28
2	0.2	25	17
3	0.3	30	17
4	0.4	20	28
5	0.5	20	20
6	0.6	18	21
7	0.7	12	18
8	0.8	16	17
9	0.9	31	18
10	1.0	29	15

The synergistic activities of all methanolic extracts tea samples diluted in Ethanol showed good activity against *S.mutans* at all concentrations. But the lemon grass extract showed the best effective result at the concentration of 0.1-0.3mg/ml. The synergistic combination of green tea showed better result as compared to the black. (Table-7)

Table 8. Synergistic Antibacterial activity of crude methanol extract of Tea samples with *Gentamycin (*Antibiotic)

Serial No	Concentration mg/ml	Zone of inhibition (mm)	
		Lemon Grass	Green Tea
1		30	28
2	0.2	25	17
3	0.3	30	17
4	0.4	20	28
5	0.5	20	20
6	0.6	18	21
7	0.7	12	18
8	0.8	16	17
9	0.9	31	18
10	1.0	29	15

As a result of this finding, the MIC value of the methanol active extracts (Diluted in ethanol) of the green tea was determined and to be bactericidal in concentration of 0.1 mg/ml. The lemon grass was not soluble in methanol. The results obtained by mixing the tea sample synergistically were poor against tested bacterial strain as compared to extract singly but mixture of green gave good response. It is suggested that do not mix the tea extract.

Table 9. MIC values and Statistical analysis of Methanolic extract of Tea samples

Serial No	Tea samples	MIC values in mg/ml	*Statistical analysis at 0.5mg/ml
1-	Green Tea(Methanolic extract diluted in methanol)	0.1	22 (±0.5)
2-	Lemon Grass (Methanolic extract diluted in methanol)	*Not Soluble	-
3-	Green Tea(Methanolic ex tract diluted in ethanol)	0.1	12 (±0.5)
4-	Lemon Grass(Methanolic extract diluted in ethanol)	0.2	25 (±0.5)

*Good activity > 22 **Moderate activity > 15 ***Poor activity < 15

Table 10. MIC values and Statistical analysis of Methanolic extract of Tea samples (Diluted in Ethanol) in synergism (With tea samples and antibiotics)

Serial No	Tea samples	MIC values in mg/ml	*Statistical analysis
1.	Green Tea + Lemon grass	-	-
2.	Green Tea +Chloroamphenical	0.1	22 (±0.5)
3.	Green Tea +Tetracycline	0.1	12 (±0.5)
4.	Green Tea +Levofloxacin	0.1	20 (±0.5)
5.	Green Tea + Gentamycin	0.1	23(±0.5)
6.	Lemon Grass +Chloroamphenical	0.1	22(±0.5)
7.	Lemon Grass +Tetracycline	0.1	8 (±0.5)
8.	Lemon Grass +Levofloxacin	0.1	25 (±0.5)
9.	Lemon Grass + Gentamycin	0.1	18 (±0.5)

The results obtained with the methanol extract were more homogenous and active the test strain (S.mutans). The results obtained with synergism with the antibiotics were best against tested bacterial strain as compared to single extract. It is suggested that tea extract with Chloramphenicol, Levofloxacin and Gentamycin prescribed by the doctors. Tetracyline only response with green tea whiles it enhance the growth of bacteria with black and lemon grass extract.

Finally, the results showed that the tea samples used in the study could be explored for possible antimicrobial agents for treating against various infections of the oral cavity.

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