ANTIBACTERIAL ACTIVITY OF BLACK TEA 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) and non-fermented tea (green tea). Methanolic extract of tea diluted in methanol and ethanol, which were tested for antibacterial activity against *Streptococcus mutans.* Methanol was found to be best antimicrobial solvent. The MIC value of methanolic extracts of Black Tea diluted in ethanol was 0.1 mg/ml. Synergistic results of antibacterial activity of methanolic extracts (diluted in ethanol) were found poor. Synergistic activity of Black tea sample with antibiotics (Chloramphenicol, Tetracycline, Levofloxacin and Gentamycin) showed best response against most of the bacteria (0.1mg/ml).

Key words: Antibacterial activity, Black Tea, *Streptococcus mutans,* synergistic activity

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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 76-78% of tea produced is consumed in the form of Black Tea, 20-22% in form of green tea and less than 2% as oolong tea. Black Tea principally consumed in Europe north America and North Africa. 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 Black tea on stomach cancer has been 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)

Citral has been used for centuries for the treatment of various ailments. Citral helps to combats the depression and bad mood and it used to treat fever cold and cough. A great remedy for stomachache 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

Friedman (2007) studied that the tea leaves produce organic compounds that may be involved in the defense of the plants against invading pathogens including insects, bacteria, fungi, and viruses. These metabolites include polyphenolic compounds, the six so-called catechins, and the methyl-xanthine alkaloids caffeine, theobromine, and theophylline. These substances impart the black color to Black Tea.

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 was 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.

Kim *et.al,* (2001) concluded the antibacterial potential of the methanolic extracts petals of *Camellia japonica* L., against food borne pathogens in microbiological media and food. The extract showed good bactericidal response against the pathogens *Salmonella typhimurium, Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*.

Various components of *Camellia sinensis* either in the form of green or Black Tea are simple catechins, which have anti-cariogenic properties in vitro. These include a direct bactericidal effect against *Streptococcus mutans* and *S. sobrinus*; prevented the bacterial adherence to teeth; inhibition of glucosyl transferase, thus limiting the biosynthesis of sticky glucan and inhibition of human and bacterial amylases (Hamilton .miller.2001)

Lakenbrink (2000) conducted the research on the polyphenolic, flavonoid, and caffeine compositions of four commercial tea bag products (typical of those used in the UK, US, continental Europe, and the Middle East) and beverages prepared from them under a range of typical consumer use conditions have been studied. Leaf composition was determined by extraction with aqueous methanol .The absolute compositions of all four products were remarkably similar in terms of most phenolic compounds. The flavonoids comprised the major proportion (93-94%) of the total phenolics estimated by the Folin-Ciocalteu method.

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. Black Tea extracts effectively inhibited the growth of *S. typhimurium 1402/84, S. typhi, S. typhi Ty2a, S. dysenteriae, Y. enterocolitica C770, and E.coli*. 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) and 5.094 mg/ml Black Tea extract (MIC 9.089 mg/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, Pakistan growing on MSB agar.

Tea samples:

Tea samples used in the present study is Black Tea and collected from different departmental store of Lahore, Pakistan. All the material was in the branded form. The botanical name of black tea is *Camellia sinensis Linn;* family Theaceae and the part of plant used were the Leaves (Post Fermented).

Extraction:

The purchased tea samples (*Black Tea)* was 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 crude 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, Tetracycline, Levofloxacin and Gentamycin) with different concentration (0.1,0.2,0.3, 0.4,0.5,0.6,0.7,0.8,0.9 and 1.0mg/ml).

Bioassay:

MIC and 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, Tetracycline, Levofloxacin and Gentamycin serving as positive control. Synergistic activity against bacterial strain was determined by taking equal amounts (0.1mg/ml) of antibiotics (Chloramphenicol, Tetracycline, Levofloxacin and Gentamycin)

RESULT AND DISCUSSION

It is 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. These results favor 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*.

Table-1 Antibacterial Activity of Methanol Extract of black Tea samples (Dilution in Methanol)

Sr. No	Concentration mg/ml	Zone of inhibition (mm) Black Tea		
		Crude Methanol Extract	Crude Ethanol extract	Synergistic antimicrobial activity of crude methanol extract (diluted in ethanol)
1	0.01	_	-	15
2	0.2	_	-	20
3	0.3	_	-	17
4	0.4	_	-	18
5	0.5	_	-	25
6	0.6	25	-	23
7	0.7	13	10	20
8	0.8	16	-	18
9	0.9	13	-	20
10	1.0	20	-	17

The crude methanolic extract of Black Tea showed full growth of bacteria between the concentrations 0.1-0.5 mg/ml while it showed moderate activity at the concentrations of 0.6- 1.0mg/ml. The crude methanolic extract of Black Tea, which was diluted in ethanol, showed good bactericidal activity nearly at all concentrations. (Table 1)

Table-2 Synergistic Antibacterial activity of crude methanol extract of black tea samples with chloramphenicol, tetracycline, levofloxacin and gentamycin *a*ntibiotics

Sr. No.	Concentrations mg/ ml	Zone of inhibition (mm) of black tea with antibiotics			
		Chloramphenicol	Tetracycline	Levofloxacin	Gentamycin
1	0.1	12	25	18	18
2	0.2	_	28	18	18
3	0.3	_	26	12	12
4	0.4	_	27	17	17
5	0.5	13	20	19	19
6	0.6	12	25	15	15
7	0.7	15	20	18	18
8	0.8	15	25	20	20
9	0.9	14	25	20	20
10	1.0	17	28	18	18

Synergistic activity with different antibiotics:

The crude methanolic extract of black tea mixed with chloramphenicol in same ratio (1:1) diluted in ethanol showed good activity against *S. mutans* at all concentrations. Mixing of extracts with chloramphenicol enhance the activities of tea samples. (Table-4)

The crude methanolic extract of Black Tea when mixed with tetracycline showed moderate results at the concentrations of 0.5-1.0 mg/ml but it gave poor results at other concentrations. The synergistic activity of crude methanolic extract of Black Tea and Levofloxcin diluted in ethanol showed best activity against *S.mutans* at all concentrations. The synergistic combination showed better result as compared to the black tea alone. (Table-1& 4)

Table 3- MIC values and Statistical analysis of Methanolic extract of Tea samples

Sr. No	Tea samples	MIC values in mg/ml	*Statistical analysis at 0.5mg/ml
1	**Black Tea**(Methanolic extract diluted in methanol)	0.6	-
2	**Black Tea**(Methanolic extract diluted in ethanol)	0.1	22 (±0.5)

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

Table 4- MIC values and Statistical analysis of Methanolic extract of Black Tea samples (Diluted in Ethanol) in synergism (With tea samples and antibiotics)

Sr. No	Tea samples	MIC values in mg/ml	*Statistical analysis
1	**Black Tea**+ Chloramphenicol	0.1	22 (±0.5)
2	**Black Tea**+ Tetracycline	0.5	18 (±0.5)
3	**Black Tea**+ Levofloxacin	0.1	18 (±0.5)
4	**Black Tea**+ Gentamycin	0.1	15 (±0.5)

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

CONCLUSION

It is concluded that methanol was the best solvent for extracting antimicrobial substances from Black tea samples. The synergistic activity of all methanolic extracts Black tea samples diluted in ethanol showed good activity against *S.mutans* at all concentrations. In synergism, the Black Tea activity was enhanced with Chloramphenicol, Levofloxacin and Gentamycin. It is suggested that Black tea samples used in the study could be explored for possible antimicrobial agents for various infections of the oral cavity.

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