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Comparative Account on Antibacterial Activity and Phytochemical Analysis of Green Tea and Black Tea

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***Abstract*:**

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the tea plant *Camellia sinensis.* The present research is conducted to study the antibacterial activity of Green tea and Black tea against bacterial pathogens such as *S. aureus* and *E.coli*. It is concluded that ethanolic and methanolic extracts of both green tea and black tea were found to be effective against all the tested bacterial pathogens. On the other hand, aqueous extract of green tea was effective against all the tested bacterial pathogens but aqueous extract of black tea was found to be effective only against 40% bacterial pathogens. When the bacterial pathogens were tested for antibiotic resistance profile, it was found that all *S.aureus* were sensitive (100% each) to Erythromycin, Gentamicin, Penicillin and Tetracycline while resistant to Oxacillin. Antibiotic resistance profile of *E.coli* showed that all *E.coli* were sensitive to Tobramycin followed by 80% was sensitive to Chloramphenicol, Trimethoprim and Vancomycin while all *E.coli* were resistant to Ofloxacin. Aqueous, ethanolic and methanolic tea extracts were checked for the presence of alkaloids, tannins, flavonoids, reducing sugars, saponins, cardiac glycosides and steroids. Qualitative analysis revealed that all the extracts of green tea and black tea showed the presence of flavonoids, tannins, alkaloids, saponins, reducing sugars and cardiac glycosides but the steroids were present only in black tea.

***Index Terms*:** Green Tea, Black Tea, Antimicrobial activity, Phytochemicals

INTRODUCTION

Tea is one of the most popular beverages worldwide. It is cultivated in more than 30 countries worldwide, and of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and 2% is oolong (Graham, 1992; Muktar and Ahmad, 2000). Tea is an aromatic beverage commonly

prepared by pouring hot or boiling water over cured leaves of the tea plant *Camellia sinensis.* After water, tea is the most widely consumed beverage in the world. It has a cooling, slightly bitter, and astringent flavour that many people enjoy. Consumption of tea (especially green) is beneficial to the health and longevity given its antioxidant, flavonoids, polyphenols and catechins content. Tea catechins have known anti-inflammatory and neuroprotective activities, help to regulate food intake. Human studies suggest that green tea may contribute to a reduce the risk of cardiovascular disease and some forms of cancer, as well as promote oral health and other physiological functions such as antihypertensive effect, body weight control, antibacterial and antiviral activity, bone mineral density increase, antifibrotic properties and neuroprotective powers (Parmar et al., 2012).

Tea is mainly produced as four varieties; white, green, oolong and black. White tea is made from young tea leaves or buds; green tea is made from mature unfermented leaves; oolong tea from partially fermented leaves and black tea from fully fermented leaves (Jigisha et al., 2012; Gupta et al., 2010). In black tea, the major polyphenols are thearubigins and theaflavins. Tea polyphenols are also known for their antibacterial activity. Antibacterial activity decreases when the extent of tea fermentation is increased, implying stronger activity in green tea than black tea. Green tea catechins, particularly EGCG and ECG, have antibacterial activity. Green tea can prevent tooth decay by inhibiting oral bacteria. The antibacterial activity of black tea has also been reported (Chan et al., 2011). Therefore present research is conducted to study the antibacterial activity of Green tea and Black tea against bacterial pathogens.

MATERIALS AND METHODS

Preparation of Extracts of Tea Samples: Aqueous, ethanolic and methanolic extracts of all green and black tea samples were prepared by dissolving 50gm tea/50ml solvent (water, ethanol and methanol) in soxhlet apparatus and subjected to extraction. Distillation was carried out for individual tea sample separately and filtrate was vaporized to dryness and weighed. The stock solutions of crude extracts were prepared by dissolving the dried extract with respective solvent to obtain the final concentration of 10mg/ml (Kaur et al., 2015).

Collection and Identification Bacterial Pathogens: To evaluate the antimicrobial activity of green and black tea samples, *Staphylococcus aureus* (n= 5) and *Escherichia coli* (n= 5) were collected from Pathology Laboratory in Nagpur and the cultures were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

Antibiotic Sensitivity Test against Bacterial pathogens: Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer et al., 1966). Five different types of antibiotics were used each for *S.aureus* and *E.coli* (Table 1). Bacterial pathogens including *Staphylococcus aureus* and *Escherichia coli*

were grown on nutrient agar at 37^0C for 24 hours. The colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5X108CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread with the help of spreader. The antibiotic discs were placed on Hi-sensitivity agar. These plates were incubated for 24 hours at 37^0C. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant or sensitive" based on standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Antibacterial Activity of Green tea and Black tea Extracts against Bacterial Pathogens: Green tea and Black tea extracts were screened against selected bacterial pathogens including *Staphylococcus aureus* and *Escherichia coli.* Selected bacterial pathogens were grown overnight on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108 CFU/ml). The suspension (100 µL) was spread over the Hi-Sensitivity agar. The wells of 6 mm diameter were cut into the agar medium with a sterilized cork borer. Then 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition around each well was measured and recorded (Bauer et al., 1966).

Phytochemical Analysis: Phytochemical Analysis of Green tea and Black Tea Extracts was carried out to test alkaloids, tannins, saponins, steroids, reducing sugars, cardiac glycosides and flavonoids (Edeoga et al., 2005).

RESULTS AND DISCUSSION

When the bacterial pathogens were tested for antibiotic resistance profile, it was found that all *S.aureus* were sensitive (100% each) to Erythromycin, Gentamicin, Penicillin and Tetracycline while resistant to Oxacillin (Table 2). Antibiotic resistance profile of *E.coli* showed that all *E.coli* were sensitive to Tobramycin followed by 80% was sensitive to Chloramphenicol, Trimethoprim and Vancomycin while all *E.coli* were resistant to Ofloxacin (Table 3).

It was found that ethanolic and methanolic extracts of both green tea and black tea were found to be effective against all the tested bacterial pathogens. On the other hand, aqueous extract of green tea was effective against all the tested bacterial pathogens but aqueous extract of black tea was found to be effective only against 40% bacterial pathogens (Table 4). According to Kumar et al., (2012) methanolic extracts showed higher zone of inhibitions as compared to aqueous and ethanolic extracts. It may be suggested that ethanolic and methanolic extracts of tea can serve as a good source for invention of new therapeutic agents to kill pathogenic bacteria. Results of this study revealed that organic solvents had greater potential for extraction of biologically active compounds and pharmacologically active substances than water and different methodologies used for processing of tea showed no effect on these compounds. It is clear that

always organic solvents exhibit the stronger efficiency in extraction of antimicrobial compounds as compared to other methods and a few studies mentioned that organic solvent extracts exhibits the superior antimicrobial activity (Ponnanikajamideen et al., 2014).

Aqueous, ethanolic and methanolic tea extracts were checked for the presence of alkaloids, tannins, flavonoids, reducing sugars, saponins, cardiac glycosides and steroids. Qualitative analysis revealed that all the extracts of green tea and black tea showed the presence of flavonoids, tannins, alkaloids, saponins, reducing sugars and cardiac glycosides but the steroids were present only in black tea (Table 5). These results were found to be similar with the results of previous study of Subhashini et al., 2010; Sharma et al., 2011. Kaur et al., (2015) reported that flavonoids possess anti glycosyl activity and can inhibit adherence of microbes. Tannins can inhibit both glucosyl transferace (GFT) activity and bacterial growth by their strong iron-binding capacity. Alkaloids interfere with the division of cells thus inhibiting their growth.

CONCLUSION

It is concluded that ethanolic and methanolic extracts of both green tea and black tea were found to be effective against all the tested bacterial pathogens. On the other hand, aqueous extract of green tea was effective against all the tested bacterial pathogens but aqueous extract of black tea was found to be effective only against 40% bacterial pathogens. When the bacterial pathogens were tested for antibiotic resistance profile, it was found that all *S.aureus* were sensitive (100% each) to Erythromycin, Gentamicin, Penicillin and Tetracycline while resistant to Oxacillin. Antibiotic resistance profile of *E.coli* showed that all *E.coli* were sensitive to Tobramycin followed by 80% was sensitive to Chloramphenicol, Trimethoprim and Vancomycin while all *E.coli* were resistant to Ofloxacin. Multiple drug resistant strains are on the rise in this era and thus complicating treatment. Moreover, the extracts of green tea and black tea were rich source antibacterials and phytoconstituents and all the tea samples were found similar on the basis of phytochemicals and antibacterials. On the other hand herbal preparations are comparatively cheaper and have lesser side effects. Further research is required to study the main active compounds in the extracts of tea.

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Table 1: Antibiotics used in the study

S. aureus		*E. coli*	
Antibiotics	**Concentration**	**Antibiotics**	**Concentration**
Erythromycin	15mcg	Chloramphenicol	30mcg
Gentamicin	10mcg	Ofloxacin	5mcg
Oxacillin	1mcg	Tobramycin	10mcg
Penicillin	10units	Trimethoprim	5mcg
Tetracycline	30mcg	Vancomycin	30mcg

Table 2: Antibiotic Resistance Profile of *S.aureus*

Bacterial Pathogens	Erythromycin	Gentamicin	Oxacillin	Penicillin	Tetracycline
S.aureus 1	18mm	18mm	R	14mm	22mm
S.aureus 2	27mm	27mm	R	26mm	25mm
S.aureus 3	21mm	22mm	R	26mm	23mm
S.aureus 4	26mm	16mm	R	22mm	23mm
S.aureus 5	26mm	21mm	R	18mm	21mm

Where, R= Resistant

Table 3: Antibiotic Resistance Profile of *E.coli*

Bacterial Pathogens	Chloramphenicol	Ofloxacin	Tobramycin	Trimethoprim	Vancomycin
E.coli 1	25mm	R	17mm	24mm	R
E.coli 2	R	R	14mm	22mm	22mm
E.coli 3	23mm	R	14mm	R	17mm
E.coli 4	21mm	R	12mm	21mm	20mm
E.coli 5	19mm	R	18mm	18mm	16mm

Table 4: Antimicrobial Activity of Green Tea and Black Tea against Bacterial Pathogens

Bacterial Pathogens	Green Tea			Black Tea		
	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
S.aureus 1	16mm	18mm	20mm	R	12mm	13mm
S.aureus 2	17mm	18mm	18mm	R	11mm	13mm
S.aureus 3	16mm	19mm	20mm	13mm	14mm	15mm
S.aureus 4	11mm	19mm	23mm	R	13mm	14mm
S.aureus 5	11mm	18mm	21mm	11mm	12mm	14mm
E.coli 1	17mm	23mm	24mm	R	16mm	15mm
E.coli 2	14mm	22mm	26mm	11mm	14mm	17mm
E.coli 3	15mm	20mm	21mm	12mm	13mm	16mm
E.coli 4	19mm	19mm	19mm	R	11mm	17mm
E.coli 5	23mm	24mm	24mm	R	11mm	17mm

Table 5: Phytochemical Analysis of Green Tea and Black Tea

Phytochemicals	Green Tea			Black Tea		
	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Reducing Sugars	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Steroids	-	-	-	+	+	+

Where, + Present, - Absent