JOURNAL OF PURE AND APPLIED MICROBIOLOGY, December 2012. Vol. **6**(4), p. 2011-2015

Comparative Profile of the Antimicrobial Activities of Assam, Dooars and Darjeeling Tea Leaves (*Camellia sinensis* L).

Debajyoti Mukherjee[1,2], Paromita G. Bhattacharjee[2] and Sutanu Samanta[1]*

[1]Lovely Professional University, NH-1, Phagwara - 144 411, India.
[2]Institute of Genetic Engineering, Badu, Kolkata - 700 128, India.

(Received: 07 May 2012; accepted: 07 June 2012)

Tea, both green tea and fermented tea, have been proved to have high antimicrobial activity against many pathogenic organisms. But status of antimicrobial activity of three tea e.g. Darjeeling tea, Dooars tea and Assam tea is yet to be known. In this study, we have shown the antimicrobial activity of three types of tea against *Vibrio cholerie, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella spp*.All types of tea have been found to have high antimicrobial activity as all three types of tea have shown at least 1.4 cm of zone of inhibition against all the pathogenic microorganisms used in this study when 100 µl of ethanolic extract of each tea was used. Among these three types of tea, Dooars tea has highest antimicrobial activity against *Pseudomonas aeroginosa and* Assam tea has highest antimicrobial activity against all other microorganisms. When minimum inhibitory concentration of each tea against each microorganism have been tested, it has been found that Assam tea has the least inhibitory concentration against all the pathogenic microorganisms and it was 20µl of ethanolic concentration. So, Assam tea can be exploited as formulating drugs against those pathogens apart from its use as beverage.

Key words: MIC, Zone of inhibition, antimicrobial activity, extract, ethanol.

Tea is a beverage and is of mixture of variously processed leaves of one of the varieties of an evergreen shrub, *Camellia sinensis* L. It is the most widely drunk beverage in the world (Stagg, et al 1980). It is refreshing, mildly stimulating, and creates a feeling of well-being. These properties may be due to caffeine and tannins. These compounds are known to have stimulant and anti-soporific actions which can elevate mood, decrease fatigue and increase capacity for work (Rall, 1990). However, other components of tea, notably, the polyphenols, may also contribute to the effects of tea with respect to their known pharmacological properties (Stagg, *et al* 1975). The complex of oxidized polyphenols in tea is often called 'tannin' (Kirk, 1980). From literature, it was observed that tea has wide range of antioxidant, anti-inflammatory, anti-carcinogenic and antibacterial activity against many pathogens (Hamilton Miller, 1975; Diker, 1991; Ryu, 1982; Todd, 1989). Several reports of the antibacterial effects of tea *in vitro* and *in vivo*, mainly against intestinal pathogens were published by many researchers (Das, 1962; Ryu, 1980; Scalbert, 1991). The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols (Sofowara, 1984; Opara, 1992). Toda *et al.* (1989a) also showed that moderate daily consumption of green tea killed *Staphylococcus aureus* and other harmful bacteria. Recent reports have revealed the tea's antibacterial and bactericidal properties on various bacterial strains isolated from patients with infected root canal (Horiba, 1991). Subsequently,

* To whom all correspondence should be addressed.
Mob.: +91-8146302486;
E-mail: sutanusamanta@gmail.com

several studies on the antimicrobial properties of Japanese tea have been reported (Toda *et al.,* 1989a; Sakanaka, *et al.,* 1989; Okubo *et al.,* 1989). The antibacterial activity of Turkish tea against *Campylobacter* sp. and *Listeria monocytogenes* is well documented. Teas are largely produced in Asian countries like India, China, and Sri Lanka. Indian teas have achieved a special status in tea global market as Indian tea is of superior quality. In India, different brands of tea e.g. Darjeeling Tea, Assam Tea and Dooars tea are produced in different geographical locations and they have different texture and aroma and different degree of popularity among tea drinkers. Darjeeling tea has often been called the "champagne of tea," and has traditionally been prized in the United Kingdom above all other black teas. Keeping the well known antimicrobial activity of tea, these three types of tea are yet to be tested against many pathogenic microorganisms especially *Salmonella* spp. which causes Diarrhea, *Vibrio cholerae* which causes cholera, *Pseudomonas aeruginosa* which is the causative agent of burn infections and *Staphylococcus aureus* which causes scaled skin syndrome, toxic shock syndrome and septicemia. Therefore, this study is aimed at comparative analysis of the antibacterial activities of three types of tea on those specified microorganisms.

MATERIALS AND METHODS

Collection of tea leaves

The air-dried leaves of green tea leaf (*Camellia sinensis*) were collected various different sources.

Darjeeling tea	:	Makaibari Tea Estates, Darjeeling, West Bengal
Assam tea	:	Chabua tea estates, Assam
Dooars tea	:	Green view tea plantation, Jalpaiguri district, West Bengal, India

Ethanol Extraction

The leaves were cut into pieces and grinded into powdery form using a sterile electric grinder. The soluble ingredients in the grounded plant part were then extracted by using ethanol (25 g of the grinded leaves were extracted using 250 ml of 99.9% ethanol). The ethanol extract of the active ingredient of the leaves was concentrated by heating at about 50-60°C and cooled at room temperature over 2 hours. This concentrated alcoholic extract was used for further experiment.

Test Pathogenic Organisms

The pathogenic bacteria used in this work were *Pseudomonas aeruginosa, Vibrio cholerae, Salmonella* spp. and *Staphylococcus aureus* obtained from laboratory stock of Institute of Genetic Engineering, Badu, Kolkata, West Bengal, India. These bacteria were maintained by weekly transfer in a nutrient agar media and were grown at 37ºC for 48 hours in incubator.

Antibacterial susceptibility testing

The antimicrobial efficacy of the leaf extracts were examined on the test pathogenic bacteria using the agar cup method (Miller, 1939). In this method, 20 ml of a 24 hours broth culture of the bacteria was aseptically introduced and evenly spread using a bent sterile glass rod on the surface of gelled sterile nutrient agar plates. Wells of about 5.0 mm diameter were aseptically punched on agar-plate using a sterile cork borer allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Different volumes (150 ml, 100 ml, 80 ml, 60 ml, and 50 ml) of the leaf extract were then introduced into the wells in the plates. A control well was created in the plate with of the extracting solvent (ethanol). The plates incubated at 37°C for 24 hours for the test bacteria. The plates were triplicated in all the experiments. Antimicrobial activity of the extract against these test bacteria was indicated by growth free "zone of inhibition" surrounds the respective well.

Measurement of minimum inhibitory concentration

For the MIC test, 20 ml of a 24 hours broth culture of the bacteria was aseptically introduced and evenly spread using bent sterile glass rod on the surface of gelled sterile Nutrient agar plates. Different volumes (10 ml, 20 ml, 30 ml, 40 ml, 50 ml) of the leaf extract were then introduced into the wells in the plates. The plates incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

Examination of antibacterial activity of three types of tea on different pathogenic bacteria

Toda *et al.* (1989b) reported that daily consumption of green tea can kill gram positive

S. aureus and other harmful bacteria. Also it has been reported (Ahn *et al.,* 1991; Wakayama *et al*., 1993; Makhtar *et al.*, 1994; Sakanaka *et al.,* 1996; Kuroda, 1999) that the green tea contains catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic properties. But status of antibacterial activity of Darjeeling tea, Assam tea and Dooars tea is yet to be examined and their comparative profile of antibacterial efficacy is not depicted as yet. Therefore, we have tried to examine the antibacterial efficacy of three different types of tea largely available in eastern part of India e.g. Darjeeling tea, Assam tea and Dooars tea on four different types of pathogenic bacteria by agar cup method. In this experiment, different concentrations of three different type's tea were used to depict a comparative antibacterial activity profile and it was shown in table 1, 3 and 5. Three types of tea have demonstrated varying antibacterial activity profile against four different types of pathogenic bacteria. Dooars tea has shown highest antibacterial activity against *Pseudomonas aeroginosa* as it produced zone of inhibition having diameter 2.4 ± 0.23 cm when 100 ml ethanolic extract of tea was used. Assam tea has shown best antimicrobial activity against *Vibrio cholerae* and diameter of zone of inhibition was 2.0 ± 0.2 cm when 100 µl ethanolic extract was used. Assam tea has also shown best antimicrobial activity against *Staphylococcus aureus* and *Salmonella* spp. So, among the three types of tea, Assam tea has been found to be best in regards of its antimicrobial activity as it has demonstrated highest antimicrobial activity against maximum number of pathogenic organisms examined in this study.

Table 1. Profile of antibacterial activity of Darjeeling Tea

Type	Extract Volumes (µl)	*Salmonella* sp (Zone of inhibition in cm)	*Vibrio cholerae* (Zone of inhibition in cm)	*Pseudomonas* (Zone of inhibition in cm)	*Staphylococcus aureus* (Zone of inhibition in cm)
Darjeeling tea extract	150	2 ± 0.21	1.8 ± 0.2	1.8 ± 0.19	2.1 ± 0.21
	100	1.5 ± 0.13	1.6 ± 0.16	1.6 ± 0.17	1.8 ± 0.17
	50	1.3 ± 0.14	1.3 ± 0.12	1.4 ± 0.12	1.5 ± 0.14
	Control	-	-	-	-

Table 2. Minimum Inhibitory Concentration of Darjeeling Tea

Type	Extract Volumes (µl)	*Salmonella* sp (Zone of inhibition in cm)	*Vibrio cholerae* (Zone of inhibition in cm)	*Pseudomonas* (Zone of inhibition in cm)	*Staphylococcus aureus* (Zone of inhibition in cm)
	10	-	-	-	-
Darjeling tea extract	20	-	1±0.12	-	1.2 ± 0.13
	30	-	1.1 ± 0.1	1.1 ± 0.11	1.3 ± 0.12
	40	1.1 ± 0.10	1.2 ± 0.12	1.3 ± 0.12	1.4 ± 0.15
	50	1.3 ± 0.16	1.3 ± 0.16	1.4 ± 0.15	1.5 ± 0.17

Table 3. Profile of antibacterial activity of DooarsTea

Type	Extract Volumes (µl)	*Salmonella* sp (Zone of inhibition in cm)	*Vibrio cholerae* (Zone of inhibition in cm)	*Pseudomons* (Zone of inhibition in cm)	*Staphylococcus aureus* (Zone of inhibition in cm)
	150	1.9 ± 0. 21	1.9 ± 0. 22	2.8 ± 0. 27	1.6 ± 0.18
Darjeling tea	100	1.7 ± 0.19	1.8 ± 0. 20	2.4 ± 0.23	1.4 ± 0.13
extract	50	1.4 ± 0.13	1.4 ± 0.16	1.8 ± 0.20	1.3 ± 0.11
	Control	-	-	-	-

Table 4. Minimum inhibitory concentration of Dooars tea

Type	Extract Volumes (µl)	*Salmonella* sp (Zone of inhibition in cm)	*Vibrio cholerae* (Zone of inhibition in cm)	*Pseudomonas* (Zone of inhibition in cm)	*Staphylococcus aureus* (Zone of inhibition in cm)
Dooars	10	-	-	-	-
tea extract	20	-	1.2 ± 0.14	1.3 ± 0.13	1 ± 0.95
	30	1.2 ± 0.14	1.3 ± 0.11	1.5 ± 0.18	1.1 ± 0.13
	40	1.3 ± 0.11	1.3 ± 0.15	1.7±0.18	1.2 ± 0.11
	50	1.4 ± 0.16	1.4 ± 0.16	1.8 ± 0.16	1.3 ± 0.12

Table 5. Profile of antibacterial activity and minimum inhibitory concentration of Assam Tea

Type	Extract Volumes (µl)	*Salmonella* sp (Zone of inhibition in cm)	*Vibrio cholerae* (Zone of inhibition in cm)	*Pseudomonas* (Zone of inhibition in cm)	*Staphylococcus aureus* (Zone of inhibition in cm)
Assam tea	10	-	-	-	-
extract	20	1.2 ± 0.11	1.3 ± 0.13	1.4 ± 0.11	1.3 ± 0.11
	40	1.3 ± 0.17	1.3 ± 0.15	1.6 ± 0. 16	1.4 ± 0.16
	60	1.6 ± 0.17	1.5 ± 0.14	1.6 ± 0.13	1.5 ± 0.18
	80	1.7 ± 0.19	1.7 ± 0.16	1.7 ± 0.19	1.7 ± 0.2
	100	1.8 ± 0.21	2.0 ± 0.2	1.9 ± 0.17	2 ± 0.22
	Control	-	-	-	-

Minimum inhibitory concentration of each tea on each pathogenic bacterium

The minimum inhibitory concentration (MIC) of the extracts is regarded as the lowest concentration of the extract that did not permit turbidity or growth of the test organism. The MIC of each type of tea on each microorganism is presented in Table 2, 4 and 5. For all types of microorganisms, Assam tea has shown the least minimum inhibitory concentration against all types of pathogenic microorganisms.

CONCLUSION

This study has assisted us to depict a clear picture about the antimicrobial activities of three types of tea produced in the eastern part of India which have gained popularity all over the world. Our study has proved that all these three types of tea have appreciable amount of antimicrobial against a battery of pathogenic organisms which are causative agent of diarrheal diseases and burn infection. Assam tea is best among three in terms of MIC. Darjeeling tea has best aroma and texture and so it is the most popular among as a drink all over the world and even as compared to Dooars tea, Assam tea is less popular, but Assam tea has been shown to have highest antimicrobial activity. Therefore, Darjeeling tea can be regarded as popular drink, but Assam tea has great potential to develop drug for diarrheal diseases and burn infection. Hence, blend of Assam tea and Darjeeling tea can meet the twin purpose e.g. drink and antimicrobial activity.

ACKNOWLEDGMENTS

We would like to express sincere gratitude to Dr. Amit Chakraborty and Dr. Sudipa Chakraborty for their suggestions. We would like to thank Ms. Preeti Singh and Ms. Sreeparna Saha for their timely help at the bench level. We are also thankful to Mr. Anup Kumar Mukherjee and Mr. Joy Chatterjee for providing the tea leaves.

REFERENCES

1. Ahn, Y.J., Kawamura, T., Kim, M., Yamamoto, T., Mitsuoka, T. Tea Polyphenols: Selective growth inhibitors of *Clostridium spp*. *Agric. Biol. Chem*., 1991; **55**:1425-1426.
2. Das, D.N.Studies on the antibiotic activity of tea. *J. Ind. Chem. Soc.*, 1962; **39**:849–854.
3. Diker, K.S., Akan, M., Hascelik, G., Yurdakok, M. The bactericidal activity of tea against *Campylobacter jejuni* and *Campylobacter coli*. *Lett. Appl.Microbiol.*, 1991; **12**: 34-35.
4. Hamilton-Miller, J.M. Antimicrobial properties of tea (Camellia sinensis L.) *Antimicrob Agents. Chemother,* 1995; **39**: 2375-2377.
5. Horiba, N., Maekawa, Y., Ito, M., Yamamoto, T. A pilot study of Japanese green tea as a medicament, antibacterial and bactericidal effects. *J.Endodonitics*, 1991; **17**: 122-124.
6. Kirk, R.E., Othmer, D.F. Encyclopedia of chemical technology, 3rd ed., vol. 22 John Wiley & Sons, Inc., New York, 1980; 628-648.
7. Kuroda, Y., Hara, Y.Antimutagenic and anticarcinogenic activity of tea polyphenols. *Mutat. Res.*, 1999; **436**(1): 60-67.
8. Makhtar, H., Hatiyar, S., Agarwal, P. Green tea and skin anti carcinogenic effect. *J. Invest Dermatol.*, 1994; **102**: 3-7.
9. Miller, R.E., Rose, S.B. Studies with the agar cup-plate method. The influence of agar on mercury antiseptics Department of Bacteriology, Woman's Medical College, Philadelphia, and the Division of Bacteriology, Philadelphia General Hospital, 1939.
10. Okubo, S., Ikigia, H., Toda, M., Shimamura, T. The anti-haemolysin activity of tea and coffee. *Lett. Appl. Microbiol*., 1989; **9**: 65-66.
11. Opara, A. A., Ansa, M.A.The antibacterial activity of tea and coffee in selected organisms. *J. Med. Lab. Sci.*, 1993; **3**: 45-48.
12. Rall, T.W.. in A. G. Gilman, T.W. Rall, A. S. Nies, P.Taylor, (ed.),Goodman and Gilman's the pharmacological basis of therapeutics, 8th ed.,Pergamon, New York, 1990; pp 618–637.
13. Ryu, E. D., Blenden, C., Wendall, D.The inhibition of growth of selected bacteria by incorporating powdered tea in the medium. *Int. J. Zoonoses*, 1982; **9**: 73-77.
14. Ryu, E. Prophylactic effect of tea on pathogenic microorganism infection to human and animals. *Int. J. Zoonos*, 1980; **7**:164-170.
15. Sakanaka, S., Kim, M. , Taniguchi, M. , Yamamoto, T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans* a cariogenic bacterium. *Agric Biol. Chem.*, 1989; **53**: 2307-2311.
16. Sakanaka, S., Aizawa, M., Kim, M., Yamamoto, T. Inhibitory effect of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. *Biosci. Biotechnol. Biochem.* 1996; **60**: 745-749.
17. Scalbert, A. Antimicrobial properties of tannins. *Phytochem,* 1991; **30**: 3875-3883.
18. Sofowara, A. A medicinal plants and Traditional medicine in Africa, John Wiley Chichester, 1998, pp 256.
19. Stagg, G. V., Millin, D. J. The nutritional and therapeutic value of tea: a review. *J. Sci. Food Agric.*, 1975; **26**: 1439-1459.
20. Stagg, G. V. Tea the elements of a cuppa.*Nutr. Bull.*, 1980, **29**: 233-245.
21. Toda, M., Okubo, S., Hiyoshi, R., Shimamura, T. Antibacterial and bactericidal activities of Japanese green tea. *Jpn. J. Bacteriol.*, 1989a; **44**(4): 669-672.
22. Toda, M., Okubo, S., Hiyoshi, R., Shimamura, T. Antibacterial and bactericidal activities of Japanese green tea. *Nippon Saikingaku Zasshi*, 1989b; **44**(4):669-672
24. Wakayama, M., Suzuki, K., Toda, M., Okubo, S., Hara, Y., Shimamura, T. Inhibition of the infectivity of influenza virus by tea polyphenols. *Antiviral Res.*, 1993; **1**: 289-299.