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IN-VITRO STUDIES ON THE INHIBITORY EFFECTS OF GREEN TEA EXTRACTS AGAINST ENTEROPATHOGENS

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Abstract

The thrust towards green medicine is continuously increasing nowadays. Numerous reports are available about the antimicrobial activity of green tea leaves. In this study, we examined antimicrobial activity of green tea leaf extracts against eight enteropathogens. All the extracts recorded antimicrobial activity, but the ethanol extracts came out with promising results. Among the eight different pathogens used, *Salmonella typhi* and *Staphylococcus aureus* showed great susceptibility towards all the extracts used in the present study. Therefore, the results of the study obviously justified the positive side of green tea consumption.

Keywords: green tea leaf, enteropathogens, antimicrobial activity.

Introduction

Since from the ancient times, plant have been extensively utilized as green medicine for treating various human ailments. In India, about 45,000 plant species are present among them thousands have been reported to possess medicinal properties (Namita *et al*., 2012). The medicinal plants have been used in Ayurveda, Unani, Siddha traditional medicines, but only very few plants have been studied chemically and pharmacologically for their potential medicinal value (Gupta *et al*., 2008; Sandhu *et al*., 2005).

Tea is the most popular beverage consumed by humans to refresh themselves, and it can contribute many beneficial health effects. Tea is prepared from the leaves of the plant *Camellia sinensis* belonged to the family Theaceae. The habit of drinking tea began about 5000 years ago in southwest China, where it was used medicinally (Adak and Gabar, 2011). Tea leaves are known for its antimicrobial activity against many microorganisms. Tea is generally divided into many categories based on how it is processed (Sharangi, 2009).

The chemical composition and difference in antimicrobial activity of green tea varies with climate, season, horticultural practices and even in the position of the leaf on the harvested shoot. The phytochemical screening of tea have been studied by many authors and the tea leaves found to contain alkaloids, saponins, tannins, catechins and polyphenols (Mbata *et al*., 2006). The polyphenols are important among them. The major flavonoids in green tea are the catechins, epicatechin (EC), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). Epigallocatechin gallate is reported to be the most active component. The leaf bud and first leaves are richest in EGCG (Sinija and Mishra, 2008; Graham, 1992; Katiyar *et al*., 2001). Toda *et al*. (1989) reported that daily consumption of green tea could be effective against most harmful bacteria including *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholerae*. It is a common practice in villages of Tamil Nadu to drink black tea to get rid of illness related with gastric discomforts like vomiting, indigestion, diarrhea, abdominal cramps etc. With this in mind, we have chosen green tea to examine antimicrobial activity of

different green tea extract against eight clinical isolates of enteropathogens.

Materials and Methods

Collection of plant materials

The green tea leaves used in this experiment were collected from Ooty tea garden, Tamil Nadu. The fresh plant leaves were washed in running tap water to remove soil, dust and ethanol for 1 minute. The washed leaves were dried in shade and ground into fine powder using mixture grinder. The powder was transferred in an airtight bottle and stored in refrigerator at 4ºC.

Preparation of plant extracts

Cold extraction method was followed in the present study. About 50.0 g of dry powder of plant leaves mixed with 200 ml of 99% ethanol. The mixture was kept at room temperature with occasional shaking for 1 week. Then the mixture was filtered through a muslin cloth for the first time and finally filtered through Whatman No. 1 filter paper. The supernatant thus obtained was transferred to an air tight bottle and stored at 4ºC until further use. The same procedure was adopted for aqueous extract preparation (100 g powder + 100 ml of distilled water).

For the preparation of tea decoction, exactly 2 g of tea powder was mixed with 100 ml of sterile distilled water and boiled for 15 minutes. Then the tea extract was filtered through the Whatman No. 1 filter paper and the extract was stored in an air tight bottle.

Test organisms used

The bacterial pathogens used in this experiment were procured from Division of Microbiology, Annamalai University, Chidambaram. The enteropathogens selected were *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter jejuni*, *Liseria monocytogenes* and *Shigella dysenteriae.*

Test for antimicrobial activity

Agar well diffusion method was adopted to assess the antimicrobial activity of green tea extracts against enteropathogens. The crude extracts were diluted so as to give the final concentration of 250 ppm, 500 ppm and 1000 ppm. About 0.2 ml of the test culture was swabbed on the surface of Muller–Hinton agar plates, and the wells (6 mm) were filled with different concentration of extracts. After incubation of 24 hrs, the antibacterial efficacy of the extracts were determined by measuring the zone of inhibition formed around the well.

Results and Discussion

Nowadays, there is an increased interest in the production of plant-based drugs for the treatment of many diseases (Aruljothi *et al*., 2014). In Tamil Nadu, majority of the people know the value of traditional plant-based medicines, hence they pick up and used to treat human ailments. In the present study, the inhibitory effect of green tea extracts against certain enteropathogens were determined. In all types of extracts, the less concentration i.e., 250 ppm recorded poor results when compared with 1000 ppm concentration. Overall results revealed that, ethanol extracts exerted good antibacterial activity than aqueous extract and tea decoction (Tables 1, 2 and 3).

Table-1 Inhibition effects of aqueous extract of green tea leaves against enteropathogens

S. No	Enteropathogens	Zone of inhibition (mm)		
		Concentration in ppm		
		250 ppm	500 ppm	1000 ppm
1	*Escherichia coli*	4.21 ± 0.14	6.23 ± 0.12	14.76 ± 0.23
2	*Salmonella typhi*	6.04 ± 0.19	12.54 ± 0.24	22.53 ± 0.39
3	*Vibrio cholera*	3.57 ± 0.07	4.23 ± 0.04	8.74 ± 0.30
4	*Staphylococcus aureus*	7.05 ± 0.14	13.54 ± 0.07	20.08 ± 0.30
5	*Bacillus cereus*	4.12 ± 0.02	9.32 ± 0.24	13.57 ± 0.12
6	*Campylobacter jejuni*	2.08 ± 0.05	3.62 ± 0.04	6.90 ± 0.08
7	*Listeria monocytogenes*	4.57 ± 0.07	7.35 ± 0.05	11.02 ± 0.23
8	*Shigella dysenteriae*	3.92 ± 0.09	7.43 ± 0.12	14.65 ± 0.27

Table-2 Inhibition effects of ethanol extracts against pathogens

S. No	Enteropathogens	Zone of inhibition (mm)		
		Concentration in ppm		
		250 ppm	500 ppm	1000 ppm
1	*Escherichia coli*	5.07 ± 0.23	7.03 ± 0.10	16.28± 0.43
2	*Salmonella typhi*	10.35 ± 0.87	16.23 ± 0.37	24.71 ± 0.36
3	*Vibrio cholera*	3.15 ± 0.09	6.74 ± 0.06	12.53 ± 0.15
4	*Staphylococcus aureus*	8.23 ± 0.12	15.06 ± 0.09	23.15 ± 0.27
5	*Bacillus cereus*	4.67 ± 0.05	9.12 ± 0.07	16.07 ± 0.11
6	*Campylobacter jejuni*	3.41 ± 0.11	5.64 ± 0.02	8.12 ± 0.06
7	*Listeria monocytogenes*	6.95 ± 0.07	9.71 ± 0.07	13.08 ± 0.10
8	*Shigella dysenteriae*	4.05 ± 0.06	8.49 ± 0.06	15.91 ± 0.22

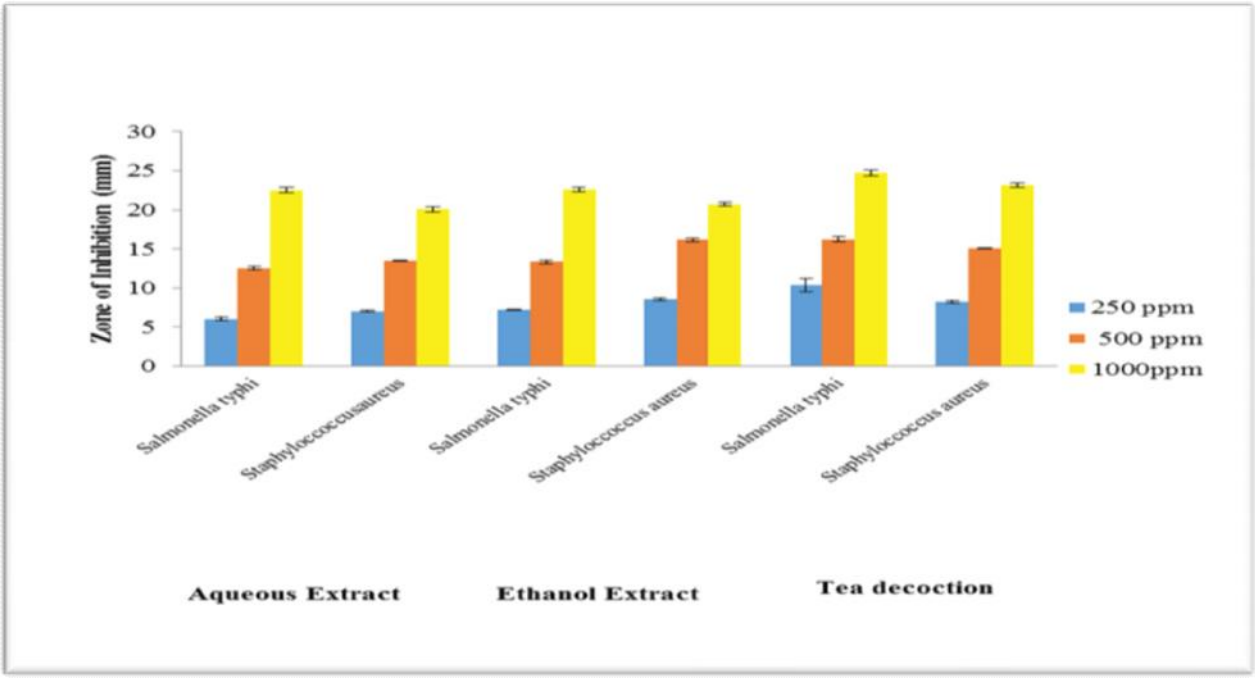
Table-3 Inhibition effects of green tea decoction against enteropathogens in agar well diffusion method

S. No	Enteropathogens	Zone of inhibition (mm)		
		Concentration in ppm		
		250 ppm	500 ppm	1000 ppm
1	*Escherichia coli*	3.84 ± 0.05	7.61 ± 0.03	13.24 ± 0.18
2	*Salmonella typhi*	7.23 ± 0.06	13.34 ± 0.24	22.61 ± 0.28
3	*Vibrio cholera*	3.62 ± 0.05	5.63 ± 0.04	9.34 ± 0.02
4	*Staphylococcus aureus*	8.56 ± 0.12	16.14 ± 0.23	20.71 ± 0.23
5	*Bacillus cereus*	4.24 ± 0.05	6.19 ± 0.05	14.64 ± 0.19
6	*Campylobacter jejuni*	4.82 ± 0.06	6.64 ± 0.04	7.54 ± 0.09
7	*Listeria monocytogenes*	5.23 ± 0.07	8.94 ± 0.06	12.71 ± 0.11
8	*Shigella dysenteriae*	4.72 ± 0.08	9.62 ± 0.13	14.43 ± 0.17

In aqueous extracts, 250 ppm concentration showed lesser inhibitory activity than 500 ppm and 1000 ppm. The pathogens, *Salmonella typhi* (22.53 ± 0.39) and *Staphylococcus aureus* (20.08 ± 0.31) get inhibited effectively at 1000 ppm concentration of aqueous extracts (Table-2). Ethanol extracts produced maximum inhibitory activity against *Salmonella typhi* (24.7 ± 0.3), and *Staphylococcus aureus* (23.15 ± 0.27) (Table-1). Both *Salmonella typhi* and *Staphylococcus aureus* were sensitive considerably to all the three extracts tested including tea decoction (Fig-1). Kiran *et al*. (2010) suggested that black tea extracts enhances immune response against bacteria, reduces expression of virulence expression factors in bacteria and also inhibits enteropathogens. Tea contains nearly 4000 bioactive compounds of which one third is polyphenols (Tariq *et al*., 2010). Ciraj *et al*. (2001) examined antibacterial activity of black tea extract against *Salmonella typhi* causing enteric fever. Alcoholic extracts showed greater inhibitory activity against *Salmonella typhi*.

Tea catechin EGCG, effectively inhibited MRSA strains (Toda *et al*., 1991). Numerous studies revealed the antibacterial activity of tea extracts against various pathogenic harmful bacteria of ocular pathogens, oral pathogens, food spoilage causing bacteria, food borne pathogens, phytopathogens *etc.* (Friedman, 2007). The polyphenols and catechins have been found to contribute antibacterial and anticarcinogenic properties. Among the catechins, EGCG is stable and can persist in the stomach for 2 hrs after ingestion. So this attribute of EGCG might be the reason behind their antimicrobial property against various enteropathogens.

Figure-1 Inhibition effects of green tea extracts against *Salmonella typhi* and *Staphylococcus aureus* at different concentrations



Daily consumption of green tea could suppress the harmful activity of enteropathogens there by reducing the risk of getting gastrointestinal disorders. Najgebauer-Lejko (2014) reported that green tea supplementation in probiotic milk could exert antimicrobial action without altering lactic acid bacterial population. Mbata *et al*. (2008) reported the antibacterial activity of green tea leaf extracts against *Liseria monocytogenes* by disc diffusion method. According to them, methanol extracts showed greater antibacterial activity than the water extract. Our study also implied that ethanol extract produced large zone of inhibition than aqueous extract. Mandal *et al.* (2010) analyzed the inhibitory effect of black tea extracts against enteropathogens including *Salmonella typhi* and *Vibro cholerae* ogawa isolates.

This study concluded that green tea extracts can minimize (or) kill the enteropathogens in *invitro* condition. Therefore, daily consumption of green tea not only exerts control over the enteropathogens but also contribute different health benefits to the consumers.

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