

Original Research Article

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Antibacterial Activity of Green Tea Leaves

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ABSTRACT

Keywords

Tea leaves, *Camellia sinensis*,
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The aim of the present investigation is to study the antibacterial activity of different green tea leaves subjected for solvent extraction against seven clinically isolated antibiotic resistant bacteria. Different types of tea leaves such as mother, scale (cataphyll), first, second and third and buds of tea plant were extracted successfully with petroleum ether, ethyl acetate and chloroform and tested for their antibacterial activity by well diffusion method. The results showed that a remarkable antibacterial activity of green tea samples of first and second leaves followed by leaf bud against the tested organisms was recorded. However zone of inhibition is moderate in third leaf and mother leaf which may be due to several factors including origin of tea leaves, vegetation habits, age of the leaves and extract preparation. Ethyl acetate extract of tea leaves was found to be the most effective against human pathogenic organisms in terms of growth inhibition.

Introduction

In recent years it has been turned the attention from chemotherapeutic agents that to focus on green pharmacy which may be due to immediate action and metabolism. According to World Health Organization (WHO) report, traditional medicine systems serve the health need of about 80% of the world's population (WHO, 2013). Traditional system of medicine is being followed in preparing novel drug products from medicinally important plants. The increasing concentration of drugs towards green pharmacy may be due to emergence of antibiotic resistance organisms, side effects

and economic concern too. The alarming situation on the steady increase of antibiotic resistance microorganisms throughout the world, which resulted increased illness followed by deaths (Levy, 2002) and highlighting the search for a novel antimicrobial agents (Stepanovic *et al.*, 2003).

It is reported that green tea leaves are known for antimicrobial activity against numerous human pathogenic microorganisms. In recent years, green and black teas are being tested against both The major difference

between green and black teas is in the fermentation practice takes place during manufacturing process which is required to produce black tea powder. In case of black tea powder production, three leaves and a bud are fermented and/or oxidized after they have been dried in manufacturing process. The phytochemicals present in tea leaves and buds are highly sensitive to oxidation process. According to Toda *et al.* (1989) daily consumption of green tea lead to kill pathogenic microorganisms like *Staphylococcus aureus*, *Vibrio parahemolyticus*, *Clostridium perfringens*, *Bacillus cereus* and *Pleisomonas shigelloides*. Green tea contains around 30-40% polyphenols and related compounds such as *Epigallocatechin gallate* (EGCG), *Epicatechin gallate* (ECG), *Epigallocatechin* (EGC) and *Epicatechin* (EC) but black tea contains only 3-7% polyphenols (Diane *et al.*, 2007). Among these EGCG is the most luxuriant component in tea extract and the most potent chemical tested for biological activity in terms of inhibiting the growth of pathogenic microorganisms (Archana and Abraham, 2011).

Tea leaves are polymorphic in nature and known for its antibacterial activity against many pathogenic microorganisms. It is being shown to have a wide range of antioxidant, anti-inflammatory, anti-carcinogenic and antimutagenic properties against pathogens. Antimicrobial activity of green tea and black tea extracts were studied using clinically important pathogenic microorganisms and compared by several Investigators (Diane *et al.*, 2007; Archana and Abraham, 2011). But no literatures are available on antibacterial activity of different green tea leaves against a wide variety of pathogenic microorganisms. Hence the present investigation made an attempt to study the antibacterial activity of different green tea leaves against selected

bacterial pathogens isolated from clinical samples.

Materials and Methods

Tea (*Camellia sinensis* (L.) O.Kuntz) leaves and buds were collected from Parry Agro tea plantations, Valparai, Tamil Nadu, India during August 2016 for the present study. Three leaves and a bud are generally used for black tea manufacturing which is used to stimulate central and autonomous nerves system of human beings to get freshness. In addition, other leaves such as mother and scale (cataphyll) leaves were also collected for the present investigation. The solvents such as petroleum ether (10.5%), ethyl acetate (15.5%) and chloroform (15.5%) were used as solvent extracts (Sawaya *et al.*, 2004). Phytochemical screening of tea leaves revealed the presence of antioxidant compounds like polyphenols and catechin in petroleum ether extract, caffeine, theaflavins and thearubigins in ethyl acetate and saponins and tannins in chloroform extracts as per the report of Diane *et al.* (2007).

A total of 7 clinical isolates of bacteria such as *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas fluorescense*, *Aeromonas hydrophilla* and *Staphylococcus aureus* were used. The selected organisms are resistant to antibiotics commonly used for treatment of various diseases caused by these bacteria and the MAR index value also calculated. Mueller Hinton agar (Himedia) plates were prepared and seeded with 16 hour old cultures of the said pathogenic microorganisms. Before seeding the inoculum, the turbidity was adjusted to the turbidity of 0.5% of McFarland solution and challenged with different concentration of tea leaf extracts by well diffusion method. The plates were incubated at 37°C under controlled condition and the zone of

inhibition of pathogenic microorganisms due to solvent extracts containing tea phytochemical compounds was recorded subsequently.

Results and Discussion

All the extracts of tea samples (leaves and buds) showed good activity against the tested pathogenic microorganisms. Among the tea leaves, first and second leaves showed greater activity than other leaves such as mother and scale followed by tea leaf bud extracts (Table 1-3). Ethyl acetate extracts of both tea leaves and buds showed a higher growth inhibition zone followed by chloroform extracts and comparatively

leaves extract was found to be more active than tea bud extracts. This tends to show that the active ingredients in the tea leaves were better extracted with ethyl acetate than chloroform and petroleum ether. The least performance in terms of growth inhibition in mother leaf may be due to minimum active participation in physiological maturity and proximity. Being an autotropic nature of tea leaves, the fully expanded mother leaf supplies adequate amount of sugars to sinks which is physiologically and biochemically active (Ponmurugan *et al.*, 2007). This gives credence to its ethnopharmacological use as a remedy to treat infections and diseases caused by above microorganisms.

Table.1 Antibacterial activity of tea mother and scale (cataphyll) leaf extracts a

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethyl acetate		Chloroform	
			1%	2%	1%	2%	1%	2%
Tea mother leaf extracts								
<i>Salmonella typhi</i>	11.3	13.3	6.5	10.0	11.0	15.5	9.3	16.7
<i>Escherichia coli</i>	13.5	14.5	2.3	04.5	6.3	14.5	5.7	12.5
<i>Bacillus subtilis</i>	10.3	13.5	6.7	14.5	8.5	18.7	8.5	16.5
<i>Klebsiella pneumonia</i>	10.7	11.0	2.3	04.3	6.7	13.0	6.0	10.0
<i>Pseudomonas fluorescense</i>	15.5	14.7	2.0	05.5	12.3	18.7	10.0	15.5
<i>Aeromonas hydrophylla</i>	16.0	16.5	4.0	06.5	10.5	14.3	8.5	16.5
<i>Staphylococcus aureus</i>	16.3	15.3	4.3	06.7	10.5	14.3	9.7	17.3
Tea scale (cataphyll) leaf extracts								
<i>Salmonella typhi</i>	11.3	13.3	2.3	4.0	4.5	6.5	2.7	4.5
<i>Escherichia coli</i>	13.5	14.7	0.5	1.3	2.3	4.3	2.5	4.7
<i>Bacillus subtilis</i>	10.7	13.5	1.5	3.3	2.7	3.5	1.5	2.7
<i>Klebsiella pneumonia</i>	10.7	11.7	1.3	1.5	3.3	5.7	2.3	5.5
<i>Pseudomonas fluorescense</i>	15.0	14.5	1.3	2.5	2.3	5.5	1.7	3.0
<i>Aeromonas hydrophylla</i>	16.3	16.7	1.5	2.7	2.3	4.5	2.7	4.5
<i>Staphylococcus aureus</i>	16.3	15.3	1.5	2.5	3.0	6.3	2.5	4.5

^a Values are the means of five replicates

Table.2 Antibacterial activity of tea first, second and third leaf extracts a

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethyl acetate		Chloroform	
			1%	2%	1%	2%	1%	2%
Tea first leaf extracts								
<i>Salmonella typhi</i>	11.3	13.5	5.5	10.0	8.5	15.0	8.7	14.7
<i>Escherichia coli</i>	13.0	14.3	5.3	8.5	5.3	10.5	5.5	11.5
<i>Bacillus subtilis</i>	10.0	13.3	4.3	9.7	8.8	16.5	5.7	10.5
<i>Klebsiella pneumonia</i>	10.0	11.7	3.5	6.5	6.6	14.7	3.5	06.3
<i>Pseudomonas fluorescense</i>	15.3	14.7	2.3	4.5	8.0	15.3	8.3	15.5
<i>Aeromonas hydrophylla</i>	16.5	16.0	3.3	7.3	8.5	14.5	6.3	12.5
<i>Staphylococcus aureus</i>	16.5	15.3	4.0	9.3	8.3	16.5	6.7	13.5
Tea second leaf extracts								
<i>Salmonella typhi</i>	11.3	13.3	5.5	12.5	9.5	15.3	6.3	12.5
<i>Escherichia coli</i>	13.5	14.7	4.3	7.5	6.3	11.3	5.7	11.3
<i>Bacillus subtilis</i>	10.5	13.3	4.7	9.5	8.7	16.5	7.5	13.5
<i>Klebsiella pneumonia</i>	10.7	11.5	3.5	7.3	6.5	11.7	5.3	11.7
<i>Pseudomonas fluorescense</i>	15.3	14.0	4.5	8.5	9.5	17.3	8.5	15.0
<i>Aeromonas hydrophylla</i>	16.0	16.3	6.3	10.7	10.0	17.5	9.3	17.0
<i>Staphylococcus aureus</i>	16.0	15.3	6.5	11.7	9.5	17.5	9.3	17.5
Tea third leaf extracts								
<i>Salmonella typhi</i>	11.5	13.5	5.5	11.0	10.0	19.3	8.5	16.3
<i>Escherichia coli</i>	13.3	14.3	4.3	07.5	10.0	17.0	7.3	16.7
<i>Bacillus subtilis</i>	10.3	13.7	6.3	10.3	08.5	17.5	7.3	15.5
<i>Klebsiella pneumonia</i>	10.7	11.5	5.5	10.7	09.3	17.0	8.5	16.5
<i>Pseudomonas fluorescense</i>	15.5	14.3	4.5	08.5	08.7	18.7	8.7	15.5
<i>Aeromonas hydrophylla</i>	16.3	16.3	5.5	10.5	10.3	19.3	10.0	17.5
<i>Staphylococcus aureus</i>	16.3	15.3	5.5	10.5	10.3	19.3	10.0	18.3

^a Values are the means of five replicates

Table.3 Antibacterial activity of tea bud extracts a

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethyl acetate		Chloroform	
			1%	2%	1%	2%	1%	2%
<i>Salmonella typhi</i>	11.3	13.3	0.5	1.0	2.3	3.5	0.5	1.5
<i>Escherichia coli</i>	13.5	14.5	0.5	1.5	2.5	3.5	1.0	2.3
<i>Bacillus subtilis</i>	10.7	13.3	1.0	1.5	1.3	2.7	0.5	1.3
<i>Klebsiella pneumonia</i>	10.3	11.7	1.0	2.3	1.7	2.5	1.0	2.0
<i>Pseudomonas fluorescense</i>	15.5	14.5	1.0	3.0	3.0	4.5	1.5	2.5
<i>Aeromonas hydrophilla</i>	16.3	16.3	0.5	1.5	2.3	4.5	1.0	2.5
<i>Staphylococcus aureus</i>	16.3	15.5	1.3	2.5	3.3	4.0	1.0	2.5

^a Values are the means of five replicates

The composition of tea leaves is highly complex and its biological activity fully depend on tea plantation hills height where it was collected and abiotic factors such as temperature, relative humidity, rainfall and sunshine. The factors may influence the antibacterial activity of green tea leaves which include origin of tea plants, genetics breeding, extract preparation and nature of tea clones and seedlings (Grange and Davey, 1990) and geographic differences (Boyanova *et al.*, 2005). Therefore the effect of biological activity of tea leaves varies according to the origin is inevitable. In view of supporting this, Brumfitt *et al.*, (1990) found no inhibition of Streptococci by tea extracts was noted but it is controversial with other reports (Nieva Moreno *et al.*, 1999). However in the current investigation, the results of six samples of tea leaf extracts revealed only minor variation on its antibacterial activity, it may be due to mixed type vegetation and the study area locality is near with one another. Further it was exhibited bacteriostatic activity in general and bactericidal activity at high

concentration in particular (Drago *et al.*, 2000) against the microbial pathogen.

The vegetation system in the study area is a mixed type, hence the minor variations were observed in the antibacterial activity which collected from different localities. Drago *et al.*, (2000) mentioned the reasons for the difficulties in comparison the results of antimicrobial properties of medicinal plants. In the present investigation, raw tea leaf materials were tested against the clinically isolated organism hence the inhibition activity may be minimal even though the high volume of extract was used (Hegazi and El Hady, 2001). Further the vast work was done on tea which is used as commercial product and this work was a new attempt in terms of using different leaf materials such as mother, scale (cataphyll), first, second, third leaves and leaf bud, hence the comparison with the earlier result was difficult. The important finding of this study is displayed the ability of antibacterial activity of Indian tea leaf against antibiotic resistant bacteria, which give a new

dimension in green pharmacy. The present investigation may be concluded that three leaves and bud of green tea were found to be effective in inhibiting the pathogenic microorganisms through antibacterial studies.

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