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## Comparative Analysis of Antimicrobial Activity of Commercial Tea Products against Human Pathogens

			
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**Keywords:** Tea, Aqueous, HPLC, *Staphylococcus aureus*, Phytochemical analysis.

### ABSTRACT

Tea is one of the most widely consumed beverages in the world, next only to water and well ahead of coffee, beer, wine and carbonated soft drinks. Tea is the agricultural product of leaves, leaf buds and internodes of *Camellia sinensis* plant. There are several health benefits of drinking tea. Green and black teas are the most popular types of tea. The present study deals with the antimicrobial activity of commercial tea powder against human pathogens. The Pathogens were isolated from the soil and identified by staining, biochemical tests. The gram-negative bacteria are such as *E.coli*, *Pseudomonas aeruginosa* gram-positive bacteria are such as *Staphylococcus aureus*, and *Bacillus subtilis* were isolated. Antibacterial activity of tea extract was prepared in term of Aqueous, Methanol and Ethanol (50%, 100%). Compared to Methanol, Ethanol, Aqueous had shown significant antibacterial activity against *Staphylococcus aureus*, in the level 50% ( $9.8 \pm 6.4$ ) followed by *E.coli*, ( $7.2 \pm 4.2$ ) *Pseudomonas aeruginosa*, ( $6.1 \pm 5.5$ ) *Bacillus subtilis* ( $6.6 \pm 5.9$ ). Phytochemical constituents such as saponins, alkaloids, tannins, steroids, flavonoids are detected. A simple HPLC gradient elution method was carried for efficient separating on compounds present in the tea samples. It may be suggested that aqueous, ethanol and methanol extracts of tea can serve as a good source for the invention of new therapeutic agents to kill pathogenic bacteria.

## INTRODUCTION

Tea has recently received the attention pharmaceutical and scientific communities due to the plethora of natural therapeutic compounds. As a result, numerous researchers have been published in a bid to validate their biological activity. Moreover, major attention has been drawn to the antimicrobial activity of tea. Being rich in phenolic compounds, tea has the preventive potential for colon, esophageal, lung cancer as well as urinary infections and dental caries. The extracts of tea origin as antimicrobial agents with new mechanisms of resistance would serve targeting the inhibition of microbial growth and the spread of antibiotic resistance with potentials are in pharmaceutical, cosmetic, food industries (Wasim Siddiqui, 2015).

Tea is an important dietary source of flavanols and flavonols. *In vitro* and animal studies provide strong evidence that tea polyphenols may possess the bioactivity to affect the pathogenesis of several chronic diseases, especially cardiovascular disease and cancer. However, the results from epidemiological and clinical studies of the relationship between tea and health are mixed. International correlations do not support this relationship although several, better-controlled case-referent and cohort studies suggest an association with a moderate reduction in the risk of chronic disease. Conflicting results from human studies may arise, in part, from confounding by socioeconomic and lifestyle factors as well as by inadequate methodology to define tea preparation and intake. Clinical trials employing putative intermediary indicators of disease, particular biomarkers of oxidative stress status, suggest tea polyphenols could play a role in the pathogenesis of cancer and heart disease. (Diane 2002).

After water, tea is the most popularly consumed beverage worldwide with a per capita consumption of 120 mL/day. Black tea is consumed principally in Europe, North America, and North Africa (except Morocco) while green tea is drunk throughout Asia; oolong tea is popular in China and Taiwan. All tea is produced from the leaves of the tropical evergreen *Camellia Sinensis*. There are three main types of tea with black tea made via a post-harvest "fermentation," an auto-oxidation catalyzed by polyphenol oxidase. Approximately 76% to 78% of the tea produced and consumed worldwide is black, 20% and 22% is green tea. Tea is a rich source of polyphenolics, particularly flavonoids. Flavonoids are phenol derivatives synthesized in substantial amounts (0.5% to 1.5%) and variety (more than 4000 identified) and widely distributed among plants (Vinson, 1995). The major flavonoids present in green

tea include catechins (flavan-2-ols) such as epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). In black tea, the polymerized catechins such as theaflavins and thearubigins predominate. The relative catechin content of tea is dependent upon how the leaves are processed prior to drying as well as geographical location and growing conditions. Hence the present study was planned to test the antibacterial activity of commercial tea powder extract against human pathogens and separation of tea biochemical by HPLC.

## **MATERIALS AND METHODS**

### **Collection of soil sample**

Soil sample was collected from the agriculture field. Then soil sample was collected from surface to 10cm depth. The serial dilution (Aneja, 2002) was performed for isolation of bacteria from the soil sample. Morphological identification by such as Gram's staining (Han's Christian Gram, 1884) and standard biochemical tests, motility test (Balaji, 2006), Indole test, MR-VP test, Oxidase test, Urease test, Triple Sugar Iron test, Carbohydrate fermentation test.

### **Collection of commercial tea**

The five several of commercial tea powder and green tea were obtained from the local supermarket.in Mannargudi, Thiruvarur District, used for all experiment hence they were represented as commercial tea product 1, commercial tea product 2, commercial tea product 3, commercial green tea product 4, and commercial green tea product 5.

Preparation of tea extracts in way of Aqueous, methanol, ethanol of solvents by the way of 10g of tea powder was soaked in 50%, 100% level.

### **Media**

Mueller Hinton Agar medium was used (pH 7).

### **Antibacterial Activity:**

**Well Diffusion Method** (Chung *et al.*, 1990 and Azoro., 2002).

Antibacterial activity was screened by agar well diffusion method. Nutrient agar plates were prepared and inoculated the test organism namely *Escherichia coli*, *Bacillus subtilis*,

*Pseudomonas aeruginosa*, *Lactobacillus*, *Staphylococcus aureus* by spread plate technique separately. Using the sterile cork borer, the well (6mm) was made into each Petri plate. After that, all Plates were incubated at 37°C for 24 hours. After incubation, the formation of the zone was measured.

#### **Disc Diffusion method** (Kirby –Bauer, 1966)

*In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia. The MHA plates were prepared by pouring 15 ml molten media into sterile Petri plates. The plates were allowed to solidify for 5min and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 min. The same procedure has been followed for the different concentrations of extracts (1, 2 and 4mg/disc) were loaded on 5mm sterile individual) discs. The loaded discs were placed on the surface of the medium and the compound was allowed to diffuse for 5min and the plates were kept for incubation at 37°C for 24h. The negative control was prepared using respective solvent. Gentamycin (100µg /disc) was used as positive control. At the end of incubation zones formed around the disc were measured with the transparent ruler in millimeter.

Statistical analysis was done by Gupta (1977). Phytochemical analysis of extracts was done by Edeoga *et al.*, (2005). Sample extraction for HPLC was done by Suematsu *et al.*, 1995, kallithraka *et al.*, 1995, Khokhar and Mangnusdottir. 2002.

#### **High performance of liquid chromatography (HPLC)**

The separation and identification of compounds were made through the High performance of liquid chromatography (HPLC). The extracts used for HPLC analysis were passed through a 0.45 µm filter (Millipore, MSI, Westboro, MA) before injection into an HPLC column of 150 mm length (Agilent technologies 1200 series). The mobile phase was acidified water containing 0.1% formic acid (A) and acidified acetonitrile containing 0.1% formic acid (B), eluted in gradient. The flow rate was 0.8 mL/min and the wavelengths of detection were set at UV 300 nm, the temperature at 30°C, injection volume = 20 µl and analysis time was 60 min. Reference substances is a mixture of gallic acid, vanillic acid, ascorbic acid, quercetin, caffeic acid, catechin and coumaric acid (solutions in methanol, each of the 0.5 mg/ml).

## Sample extraction

The ground tea samples were extracted using solvent and different extraction times. Among the solvents used for extraction was methanol (15% and 70%). These solvents have been used for extraction of flavanols in various studies (Suematsu *et al.*, 1995; Kallithraka *et al.*, 1995; Khokhar and Mangnusdottir, 2002). Tea sample (0.2 g) was extracted with 5 mL solvent with intermittent shaking (30s on vortex mixer). The sample was then centrifuged at 1400g for 10 min at 16C. The supernatant was taken into a 10 mL volumetric flask and the extraction steps repeated to reach the final volume of 10 mL. The extracts were filtered through a 0.5mm Millipore filter before the injection was made. The extract is stable for 24 h if stored at 4C. Different extraction times tried were 10, 20 and 30 min and 10+10+10 min.

## RESULTS

The present study has been undertaken for the antibacterial activity of commercial tea powder against human pathogens. Isolation and identification were done by the standard method; morphological characteristics of isolated by organisms are listed in the *Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

### Antibacterial activity

The antibacterial potency of aqueous, ethanol, methanol extracts of commercial tea was tested against some human pathogenic bacteria for the presence or absence of the zone of inhibition. The result was relative to antimicrobial activity by measuring the diameter of the zone of inhibition.

### Antibacterial activity of commercial tea

#### Aqueous extracts of commercial tea powder 1

In 100% level of Aqueous extract of CT<sub>1</sub> was showed the minimum zone of inhibition of *staphylococcus aureus* 9.3±7.4, *E.coli* 8.3±6.1, *P. aeruginosa* 5.4±3.7, and *Bacillus subtilis* 4.8±1.2. Next 50% level of aqueous extract of CT<sub>1</sub> had shown minimum zone of inhibition of *staphylococcus aureus* 7.2±2.3, *E.coli* 6.3±1.5, *P. aeruginosa* 7.1±5.8, and *Bacillus subtilis* 8.1±6.7.

### **Aqueous extracts of commercial tea powder 2**

In 100% level of the Aqueous extract of CT<sub>2</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 9.5±4.6, *E.coli* 7.4±3.6, *P. aeruginosa* 5.9±1.7, and *Bacillus subtilis* 7.0±6.1. Although, 50% level of aqueous extract of CT<sub>2</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 8.1±5.6, *E.coli* 6.4±3.5, *P. aeruginosa* 7.3±2.8, and *Bacillus subtilis* at 5.2±2.7.

### **Aqueous extracts of commercial tea powder 3**

In 100% level of Aqueous extract of CT<sub>3</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 7.4±4.7, *E.coli* 8.2±3.1, *P. aeruginosa* 5.7±4.4, and *Bacillus subtilis* 4.9±5.0. Similarly, 50% level of aqueous extract of CT<sub>3</sub> had shown minimum zone of inhibition of *Staphylococcus aureus* 6.5±4.5, *E.coli* 3.2±2.1, *P. aeruginosa* 7.9±4.6, and *Bacillus subtilis* at 4.8±3.2.

### **Aqueous extracts of commercial green tea 4**

In 100% level of Aqueous extract of CGT<sub>4</sub> was showed minimum zone of inhibition of *Staphylococcus aureus* 8.8±5.9, *E.coli* 7.4±4.8, *P. aeruginosa* 6.8±3.2, and *Bacillus subtilis* at 3.7±5.1. Likewise, 50% level of aqueous extract of CGT<sub>4</sub> had shown minimum zone of inhibition of *Staphylococcus aureus* 9.8±6.4, *E.coli* 7.2±4.2, *P. aeruginosa* 8.1±4.3, and *Bacillus subtilis* 6.0±2.8.

### **Aqueous extracts of commercial green tea 5**

In 100% level of Aqueous extract of CGT<sub>5</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 8.6±6.5, *E.coli* 7.3±4.1, *P. aeruginosa* 9.5±3.1, and *Bacillus subtilis* 5.5±2.8. Next 50% level of aqueous extract of CGT<sub>5</sub> had shown minimum zone of inhibition of *Staphylococcus aureus* 6.4±4.2, *E.coli* 7.4±3.6, *P. aeruginosa* 7.7±2.5, and *Bacillus subtilis* 6.9±3.6.

**Table.1: Antibacterial activity of aqueous extracts of commercial tea powder sample**

Sr. No.	Organisms	Tea Sample	Extract	Zone of Inhibition(mm)	
				50%	100%
1	<i>S.aureus</i>	Commercial Tea Sample1	Aqueous	7.2±2.3	9.3±7.4
	<i>E.coli</i>			6.3±1.5	8.3±6.1
	<i>P.aeruginosa</i>			7.1±5.8	5.4±3.7
	<i>B. Subtilis</i>			8.1±6.7	4.8±1.2
2	<i>S.aureus</i>	Commercial Tea Sample2	Aqueous	8.1±5.6	9.5±4.6
	<i>E.coli</i>			6.4±3.5	7.4±3.6
	<i>P.aeruginosa</i>			7.3±2.8	5.9±1.7
	<i>B. Subtilis</i>			5.7±2.7	7.0±6.1
3	<i>S.aureus</i>	Commercial Tea Sample3	Aqueous	6.5±4.5	7.4±4.7
	<i>E.coli</i>			3.2±2.1	8.2±3.1
	<i>P.aeruginosa</i>			7.9±4.6	6.3±4.4
	<i>B. Subtilis</i>			4.8±3.2	4.9±5.0
4	<i>S.aureus</i>	Commercial Green Tea Sample4	Aqueous	9.8±6.4	8.8±5.9
	<i>E.coli</i>			7.2±4.2	7.4±4.8
	<i>P.aeruginosa</i>			8.1±4.3	6.8±3.2
	<i>B. Subtilis</i>			6.0±28	3.7±5.1
5	<i>S.aureus</i>	Commercial Green Tea Sample5	Aqueous	6.4±4.2	8.6±6.5
	<i>E.coli</i>			7.4±3.6	7.3±4.1
	<i>P.aeruginosa</i>			7.7±2.5	9.5±3.1
	<i>B. Subtilis</i>			6.9±3.6	5.5±2.8

Values are triplicates and represented as mean ± standard deviation

### **Methanol extracts of commercial tea powder 1**

In 100% level of Methanol extract of CT<sub>1</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 7.1±5.4, *E.coli* at 4.6±3.9, *P. aeruginosa* 7.4±6.1, and *Bacillus subtilis* at 2.1±4.2. Moreover, 50% level of methanol extract of CT<sub>1</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 5.3±3.2, *E.coli* 4.1±1.3, *P. aeruginosa* 5.9±1.7, and *Bacillus subtilis* at 6.2±3.4.

### **Methanol extracts of commercial tea powder 2**

In 100% level of Methanol extract of CT<sub>2</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 6.3±5.8, *E.coli* 7.9±5.2, *P. aeruginosa* 6.3±7.7, and *Bacillus subtilis* 6.0±5.2. Although 50% level of methanol extract of CT<sub>2</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 5.3±6.8, *E.coli* 7.5±5.6, *P. aeruginosa* 5.2±7.4, and *Bacillus subtilis* 4.6±2.9.

### **Methanol extracts of commercial tea powder 3**

In 100% level of Methanol extract of CT<sub>3</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 3.8±6.5, *E.coli* 7.5±5.3, *P. aeruginosa* 6.4±2.6, and *Bacillus subtilis* 5.7±6.5. From the 50% level of methanol extract of CT<sub>3</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 6.7±5.3, *E. coli* 8.3±3.5, *P. aeruginosa* 5.3±4.2, and *Bacillus subtilis* 5.1±3.3.

### **Methanol extracts of commercial green tea powder 4**

In 100% level of Methanol extract of CGT<sub>4</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 4.8±5.6, *E.coli* 7.4±6.4, *P. aeruginosa* 5.3±4.1, and *Bacillus subtilis* 6.7±4.2. Next 50% level of methanol extract of CGT<sub>4</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 7.6±5.4, *E.coli* 6.7±3.2, *P. aeruginosa* 6.4±5.6, and *Bacillus subtilis* 6.7±4.2.

### **Methanol extracts of commercial green tea powder 5**

In 100% level of Methanol extract of CGT<sub>5</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 7.3±4.7, *E.coli* 8.3±4.1, *P. aeruginosa* 6.3±3.2, and *Bacillus subtilis* 6.9±7.8. Followed by 50% level of methanol extract of CGT<sub>5</sub> had shown the minimum zone

of inhibition of *Staphylococcus aureus* 7.3±4.2, *E.coli* 2.4±5.2, *P. aeruginosa* 6.4±5.6, and *Bacillus subtilis* 7.2±4.3.

**Table. 2: Antibacterial Activity of Methanol Extracts of Commercial Tea Powder**

Sr. No.	Organisms	Tea Sample	Extract	Zone of Inhibition(mm)	
				50%	100%
1	<i>S.aureus</i>	Commercial Tea Sample1	Methanol	5.3±3.2	7.1±5.4
	<i>E.coli</i>			4.1±1.3	4.6±3.9
	<i>P.aeruginosa</i>			5.9±1.7	7.4±6.1
	<i>B. Subtilis</i>			6.2±3.4	2.1±4.2
2	<i>S.aureus</i>	Commercial Tea Sample2	Methanol	5.3±6.8	6.3±5.8
	<i>E.coli</i>			7.5±5.6	7.9±5.2
	<i>P.aeruginosa</i>			5.2±7.4	6.3±7.7
	<i>B. Subtilis</i>			4.6±2.9	6.0±5.2
3	<i>S.aureus</i>	Commercial Tea Sample3	Methanol	6.7±5.3	3.8±6.5
	<i>E.coli</i>			8.3±3.5	7.5±5.3
	<i>P.aeruginosa</i>			5.3±4.2	6.4±2.6
	<i>B. Subtilis</i>			5.1±3.3	5.7±6.5
4	<i>S.aureus</i>	Commercial Green Tea Sample4	Methanol	7.6±5.4	4.8±5.6
	<i>E.coli</i>			6.7±3.2	7.4±6.4
	<i>P.aeruginosa</i>			6.4±5.6	5.3±4.1
	<i>B. Subtilis</i>			7.5±5.4	6.7±4.2
5	<i>S.aureus</i>	Commercial Green Tea Sample5	Methanol	7.3±4.2	7.3±4.7
	<i>E.coli</i>			2.4±5.2	8.3±4.1
	<i>P.aeruginosa</i>			6.4±5.6	6.3±3.2
	<i>B. Subtilis</i>			7.2±4.3	6.9±7.8

Values are triplicates and represented as mean ± standard deviation

### **Ethanol extracts of commercial tea powder 1**

In 100% level of Ethanol extract of CT<sub>1</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 2.8±1.4, *E.coli* 5.3±2.1, *P. aeruginosa* 5.4±1.7, and *Bacillus subtilis* 3.8±2.2. Similar to 50% level of ethanol extract of CT<sub>1</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 4.3±2.3, *E.coli* 5.2±1.5, *P. aeruginosa* 4.1±1.3, and *Bacillus subtilis* 5.7±1.9.

### **Ethanol extracts of commercial tea powder 2**

In 100% level of Ethanol extract of CT<sub>2</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 6.4±4.3, *E.coli* 7.4±5.2, *P. aeruginosa* 4.3±7.5, and *Bacillus subtilis* 6.3±6.1. Next 50% level of ethanol extract of CT<sub>2</sub> had the minimum zone of inhibition of *Staphylococcus aureus* 2.6±2.2, *E.coli* 3.4±5.8, *P. aeruginosa* 5.2±4.2, and *Bacillus subtilis* 6.1±3.2.

### **Ethanol extracts of commercial tea powder 3**

In 100% level of Ethanol extract of CT<sub>3</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 4.7±2.3, *E.coli* 2.6±3.1, *P. aeruginosa* 3.1±4.6, and *Bacillus subtilis* 5.3±5.2. 50% level of ethanol extract of CT<sub>3</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 7.2±3.2, *E.coli* 4.7±5.7, *P. aeruginosa* 2.9±3.1, and *Bacillus subtilis* 2.2±4.6.

### **Ethanol extracts of commercial green tea powder 4**

In 100% level of Ethanol extract of CGT<sub>4</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 4.5±3.5, *E.coli* 3.2±4.1, *P. aeruginosa* 4.3±5.1, and *Bacillus subtilis* 5.4±3.7. Moreover, 50% level of ethanol extract of CGT<sub>4</sub> had shown the minimum zone of inhibition of *Staphylococcus aureus* 5.3±4.2, *E.coli* 3.1±3.2, *P. aeruginosa* 5.6±6.3, *Bacillus subtilis* 6.2±3.6.

### **Ethanol extracts of commercial green tea powder 5**

In 100% level of Ethanol extract of CGT<sub>5</sub> was showed the minimum zone of inhibition of *Staphylococcus aureus* 6.4±4.5, *E.coli* 2.5±3.1, *P. aeruginosa* 5.3±2.2, and *Bacillus subtilis* 7.5±4.2. Next 50% level of ethanol extract of CGT<sub>5</sub> had shown the minimum zone of

inhibition of *Staphylococcus aureus* 2.6±5.3, *E.coli* 4.2±2.3, *P. aeruginosa* 5.3±4.7, and *Bacillus subtilis* 2.7±4.8.

**Table.3: Antibacterial Activity of Ethanol Extracts of Commercial Tea Powder**

Sr. No.	Organisms	Tea Sample	Extract	Zone of Inhibition(mm)	
				50%	100%
1	<i>S.aureus</i>	Commercial	Ethanol	4.3±2.3	2.8±1.4
	<i>E.coli</i>	Tea Sample1		5.2±1.5	5.3±2.1
	<i>P.aeruginosa</i>			4.1±1.3	5.4±1.7
	<i>B. Subtilis</i>			5.7±1.9	3.8±2.2
2	<i>S.aureus</i>	Commercial	Ethanol	2.6±2.2	6.4±4.3
	<i>E.coli</i>	Tea Sample2		3.4±5.8	7.4±5.2
	<i>P.aeruginosa</i>			5.2±4.2	4.3±7.5
	<i>B. Subtilis</i>			6.1±3.2	6.3±6.1
3	<i>S.aureus</i>	Commercial	Ethanol	7.2±3.2	4.7±2.3
	<i>E.coli</i>	Tea Sample3		4.7±5.7	2.6±3.1
	<i>P.aeruginosa</i>			2.9±3.1	3.1±4.6
	<i>Bacillus Subtilis</i>			2.2±4.6	5.3±5.2
4	<i>S.aureus</i>	Commercial	Ethanol	5.3±4.2	4.5±3.5
	<i>E.coli</i>	Green Tea		3.1±3.2	3.2±4.1
	<i>P.aeruginosa</i>	Sample4		5.6±6.3	4.3±5.1
	<i>B. Subtilis</i>			6.2±3.6	5.4±3.7
5	<i>S.aureus</i>	Commercial	Ethanol	2.6±5.3	6.4±4.5
	<i>E.coli</i>	Green Tea		4.2±2.3	2.5±3.1
	<i>P.aeruginosa</i>	Sample5		5.3±4.7	5.3±2.2
	<i>B. Subtilis</i>			2.7±4.8	7.5±4.2

Values are triplicates and represented as mean ± standard deviation

### Phytochemical screening of commercial tea product

In the phytochemical screening of steroids are absent in all tested commercial tea samples. Saponins, Alkaloids, Tannins, Flavonoids are present in all tea sample.

**Table-4. Phytochemical Screening Of Commercial Tea Product**

Tea product	Steroids	Alkaloids	Tannins	Flavonoids	Saponins
CT <sub>1</sub>	–	+	+	+	+
CT <sub>2</sub>	–	+	+	+	+
CT <sub>3</sub>	–	+	+	+	+
CGT <sub>4</sub>	–	+	+	+	+
CGT <sub>5</sub>	–	+	+	+	+

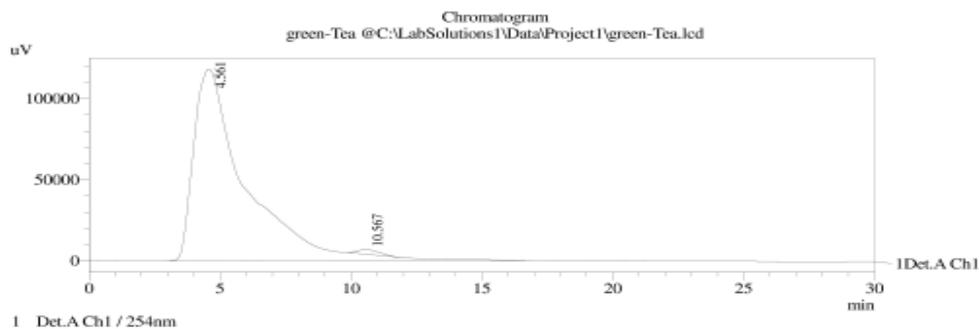
### HPLC analysis

A simple HPLC gradient elution method was carried for efficient separate from various CGT<sub>4</sub> samples using methanol as solvent biochemical. This method is efficient with high reproducibility and accuracy (Figure 1).

In our present study was clearly highlighted that aqueous extract of CGT<sub>4</sub> is effectively control all the pathogens at 50% level by good diffusion.

HPLC ANALYSIS REPORT

Sample Information  
 Acquired by : Admin  
 Sample Name : green-Tea  
 Sample ID :  
 Vial# :  
 Injection Volume : 20 uL  
 Data Filename : green-Tea.lcd  
 Method Filename : SJC-Law-Method.lcm  
 Batch Filename :  
 Report Filename : Default.lcr  
 Date Acquired : 30-03-2017 PM 03:57:41  
 Data Processed : 30-03-2017 PM 04:27:42



1 Det.A.Ch1 / 254nm

PeakTable					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.561	16371631	118396	98.980	97.590
2	10.567	168742	2923	1.020	2.410
Total		16540373	121319	100.000	100.000

ANALYSED BY

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Figure 1: HPLC analysis

DISCUSSION

Our study reports were similar to the findings of (Kaur,2015) revealed that zone of inhibition (ZOI) of methanolic extract of green tea, black tea and highest antimicrobial activity (ZOI 19.2, 13.6, 15.8 respectively;  $-1.067 \pm 0.006$ ,  $0.756 \pm 0.005$ ,  $0.878 \pm 0.0010$  respectively) against *Bacillus* and *E.coli* showed resistance to aqueous extracts of all tea samples. In our present study *E.coli*, *Staphylococcus* are showed the zone of inhibition from the aqueous extract of green tea, black tea (25, 19mm respectively;  $- 3.051 \pm 1.005$ ,  $2.076 \pm 0.006$ ,  $1.087 \pm 0.011$  respectively).

Our study was correlated to the findings of (Abhishek Mehta 2016). All tea extracts have shown significant antibacterial activity against *S.aureus* ATCC 25922 with aqueous extract of Green tea exhibiting highest activity. In our present study, all tea extracts exhibited

significant activity against *S. aureus*, especially aqueous extract of green tea exhibiting highest activity.

Our study was agreed with (Macys Jamal Mageed, 2015). Both green tea extracts were effective in inhibition of *Porphyromonas gingivalis* growth on agar plates but the alcoholic extract showed larger inhibition zones. In our present study as green tea extract were effective inhibition of aqueous extract showed maximum zones of inhibition.

Our study was correlated with (Chenielle Delahaye, 2009). The methanol extract's average zone of inhibition for MRSA [ $25.61 \pm 2.11$ mm] was larger than that of non-methicillin resistant *S. aureus* [ $17.41 \pm 1.10$  mm] in our present study ethanol showed the average zone of inhibition for *Bacillus subtilis* ( $20.16 \pm 1.06$ mm).

Our study was agreed by (May Flayyih, 2013) with the synergistic activity of tea extract with antibiotics had changed the resistance of *P. aeruginosa* (without the tea) to sensitive (in presence of tea extract). In our present study of antibacterial activity of tea extract with antibiotic had changed the resistance of *E.coli*

Our study was correlated (Saif Saliem Juma, 2015). Aqueous and alcoholic green tea extracts showed increase in the diameter of thus inhibition zones as their concentration was increased.90% and 100%. In our present study of aqueous and methanolic extracts showed increases in the diameter of their zone of inhibition at 50% and 100%.

Our study was correlated (Turkmen Erol 2009). The extract yield in FTL , the highest yields (23,03-50.09%) were achieved by using methanol as an initial extraction solvent, followed by water and ethanol, respectively. In our present study reported that commercial tea powder highest zone formation in (25,22mm) the aqueous solvent.

## CONCLUSION

It is clear that are 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 exhibit the superior antimicrobial activity. This study in addition to that this antibacterial activity is dependent not only on the type of the tea product but also on different processing techniques involved in preparing tea extracts which determine the concentration of polyphenols particularly catechins responsible for this activity.

Further research is required for isolation and identification of main active compounds in the extract of tea. The combined use of tea and antibiotics could be also useful in fighting emerging drug-resistant problem, especially among human pathogens.

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## REFERENCES

1. Abhishek Mehta, Comparative analysis of the antibacterial activity of aqueous, ethanolic, methanolic and acetone extracts of commercial green tea and black tea against standard bacterial strains. *International Journal of Current Microbiology and Applied Science* 2016, Vol,5(11), 145-152.
2. Aneja, Biochemical activities of Microorganisms. In: *Experiments in Microbiology*, New age international (p) Ltd, New Delhi, 2002, vol 1(5) pp: 265-270.
3. Archana, Comparative analysis of the antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *Journal of Applied Pharmaceutical Science* vol 2011, 1 (8), 149-152.
4. Arts ICW, Van de Putte B, Hollman PCH Catechin contents of foods commonly consumed in the Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J Agric Food Chem* 48: 2000, p1746–1751.
5. AW Bauer, WMM. Kirby, JC Sherris, M.Tuck, Antibacterial activity of plant extract against bacterial pathogen. *Am J Clin Pathol* 1966, 45: 493-6.
6. Balaji, P., Hariharan G.N. In the vitro antimicrobial activity of *Parmotrema praesorediosum* thallus extracts. *Res. J. Bot.* 2000. 2(1), 54-59.
7. Bipul Biswas, Antimicrobial activities of leaf extracts of guava gram positive bacteria. *International Journal of Microbiology* 2013, Vol (7), 127-130.
8. Cappuccino, J.G., and Sherman, *Microbiology: A laboratory manual*. New York 1999, 125-179.
9. Chacko, Beneficial effect of green tea, *Journals Database record for the journal*. 2010 Vol 5(13).
10. Chen J, Purification and characterization of the 1.0 MDa CCR4-NOT complex identifies two novel components of the complex. *J Mol Biol* vol 2001, 314(4):683-94.
11. Cheniella, Antibacterial and antifungal analysis of crude extracts from the leaves. *Journal of medical and biological science* 2009, vol 3(1), p 1-7.
12. Chung M, Raman G., Breastfeeding and maternal and infant health outcomes in developed countries. 2007, vol 2(153):1-186.
13. Diane, The Role of Tea in Human Health .*journal of the American college of nutrition*, 2002.vol.21(1),1-13.
14. Flayyih, antimicrobial effects of black tea (*Camellia sinensis*) on *Pseudomonas* isolated from eye infection. *World Journal of Pharmacy and Pharmaceutical Sciences* 2013, Vol 54(2), 255-265.
15. Halder, *Applied Soil Ecology* 34, Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, 2005, 33–41.
16. Jamal Nageed, Prevalence and Comparative Analysis of Cutaneous *Leishmaniasis* in Dargai Region in Pakistan *J. Zool.*, 2013, vol. 45(2), 537-541.
17. Kaur, antibacterial activity and phytochemical profile of green tea, black tea. *Int. J. Pure App. Biosci.* 2015, 3 (3):117-123.
18. Kallithraka, S, Survey of solvents for the extraction of grape seed phenolics. *Phytochemical analysis*.1995, (6), 265-267.
19. Khokhar, S., Mangnusdottir, S.G.M., total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agriculture and Food Chemistry* 2002, (50), p: 565-570.

20. Liu, Inhibitory activity of tea polyphenol and *Hansenpora uvarum* against *Botrytis cinera* infection. Journal compilation, the Society for Applied Microbiology, 2010, 257-267.
21. Maksum Radji, Asian Pac J Trop Biomed, Antimicrobial activity of green tea extract against isolates of methicillin resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa* .2013, 663-667.
22. Minakshi, Preparation of organic compost using waste tea powder National Conference on Biodiversity: Status and Challenges in Conservation, 2013, p.97-99.
23. Navas, Acien A, Silbergeld EK, Sharrett R, Calderon-Aranda E, Selvin E, Guallar E. Metals in urine and peripheral arterial disease. Environ Health Prospect. 2005, (13):164–169.
24. Siniya, Green tea: Health benefits. Journal of Nutritional & Environmental Medicine 2008, 17(4): 232-242.
25. Suematsu, S., Hisanobu, Y., Saigo, H., Matsudo, R., Komatsu, Y. A new extraction procedure for determination of caffeine and catechins in green tea. Nippon Shokuhin Kagaku Kaishi, 1995, 42, 419–424.
26. Vaishali, Toxic metals in black tea, Asian Jr. of Microbiol. Biotech. Env. Sc.J. 2012. Vol. 14 (4):557-560.
27. Vaishali Sharma, A simple and convenient method for analysis of tea biochemical by reverse phase HPLC. Journal of food composition and analysis, 2004, (18).p; 583-594.
28. Wasim Siddique, Antimicrobial property of tea and three extract *in vitro* critical reviews in Food Science and nutrition, 2015, vol, 56(9), 1428-1439.
29. Wu, Effect of instant tea powder with high-catechins content on shelf life. Journal of agriculture science technology. 2013, vol, 15:537-544.
30. Xue Jun wars, Association between green tea and colorectal cancer risk. Asian Pacific Journal of cancer prevention, 2012, vol 13, p.3123-3127.
31. Yujie Ai, Rapid determination of the monosaccharide composition and contents in tea polysaccharides from green tea by pre-column derivatization HPLC, journal of chemistry, 2016 (5).
32. Zhu, Differential regulation of mesodermal gene expression by *Drosophila* cell type-specific Forkhead transcription factors. 2005, vol:139(8):1457--1466.

