Antibacterial and Phytomical Potential of Green Tea Combination with Natural Herbs against Diarrheal Pathogens

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Abstract

This study was performed in order to produce green tea with new formulation and to identify antibacterial, antioxidant against diarrheal pathogens. Totally five strains of *E. coli* were isolated from diarrheal pathogens. Identification was carried out using biochemical tests. Branded Organic Green Tea, *Citrus lemon, Gingiber officinale*, *Hibiscus rosa sinensis,* Brown sugar and Branded Honey samples were choosen for tea preparation. From the results it can be concluded that the Herbal tea extract (*Hibiscus rosasinensis, Camellia sinensis*, Brown sugar, *Zingiber officinale*, *Citrus lemon*, Honey) was rich in phytonutrients and have the high efficacy against diarrheal pathogens. The results suggest that aqueous extract would be very promising natural antioxidant honey as well as alternative antibacterial agent in therapeutics.

Keywords: *Citrus lemon,* Diarrheal Pathogens*, Gingiber officinale,* Green Tea*, Hibiscus rosa sinensis*

1. Introduction

For more than a thousand years green tea has been the second most popular drink worldwide after plain water and it has been renowned as a health product due to its massive health benefits[1]. Among the various types of green tea consumed in China, Japan, India and Thailand, *Camillia sinensis* posses more health benefits.

Of late the focus on green tea has skyrocketed more so its potentials towards, treatment of inflammatory illness[2], anti-cancer prevention[3], and anti-mutagenis treatment[4]. This is due to the availability of pytochemicals such as polyphenols which act as antioxidants. The green tea on top of that also has catechins that have potentials towards the treatment of antibacterial[5]. Venkateswara, et al., reported on catechins improved chronic periodontal after treatment with green tea extract[6].

Enteropathogenic *E. coli* (EPEC) is known to be the major causing agent towards diarrhea in children below 5 years[7]. Research carried out in India, Delhi showed that 50% of persistent diarrhea was caused by bacterium Enteroaggregative *E. coli* [8].

Antibiotics are used in the treatment of various bacterial related infections but of late due to the overuse and misuse of the antibiotics it has lead to the development of antibiotic resistance for instance towards treatment of diarrhea related bacteria[9]. There are various causing agents for diarrhea, for instance bacterial, oral parasitic pathogens or multiple pathogens[10].

Medicinal plants usage of late has gained much interest in various cosmetic, pharmaceutical and nutritional applications[11]. In the pharmaceutical sector, medicinal plants provide natural drug with low cost and less side effects[12].

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The present study was carried out to produce green tea with new formulation and to identify antibacterial, antioxidant against diarrheal pathogens.

2. Materials and Methods

2.1 Sample Collection

Branded Organic Green tea, *Citrus Lemon, Gingiber Officinale*, Brown sugar and Branded
Honey sample were purchased in super market in Thindal, Erode District.

2.2 Preparation of *Hibiscus rosasinensis* Powder

Hibiscus rosasinensis flowers were collected from nearby village, Sivagiri. The flowers were cut into small pieces, dried under shade for 3 days. Then it was crushed and made in a fine powdered. This powder was used for further studies.

2.3 Preparation of Distinct Tea Extracts

2.3.1 Hot Tea extraction

The extraction of green tea was performed by Robert's method. Dried Organic green tea leaves
 (2 g) were soaked in boiling distilled water (100 ml) and boiled for 5 minutes. The water
 extract was filtered for further study.

2.3.2 Preparation of Citrus lemon

The *Citrus lemon* were washed in running water in research center, surface cleaned with 70% ethanol and cut open with a sterile blade and the juice squeezed out into a clean all inclusive compartment independently and after that sifted into another sterile holder to expel the seeds and different tissues. Containers were aseptically opened and diverse focuses (1, 2, 3 and 4%) of lemon were set up for antibacterial activity.

2.4 Identification of Pure Honey

In order to confirm the honey is pure, several studies were conducted. For instance inserting match stick into honey and striking to see if it will burn which confirms to be

pure. The honey was then filtered and stored in sterile bottle for before use. Different Concentrations (1, 2, 3, 4%) of honey were prepared for Antibacterial activity.

2.5 Isolation of Bacteria

A total five strains of Diarrheal pathogens such as *E. coli* strain was isolated from
Government Hospital, Erode.

2.6 Identification of Pathogens

The isolated pathogens on the basis of Grams staining and biochemical characteristics were identified and results interpreted using Bergeys manual of systematic Bacteriology.

2.7 Antibiotic Susceptibility Test[7]

The antibacterial activity of the isolated bacterial strains was performed using commercially
    available antibiotics such as Ampicillin (10 μg), Kanamycin (5 μg), of loxacin (15 μg),
    Erythromycin (5 μg), Norfloxin (25 μg). A suspension of each strain was swabbed uniformly on each agar plates. Commercially available antibiotics discs were impregnated on the plates and it was maintained at 37ºC for 24 h. The inhibition zone formed was measured.

3. Optimization of Tea Extraction Preparation

3.1 Hot Extraction

2 gram of dried green tea leaves were soaked with 100 ml of hot water and heated at 5 minutes. After maceration, the mixtures were filtered. There are 4 different tea extract were selected for this study.

1. 2% Camellia sinensis + 4% Honey + 0.1% Hibiscus rosasinensis + 1% Zingiber officinale + 1% Brown sugar +1% Citrus lemon.
2. 2% Camellia sinensis + 4% Honey + 0.1% Hibiscus rosasinensis + 1% Zingiber officinale + 2% Brown sugar +2% Citrus lemon.
3. 2% Camellia sinensis + 4% Honey + 0.1% Hibiscus rosasinensis + 1% Zingiber officinale + 3% Brown sugar+ 3% Citrus lemon.

4. 2% Camellia sinensis + 4% Honey + 0.1% Hibiscus rosasinensis + 1% Zingiber officinale + 4% Brown sugar + 4% Citrus lemon.

3.2 Agar Well Diffusion Method

Antibacterial activity was carried out for the fish pathogenic bacteria strain *Escherichia coli* by Agar-well diffusion method. The bacterial pathogens were sub cultured on 20 ml of nutrient broth and were poured into petriplates. Wells (6 mm) diameters were made on the nutrient agar plate using a sterile cork borer under aseptic condition. The bacteria culture to be tested was swabbed on the nutrient agar plate using sterile cotton swab and 50 and 100 μl of plant extract was added separately to each well. All the plates were incubated at 37°C for 24 hrs. The different zones of inhibition were measured.

3.3 Determination of Minimum Inhibition Concentration (MIC)

Minimum Inhibition Concentration was done by the lowest cost of the samples where it can show the bactericidal and bacteriostatic effect. The test was performed in 96 well microtiter plate. Microtiter plate wells from each column in first row added with 100 μl (500 mg/ml) of stock was added. 50 μl of steriled distilled water was put in rows 2 up to 12. From row 1 to 2, two fold dilutions were done by transferring 50 μl of solution from row 1 to 2, using a multichannel pipette.

4. Results and Discussion

Isolation and identification of pathogens from diarrheal samples are shown in Table 1. Totally 5 *E. coli* strains were

Table 1. Biochemical characterization of selected isolates

S. no	Biochemical Test	Isolates				
		Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
1	Gram's staining	-	+	+	-	-
2	Methyl Red	+	+	-	-	-
3	Voges Proskauer	+	+	+	+	+
4	Indole	+	-	-	-	+
5	Citrate	-	-	+	-	-
6	Urease	-	+	-	+	-
7	TSI	A/A	A/A	A	A/A	A
8	Lactose	+	+	+	+	+
9	Sucrose	+	+	+	+	+
10	Glucose	+	+	+	+	+
	Results	*E.coli* 1	*E.coli* 2	*E.coli* 3	*E.coli* 4	*E.coli* 5

+ refers to positive results, - indicates negative results, A indicates Acid, A/A

Indicates Acid/Alkali

Table 2. Phytochemical analysis of tea ingredient

S.No	Phytochemical analysis	Camellia sinensis	Hibiscus rosasinensis	Citrus lemon	Honey	Zingiberoffinale	Brown sugar
1	Alkaloids	+	+	+	+	+	+
2	Flavanoids	+	-	+	-	+	+
3	Saponins	-	-	+	-	-	-
4	Terpenoids	-	+	+	-	+	-
5	Tannins	+	+	+	-	+	-
6	Cardiac glycosides	+	-	-	-	-	-
7	Phylobatannins	-	+	-	-	-	+
8	Steroids	+	+	+	+	-	+
9	Carbohydrates	+	+	-	+	+	+
10	Protein	+	+	+	+	+	+

+ = indicates present and - = indicates negative Qualitative

Table 3. Antibiotic sensitivity test for isolates

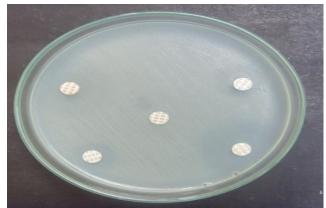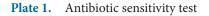| S.no	Isolates	Zone of Inhibition (mm)				
		Cloxacillin (10 mcg)	Rifampicin (10 mcg)	Streptomycin (25 mcg)	Penicillin (10 units)	Ceftidixime (5 mcg)
1	E. coli I	10	R	10	R	11
2	E. coli II	13	12	R	13	10
3	E. coli III	13	R	R	11	15
4	E. coli IV	08	10	R	10	13
5	E. coli V	12	12	R	11	09

R= indicates Resistant, mm = indicates millimeter

isolated. These strains were identified by biochemical tests following the method of Bergey's manual.

Camellia sinensis, *Hibiscus rosasinensis*, *Citrus lemon*, Honey, *Zingiber officinale* and Brown sugar used for the Qualitative Phytochemical analysis. Alkaloids, Carbohydrates and protein were present in all the plants like wise. Saponins and Phylobatannins were absent in all the plants (Table 2).

Plate 1. Antibiotic sensitivity test

Table 4. Antibacterial activity of *Camellia sinensis*

S.no	Isolates	Zone of Inhibition (mm)			
		1g	2g	3g	4g
1	*E .coli* 1	09	10	12	11
2	E. coli 2	12	12	13	13
3	E .coli 3s	13	11	12	13
4	*E. coli* 4	10	12	14	15
5	*E. coli* 5	11	13	15	15

mm = indicates millimeter

Table 5. Minimum Inhibitory Concentration of aqueous extract of 1% *Citrus Lemon*

S.no	Isolates	100 µl	50 µl	25 µl	12.5 µl	6.25 µl	3.12 µl	1.56 µl	0.78 µl	0.39 µl	0.19 µl	0.09 µl	0.04 µl
1	**E. coli** 1	-	-	-	+	+	+	+	+	+	+	+	+
2	**E. coli** 2	-	-	+	+	+	+	+	+	+	+	+	+
3	**E. coli** 3	-	-	-	+	+	+	+	+	+	+	+	+
4	**E. coli** 4	-	-	-	+	+	+	+	+	+	+	+	+
5	**E. coli** 5	-	-	+	+	+	+	+	+	+	+	+	+

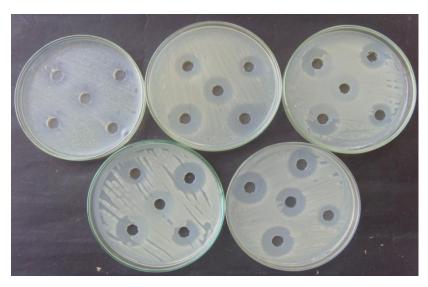+ Indicates Presence of bacteria - Indicates Absence of bacteria

Plate 2. Antibacterial activity of *Citrus lemon* extract against *E. coli* cultures
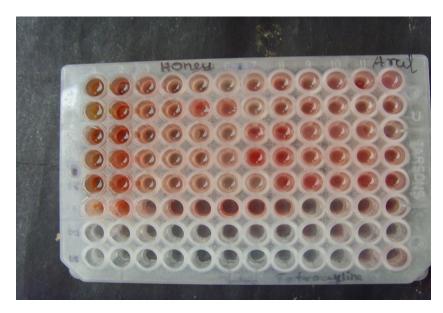

Plate 3. Antibacterial activity of *Citrus lemon* extract against *E. coli* cultures

Table 3 and 4, plate 1and 2 showed antibacterial activity of individual plants against *E. coli* strains. *Camellia sinensis* showed a maximum inhibition zone of (15 mm) against *E. coli* 5 at 5 g with minimum a inhibition zone of (09 mm) against *E. coli* 1 at 1g (Table 5)[13]. Studies on the antimicrobial activities of dust tea methanol extract, commercial green tea and fresh tea leaves. The fresh green tea leaves had a maximum inhibition zone against fungi (30 mm)[14]. Reported the antibacterial activity of boiled ginger extract against food borne pathogens.

5. Summary and Conclusion

Totally five strains of *E. coli* were isolated from diarrheal pathogens. Identification was carried out using biochemical tests. Branded Organic Green TEA, *Citrus lemon, Gingiber officinale, Hibiscus rosa sinensis,* Brown sugar and Branded Honey samples were chosen for tea preparation.

2% *Camellia sinensis*, 4% Honey, 0.1% *Hibiscus rosa sinensis*, 1% *Zingiber officinale,* 1% Brown sugar and 1%, 2%, 3%, 4% *Citrus lemon* chosen for tea preparation.

Antibacterial activity of individual compounds were studied using agar well diffusion method and minimum inhibitory concentration of *Camellia sinensis, Citrus sinensis* showed maximum antibacterial activity against selected pathogens (22 mm).

The phytochemical analysis was carried out for the selected herbals. Alkaloids, Carbohydrates and protein were present in all the plants like wise Saponins and Phylobatannins were absent in all the plants.

From the results it can be concluded that the Herbal tea extract (*Hibiscus rosasinensis, Camellia sinensis*, Brown sugar, *Zingiber officinale*, *Citrus limon*, Honey) was rich in phytonutrients and have the high efficacy against diarrheal pathogens. The results suggest that aqueous extract would be very promising natural antioxidant honey as well as alternative antibacterial agent in therapeutics.

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