Phytochemical analysis and Antibacterial activity of Three different Green Tea Extracts

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Abstract

Camellia sinensis, is consumed in different part of the world as green tea. A set of three green tea dust, flavored green tea dust in a combination of green tea with honey and fruits, green tea with honey and lemon were used for this study. Phytochemical screening was done to identify the presence of phytochemicals in three different green tea extracts. This study indicated the presence of phenolics, alkaloids, flavonoids, steroids, saponins, tannin in green tea extracts. Highest antibacterial activity (14mm) was observed in the extract of green tea dust against Enterobacter. Moderate zone of inhibition was recorded in flavored green tea dust (honey and fruits) against Enterobacter and Pseudomonas sps. Lowest antibacterial activity was shown in flavored green tea dust (honey and lemon) against Staphylococcus aureus.

Key words: *Green tea extract, phytochemical analysis, antibacterial activity*

1. Introduction

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different part of the world as green, black, or oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea. The first green tea was exported from India to Japan during the 17th century (Chen *etal*., 2001). It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea, which is mainly consumed in Asia, some part of North Africa, the United States, and Europe. The association between tea consumption, especially green tea, and human health has long been appreciated. (Vanessa and Gary 2004). The green tea and black tea are processed differently during manufacturing.

Camellia sinensis is an evergreen tree or shrub with yellow-white flowers and long, serrated leaves. Flowers are axillary, solitary, or up to three in a cluster. They are 2.53.5 cm in diameter and have six to eight petals. The outer petals are sepaloid and the inner petals are obovate to broadly obovate. There are numerous stamens 0.8-1.3 cm in length. Flowering of *Camellia sinensis* occurs from October through February and fruiting occurs from August to October Young leaves have short white hairs on their underside and young branches are grayish yellow and glabrous. Current year branchlets are purplish red. Terminal buds are silvery gray and sericeous. Petioles are 4-7 mm in length, pubescent, and glabrescent. Leaf blades are elliptic, oblong-elliptic, or oblong. Seeds are brown, sub globose, and 1-1.4 cm in diameter (Shim *etal.,* 1995).

There are so many health benefits of green tea in humans and animals are studies using animal models show that green tea catechins provide some protection against degenerative diseases (Sano *etal.,* 1995). Some studies indicated that green has an anti-proliferative activity in hematoma cells and a hypolipidemic activity in hematoma treated rats, as well as the prevention of hepatoxicity and as a preventive agent against mammary cancer post-initiation (Pan *etal.,* 2003). Green tea catechins could also act as antitumorigenic agents and as immune modulators in immune dysfunction caused by transplanted tumors or by carcinogenic treatment (Sagesaka-Mitane.*etal.,* 1998). Moreover, green tea, its extract, and its isolated constituents were also found to be effective in preventing oxidative stress and neurological problems (Koo and Cho, 2004).

2. Materials and methods

2.1. Collection of green tea

A set of three, green tea dust, flavored green tea dust in a combination of green tea with honey and fruits, green tea with honey and lemon were used for this study. Tea samples were obtained from a local supermarket at Tholayavttam, Kanyakumari district, Tamil Nadu.

2.2. Solvent extraction

Green tea samples were extracted by dissolving 50gm powdered tea in 250 ml of methanol. It was placed in a shaker for 3-5 days, and the extract was collected from the conical flask by filtration. Then the extract was kept in the water bath at 60º C to evaporate the solvent. The extract thus obtained was concentrated using a vacuum evaporation and stored in reagent bottle.

2.3. Phytochemical screening

The samples were tested for the presence of various phytochemicals. Three different green tea extracts were used to study the phytochemical constituents.

i) Test for alkaloids

To 1 ml of the filtrate, a drop of Mayer's reagent was added along the side of the test tube. The test solution was observed for the presence of black or green precipitate.

ii) Test for tannin

1 ml of sample was taken and 1 ml of ferric chloride solution was added. The test solution was then observed for the presence of black or green precipitate.

iii) Test for saponin

2 ml of sample was added to 5 ml distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and observed for the formation of emulsion.

iv) Test for carbohydrate

1 ml of the extract was treated with 2-3 drops of 1 % alcoholic alpha naphthol and 2 ml of conc. Sulfuric acid. This was added along the test tube (violet ring at the junction of two layers)

v) Test for terpenoids

5 ml of the sample was mixed with 2 ml of chloroform and concentrate sulphuric acid was carefully added to form layer. It was observed for the formation of reddish-brown coloration at the interphase.

vi) Test for flavonoid

5 ml of dilute ammonia solution was added to a protein of the aqueous filtrate of each sample followed by addition of concentrated sulfuric acid. It was observed for the formation of yellow coloration.

vii) Test for steroids

1 ml of aqueous extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added along the side of the test tube. The upper layer turns red, sulfuric acid layer show yellow with green fluorescence. This indicates the presence of steroids.

viii) Test for amino acids

1 ml of extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of amino acid.

ix) Test for phenols

1 ml of each extract was dissolved in alcohol or water was separately with a few ml of neutral ferric chloride solution. Any change in color indicate the presence of phenolic compound.

x) Test for protein

1ml of diluted extract, 1ml of 5% CuSO4 and 1% of NAOH solution was added. Deep blue color and confirmed the presence of protein.

2.4. Antibacterial activity

Antibacterial activities of the different green tea extract were tested using well diffusion method. About 25 ml of nutrient agar medium was poured into a sterile petri plate. The plates were allowed to solidify, after that bacterial pathogens are swabbed using sterile cotton swab. Wells were made on the agar surface with 6mm corkborer. Green tea extracts were poured into the well using sterile pipette. The plates were incubated at 37^0 C ± 2^0 C for 24 hours for antibacterial activity. After the incubation petriplates were observed for the zone of inhibition around the well.

3. Result and Discussion

Phytochemical screening is a mandatory study to known the occurrence of type of chemical group present various extract obtained from successive solvent extraction procedure. Phytochemical screening was done to identify the presence of phytochemicals in three different green tea extracts and it is represented in Table 3.1. This study indicated the presence of phenolics, alkaloids, flavonoids, steroids, saponins, tannin in green tea extracts and indicated the absence of compounds such as carbohydrate, amino acid, protein and terpenoids are shown in *Fig*.3.1. The tannin, alkaloid, saponin, flavonoid, steroids and phenols are present in rich amount. The carbohydrate, amino acid, protein and terpenoids are present in little amount (Zaveri, 2006). Saponins which act as bioactive antibacterial agents in plants.

Table:3.1 Phytochemical analysis of different green tea Extracts

S. No	Phytochemical	Green tea dust	Flavored Green tea dust (honey & fruits)	Flavored Green tea dust (honey & lemon)
1	Phenols	+	+	+
2	Tannin	+	+	+
3	Saponin	+	+	+
4	Carbohydrates	-	-	-
5	Terpenoids	-	-	-
6	Flavonoid	+	+	+
7	Alkaloids	+	+	+
8	Amino acids	-	-	-
9	Protein	-	-	-
10	Steroids	+	+	+

+ indicates present; - indicates absent

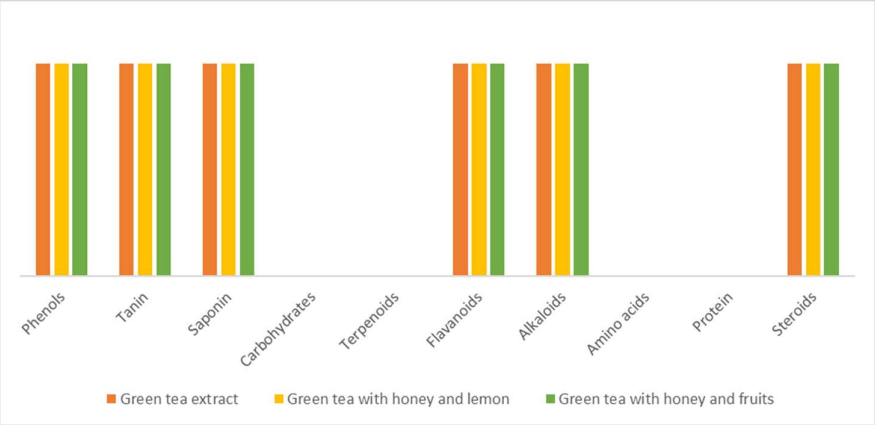

Fig.3.1 Phytochemical Analysis of Different Green Tea Extracts

Green tea extracts are analyzed further for an antibacterial activity. The tea extracts were treated against four potentially pathogenic clinical microorganisms such as *Enterobacter, Pseudomonas sps, Staphylococcus aureus, E. coli,* at different concentrations to understand the most effective activity. Zone of inhibition against clinical pathogens are indicated in Table 3.2.

Extracts of green tea dust shown maximum zone of inhibition of 14mm against *Enterobacter*, 10 mm against *Pseudomonas sps*, 9mm against *E. coli*, 10 mm against *Staphylococcus aureus* respectively. Extracts of Flavored green tea dust (honey and fruits) indicated highest zone of inhibition of 13mm against *Enterobacter*, 12 mm against *Pseudomonas sps*, 8 mm against *E. coli*, 8 mm against *Staphylococcus aureus* respectively. Extracts of flavored green tea dust (honey and lemon) exhibited zone of inhibition of 10mm against *Enterobacter*, 9 mm against *Pseudomonas sps*, 10 mm against *E. coli* and 7 mm against *Staphylococcus aureus*.

Table 3.2 Antibacterial activity of various green tea Extracts against Bacterial pathogens

S.No	Bacterial pathogens	Zone of inhibition (mm)		
		Green tea dust	Flavored Green tea dust (honey & fruits)	Flavored Green tea dust (honey & lemon)
1.	*Enterobacter sps*	14	13	10
2.	*Pseudomonas sps*	10	12	9

3.	*E. coli*	9	8	10
4.	*Staphylococcus aureus*	10	8	7

Highest antibacterial activity (14mm) was observed in the extract of green tea dust against *Enterobacter*. Moderate zone of inhibition was recorded in flavored green tea dust (honey and fruits) against *Enterobacter* and *Pseudomonas sps*. Lowest antibacterial activity was shown in flavored green tea dust (honey and lemon) against *Staphylococcus aureus* (*Fig*.3.2).

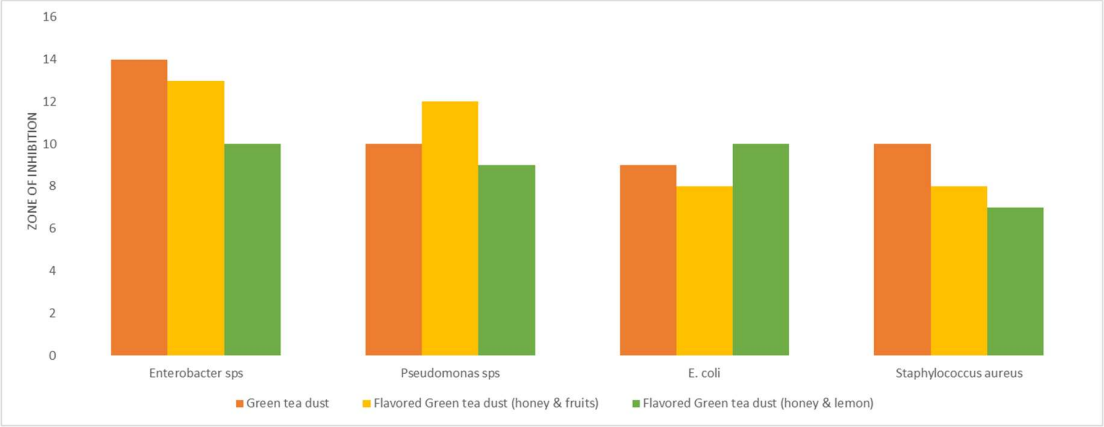

Fig.3.2 Antibacterial activity of various green tea Extracts against Bacterial pathogens

Hamilton-Miller and Shah, (2002) Reported that inhibitory effect of green tea extracts on some strains of bacteria (*E. coli, S. aureus,* MRSA*, S. flexneri and* ETEC). In that study, crude ethanolic and methanolic extracts of green tea exerted the greatest inhibitory activity on all test strains including MRSA, while aqueous extract exhibited the least. Ethanolic extract exercised antibacterial properties even at lower concentrations of 80µg/µl, yielding larger clear zones than methanolic or aqueous extracts of the same concentration (Tsuneki *etal.,* 2004). These findings are significant because *S. aureus* is one of the major causes of both nosocomial and community-acquired infections globally (Hara, 1990). The antibacterial activity of green tea extract is comparable to standard antibiotic. The results of this study are consistent with other studies that reported that green tea extract showed activity against both MRSA and *Staphylococcus aureus*.

4. Conclusion

The results of this study suggest that *Camellia sinensis* extracts are rich in phenolic compounds and have potent antimicrobial and antioxidant activity. Hence, green tea can be used as a natural source of antioxidants to prevent the progression of many diseases. It can be concluded that the method of extraction and the type of solvent used to determine the efficacy of antibacterial properties of green tea extracts. It can be concluded that the extracts of *Camellia sinensis* are effective against both gram-positive and gram-negative bacteria, including multi drug-resistant clinical isolates.

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6. References

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