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Synergic Antimicrobial Impacts of Green tea, Honey and Ginger on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

***Mayur Rohi, Rupam Nashine, Ashok V. Gomashe**
Dept. of Microbiology, Shivaji Science College, Congress Nagar, Nagpur-440012, MS, India
mayurohi@gmail.com

Abstract
Green tea (*Camellia sinensis*) is non fermented tea. The tea is an infusion of leaves that has been consumed for countries as beverages. It has many health benefits. Therefore the present research work was carried out to evaluate the antimicrobial activity of green tea extracts in combination with honey and ginger against the tested bacterial pathogens i.e. *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antimicrobial activity was analyzed by agar well diffusion method. It has been reported that green tea in combination with honey and ginger (methanol extract) was found to be effective against both tested bacterial pathogens while the green tea distilled water extracts were less effective. The Phytochemical analysis revealed the presence of Alkaloids, Saponin, Tannin and Reducing sugars.
Keywords: Green tea, Antimicrobial activity, Phytochemicals, Ginger, Honey

Introduction

Green tea is non fermented tea. Green tea is generally safe, non toxic and with no probable side effects on consumption (Der, 1999). The second most popular beverage in the world after water is believed to be tea. There are different kinds of tea, but green tea carries with it a slew of promised health benefits. And now, scientists have made a new discovery within a simple cup of green tea. The primary difference between green tea and black tea is in the fermentation process required to produce tea. In case of black tea the leaves and buds are fermented or oxidized after they have been dried. In green tea, the leaves are steamed after they are dried. The Phytochemicals present in tea leaves are highly sensitive to oxidation process (Toda *et al.,* 1989).

Epigallocatechin gallate (EGCG), Epicatechin gallate (ECG), Epigallocatechin (EGC) and Epicatechin (EC) are significant antioxidants constituents (Diane *et al.,* 2007). EGCG is the most luxuriant component in tea extract and the most potent chemical tested for biological activity. The combined use of tea and antibiotics could be useful in fighting emerging drug-resistance problem especially among enteropathogens (Archana and Jayanthi, 2011). Numerous studies in humans and a variety of experimental animal models have demonstrated that green tea possess antioxidant, antdiabetic, anti-inflammatory, antibacterial, and anticarcinogenic effects (Chang *et al.,* 1999).

Bacteria that causes the infection was resistant to first-line antibiotics, treatment options are usually replaced with a second or third choice of antibiotics, which are generally much more expensive. Microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the common pathogens of human infection. *Staphylococcus aureus* is an opportunistic pathogen of human skin. *Pseudomonas aeruginosa* is a pathogen associated with pyogenic infection and urinary tract infection. These microorganisms are highly pathogenic and rate of infection caused by these microorganisms are considerably increasing in recent years. Hence the use of plant products has been increased worldwide to minimize the side effects (Padmini *et al.*, 2011).

Therefore, alternative antimicrobial agents are needed to be developed and employed to control multi-drug resistant bacteria. To face this challenge, there has been growing interests to find antimicrobial compounds from medicinal plant extracts as an alternative approach to discover new antimicrobial compounds (Rios and Recio, 2005). The antimicrobial activities of some herbal medicines against different pathogens have been reported from different countries.

Scientifically Ginger is known as Zingiber officinale. Ginger is also a fluently used flavoring material in green tea due to its test, aroma and medicinal properties, the use of drug is mentioned in form of Trikatu, a famous Ayurvedic remedy for the treatment of digestive disorders. In Ashtanga Hridaya, the plant has been used in Rasna Saptak Quath (a decoction based on seven medicinal herbs), and a traditional remedy for arthritis (Sushruta Samhita of Sushruta). Pharma-cologically, the drug in Ayurveda has been described as appetizer. It is also indicated in ointment form for local application in pains (Kikuzaki *et al.*, 1991).

Honey has been used as medicine in many cultures for a long time. However, it has limited use in medicine due to lack of scientific report. In recent days, honey is becoming acceptable as a reputable and effective therapeutic agent. Its beneficial role has been endorsed to its

antimicrobial, anti-inflammatory and anti-oxidant activities as well as boosting of the immune systems.

Green tea is also lauded for its immune-boosting properties and is linked to a decreased risk for serious health issues, including cancer and heart disease. Some researchers believe it can lower the risk for Alzheimer's disease because it helps reduce plaque deposits in the brain. Many people use it as a weight control aid since it promotes fat oxidation. In order to capture the catechins and flavonoids from tea, you should drink it freshly brewed. Green tea is delicious hot and also makes a refreshing iced drink on a hot summer day (Radiji *et al.*, 2013). Green tea is available as a nutritional supplement for people who are not inclined to drink several cups per day. The supplements are available in tablet, liquid and powdered form and can be purchased online and at natural health stores. Although prescription antibiotics are not always the best choice, there are some cases where they are required to deal with serious illnesses or injuries where infections persist. If it becomes necessary for you to take prescription antibiotics consider adding green tea to your bacteria-fighting arsenal. Therefore the current investigation was carried to study the antimicrobial activity of green tea in combination with ginger and honey against *Staphylococcus aureus and Pseudomonas aeruginosa.*

Materials and Method

Preparations of plant extract: C. sinensis leaves were collected from the local market of Nagpur region, Maharashtra (India).

Aqueous extraction: A 10g of each of the grounded leaves were extracted by soaking for 2 days using 100ml of distilled water in a 250ml sterile conical flask. The extracts were filtered using Whatman filter paper No 1 and stored in universal bottles and refrigerated at 4°C prior to use.

Methanol extraction: A 10g powdered sample extracted with 50% methanol to produce crude extracts containing wide range of active compounds. The extracts were prepared by maceration of the plant material with the solvents in a shaker for 2 days. The respective extracts were filtered using Whatman No.1 filter paper and stored in universal bottles and refrigerated at 4°C prior to use.

Antibacterial activity: In these methods, a 15-20ml of Mueller Hinton agar was poured on sterile glass Petri dish (90mm) and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with reference bacterial strain. Agar plate was punched with sterile cork borer of 4mm size and 100 µl of each sample was poured with micropipette in the bore. The plates were incubated at 37°c for 24 hrs (Bauer *et al.*, 1966).

Phytochemical analysis: The Phytochemical analysis was performed to check the presence of following Phytochemicals - Alkaloids, Tannin, Reducing Sugar and Saponin.

Alkaloids: - Filtrates were treated with iodine in potassium iodide. Formation of brown/radish precipitate indicates the presence of alkaloids.

Tannin: - 5ml of extract and 5ml of distilled water was mixed and heated at 80-100°c. For 10 min. in water bath 1% ferric chloride (5-6 drops) was added Dark green color indicated the presence of tannins.

Reducing Sugar: -Extract was shaken with distilled water Boiled with the Fehling's solution A and B for 10min Orange and red colored precipitate indicated the presence of reducing sugar.

Saponin: - 0.2ml of sample was taken and 4.3ml of distilled water was added to it than heated to boil, frothing showed the presence of saponins.

Result:

Antimicrobial activity of green tea extracts was carried out against five different strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Out of that 40% of *S. aureus* as well as *pseudomonas aeruginosa* were found to be sensitive while 60% strains were found to be resistant to ginger, honey and green tea extract prepared in methanol extracts (Table 1, 2).

Antimicrobial activity of green tea extract was carried out against five different strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* out of that 20% strains were found to be sensitive while 80% strains were found to be resistant to Ginger, Honey and Green tea extract prepared in Distilled water (Table 3, 4).

Antibiotic resistance profile showed that all the strains of *Staphylococcus aureus* were found to be sensitive to Levofloxacin and Clindamycin (Table 5), while all the strains (100%) of *Pseudomonas aeroginosa* were sensitive to Levofloxacin and Azithromycin. However 40% *Pseudomonas aeroginosa* were sensitive to Clandamycin (Table 5). Phytochemical analysis revealed the presence of Alkaloids, Tannin, and Saponin. While the reducing sugars were found to be absent (Table 6).

Discussion

The Current study showed that the leaves extract of *Camellia sinensis* posses potent antimicrobial activity. This confirms its use against infection. The organisms found to be

sensitive to fresh green tea extracts were *Staphylococcus aureus and Pseudomonas aeruginosa.* The results were similar with Previous finding in which the capability of green tea was evaluated. It was reported that the growth of *Staphylococcus aureus and Pseudomonas aeruginosa* (Boran *et al.*, 2015). Among these, the methanolic extract of fresh green tea exhibited greater antimicrobial activity. These observations may be attributed to green tea catechin compounds and polyphenols. These compounds have been found to possess antibacterial action (Saikia *et al.*, 2006).

The fresh green tea extract with methanol was found to posses highest antimicrobial activity. It has been documented that green tea contains catechin and polyphenols which are highly sensitive to the oxidation process. The catechin and polyphenols have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties. These compounds could be responsible for the inhibition of pathogens. The antibacterial effects of tea polyphenols (TPP) extracted from Korean green tea (Camellia sinensis) against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) were evaluated. In a similar study, the liquid extracts of green tea seed have shown inhibitory effects on some Gram-positive as well as Gram-negative bacteria (Gadang *et al.*, 2008).

Green tea extracts demonstrated inhibitory properties against major pathogens including *L.monocytogenes*, *E. coli*, *Salmonella typhimurium*, *Campylobacter jejuni*, and others including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella Enteriditis*, *Shigellaflexneri*, *Shigella dysenteriae*, and *V. cholera* (Toda *et al.*, 1991; Gadang*et al.*, 2008).

In present study alkaloids, Saponin, tannin and reducing sugar were present which were similar with the findings of Rasheed and Haider, (1998).

Table1*:* Antimicrobial activity of green tea Extracts in methanol against *Staphylococcus aureus*

Staphylococcus aureus	G.T+M	G.T+M+G	G.T+M+H	G.T+M+G+H
1	S	S	S	S
2	R	R	R	R
3	R	R	R	R
4	R	R	R	R
5	S	S	S	S

Where, G.T-Green Tea, M-Methanol, G-Ginger, H-Honey
R-Resistance ,S-sensitive

Table2*:* Antimicrobial activity of green tea Extracts in methanol against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	G.T+M	G.T+M+G	G.T+M+H	G.T+M+G+H
1	R	R	R	R
2	S	S	S	S
3	R	R	R	R
4	R	R	R	R
5	S	S	S	S

Table3: Antimicrobial activity of green tea Extracts in distilled water against *Staphylococcus aureus*

Staphylococcus aureus	G.T+D/W	G.T+D/W+G	G.T+D/W+H	G.T+D/W+G+H
1	R	R	R	R
2	R	R	R	R
3	S	S	S	S
4	R	R	R	R
5	R	R	R	R

Table 4: Antimicrobial activity of green tea Extracts in distilled water against *Pseudomonas aeroginosa*

Pseudomonas aeruginosa	G.T+D/W	G.T+D/W+G	G.T+D/W+H	G.T+D/W+G+H
1	R	R	R	R
2	R	R	R	R
3	R	R	R	R
4	S	S	S	S
5	R	R	R	R

Where, D/W-Distilled water

Table5: Antibiotic Resistance Profile of *Staphylococcus aureus*

Staphylococcus aureus	Zone of inhibition in mm				
	Levofloxacin	Piperacilin	Amoxyclave	Azithromycin	Clindamycin
1	S	R	R	R	S
2	S	R	R	R	S
3	S	R	R	R	S

4	S	R	R	R	S
5	S	R	R	R	S

Table6:- Antibiotic Resistance Profile of *Pseudomonas aeruginosa*

Pseudomonas Aeruginosa	Zone of inhibition in mm				
	Levofloxacin	Piperacilin	Amoxyclave	Azithromycin	Clindamycin
1	S	R	R	S	S
5	S	R	R	S	S
6	S	R	R	S	R
7	S	R	R	S	R
8	S	R	R	S	R

Table7: Phytochemical analysis of Green tea extract

Sr. No.	Sample	Phytochemical test			
		Alkaloids	Saponin	Tannins	Reducing Sugars
1.	50 % methanol extract	+	+	+	-
2.	100 % Distilled water extract	+	+	+	-

Where, + = Present, - = absent

Conclusion

In the present study it was concluded that green tea along with combination of ginger, honey and green tea was found to be effective against *S. aureus* and *P. aeruginosa*. It can be hoped that this may help to avoid the side effects of antibiotics. In future, the combined use of tea and antibiotics could be also useful in fighting various kinds of infections.

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