



## **Estimation of some Phenolic compound and Antimicrobial activity of Green Tea (*Camellia sinensis* L.) extract**

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### **Keywords**

Antimicrobial activity, green tea extract, paper disc diffusion.

### **Abstract**

In this article, the activity of antibacterial of methanolic owns a green tea leaves (*Camellia sinensis*) extracted on different pathogenic strains has been investigated with the use of paper disc diffusion technique. the tested strains were Enterococcus faecalis , Escherichia coli , Staphylococcus aureus ,Methicillin –resistant staphylococcus aureus (MRSA) and pseudomonas aeruginosa. The results were showed that the extract produced inhibition zones in all tested bacteria ranging from 18 mm in S.aureus to 12mm in p.aeruginosa . we can concluded that The extract from green tea leaves may be helpful in the fight against new drug resistance brought on by the germs mentioned above. Some phenolic materials were determined using a high-performance liquid chromatograph(hplc), and three extraction methods (aqueous, methanol and ethanol) were used. The following phenolic substances were estimated) Epicatechin, Gallic acid, Chlorogenic, Caffeine, Quercetin, Kaempferol). The methanol extraction method significantly outperformed the other of the methods and recorded the highest averages of ( Epicatechin, Gallic acid, Chlorogenic, Caffeine, Quercetin, Kaempferol) reached) 30.367, 26.467, 37.533, 34.800, 25.533, 42.700) mg.g sequentially.

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## **1. Introduction**

The plant leaves of (*Camellia sinensis*) are infused in hot water to create green tea, a form of non-fermented tea. This beverage has been enjoyed for many years and is prized for its therapeutic qualities. Tea has been phytochemically analyzed and has been shown to contain a variety of substances, including alkaloids, saponins, tannins, catechin, and polyphenols (1). It has been discovered that tea leaves exhibit antibacterial action against a variety of microbes.

The 1st distinction between (black and green) tea is the technique of fermentation utilized to t eache production for each. Black tea is created by letting the dried leaves to oxidize or ferment as opposed to (greentea), which is generated by the

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dried leaves with heat. (Greentea) retains more of its active components than black tea because the phytochemicals in leaves of the tea are far more (sensitive-to-oxidation). According to earlier studies (2), *Pleisomonas shigelloides*, *Clostridium perfringens*, *Vibrio parahemolyticus*, *Staphylococcus aureus*, and *Clostridium perfringens* can all be eliminated by drinking green tea on a regular basis. Between (30%-40%) of water-extractable polyphenols are exists in (greentea) as a comparsion to (3%-10%) in (blacktea). (Greentea) owns between (30-40)% of water-extractable polyphenols, as opposed to 3 to 10 percent in black tea. Four polyphenols found in green tea—epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), and epicatechin (EC)—are well-known for their potent antioxidant capabilities.(3)

Beginning in year 1980, Tea can be destroy for a (range) of dangerous bacteria, or below slightly (the usual quantities) of % in brewed tea, according to comprehensive studies on the antimicrobial effects of green tea (4). Alpha-haemolytic streptococci, which are the main cause of dental cavities, and *Helicobacter pylori*, which can cause gastritis and increase the risk of stomach cancer, are other bacterial species against which the antibacterial effect of green tea extracts has been shown. Green tea's chemical composition is intricate and little studied.

The current study's goal is to study the antibacterial abilities of a methanolic of (greentea) extraction, *Camellia sinensis*, against a range of clinical bacterial isolates. The presence of polyphenolic compounds in green tea, particularly flavonoids like catechins, catechin gallates, and proanthocyanidins, is widely documented. Free amino acids (1-5.5%), lignin (6.5%), organic acids (1.5%), chlorophyll (0.5%), caffeine (about 3.5% of the total dry weight, or approximately 50 mg per cup when brewed), theophylline (0.02-0.04%), theobromine (0.15-0.2%), and other methylxanthines are all present in the fresh leaves of green tea. Various "flavor compounds" are also present, but in considerably lower amounts (5). Previous research has demonstrated the antibacterial effects of green tea against a range of microorganisms, and its primary mechanism of action is believed to be owing to its polyphenolic components (2). The methanolic extract of green tea has been shown to dramatically reduce the development of various bacterial species, including *Pleisomonas shigelloides*, *Vibrio parahemolyticus*, *Clostridium perfringens*, *Bacillus cereus*, and *Staphylococcus aureus* (3). In this study, we intended to determine how well methanolic green tea extract inhibited the growth of different clinical isolates of bacteria.

## 2. Materials and methods

1 -Plant collection: To begin the experiment, 100 grams of fresh green tea leaves were collected from a local market and dried before being ground into a fine powder.

2 -Methanol extraction: The methanolic extraction of the green tea powder was performed by soaking the powder in 250 ml of 95% methanol for a period of two weeks(6).

3 -Test organisms: The pathogens employed in this investigation comprised the bacterial strains *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*,

*Pseudomonas aeruginosa*, and Methicillin-resistant *Staphylococcus aureus*, which were all obtained from the medical laboratory of AL-Yarmook hospital.

4 -Antibacterial activity of green tea extract: Using a high-performance liquid chromatograph (hplc) and three different extraction techniques (aqueous, methanol, and ethanol), certain phenolic compounds were identified. The amounts of the phenolic compounds epicatechin, gallic acid, chlorogenic, caffeine, quercetin, and kaempferol were estimated. The arithmetic means were compared using the least significant difference (L.S.D.) after the data were statistically analyzed using the complete randomized design (CRD).

5 -Estimation of some phenolic substances in green tea leaves: Some phenolic materials were determined using a high-performance liquid chromatograph(hplc), and three extraction methods (aqueous, methanol and ethanol) were used. The following phenolic substances were estimated) Epicatechin, Gallic acid, Chlorogenic, Caffeine, Quercetin, Kaempferol) The data were statistically analyzed according to the complete randomized design(CRD), and the arithmetic means were compared on the basis of the least significant difference L.S.D.

### 3. Results

The findings of this investigation showed that green tea (*Camellia sinensis*) methanolic extract has strong antibacterial activity. To do this, gelled sterile Mueller-Hinton agar plates with a broth culture of the test bacteria were put on top, and inhibition zones produced around the paper discs were measured. In comparison to other extracts, the methanolic extract of fresh green tea exhibited greater antibacterial activity. *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Methicillin-resistant *Staphylococcus aureus* were among the test organisms that were discovered to be sensitive to the extract, albeit there were some variances in their sensitivity to the extract. For instance, *Pseudomonas aeruginosa* exhibited the lowest inhibition zone, whereas *Staphylococcus aureus* displayed the biggest inhibition zone (18 mm) (13 mm). The inhibition zone diameters of the green tea extract against the selected bacterial strains are shown in Table 1.

**Table 1.** Antibacterial activity of (greentea) extract on selected strains.

Bacterial strains	Zone of inhibition(mm)
<i>Staph.aureus</i>	18
<i>MRSA</i>	17
<i>E.faecalis</i>	15
<i>E.coli</i>	15
<i>P.aeruginosa</i>	12

The shown results above from Table (2) investigate that there are differences in a significant manner in the (greentea) leaves content of some phenolic substances due to the effect of the extract technique. The methanol extraction method significantly outperformed the rest of the methods and recorded the highest averages of ( Epicatechin, Gallic acid, Chlorogenic, Caffeine, Quercetin, Kaempferol) reached ( 30.367, 26.467, 37.533, 34.800, 25.533, 42.700) mg.g sequentially.

**Table 2.** The effect of the extraction method on the content of green tea leaves from some phenolic substances

Treatment. (Extract Method)	Epicatechin (mg.g)		Gallic acid (mg.g)		Chlorogenic (mg.g)		Caffeine (mg.g)		Quercetin (mg.g)		Kaempferol (mg.g)	
	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean
Water	1.267	21.733	1.474	20.800	1.893	32.500	0.913	26.367	1.468	20.767	0.601	36.167
Methanol	1.450	30.367	0.867	26.467	0.742	37.533	0.987	34.800	0.742	25.533	0.889	42.700
Ethanol	0.606	24.033	1.213	24.067	1.041	23.500	1.014	22.167	0.696	22.133	0.928	37.833
L.S.D		4.111		4.270		4.652		3.430		3.638		2.889
SE(m)		0.819		1.031		0.972		1.319		1.210		1.165
SE(d)		1.158		1.458		1.375		1.865		1.712		1.648
C.V.		3.647		7.830		6.062		7.327		8.817		7.953

#### 4. Discussion

The results founded in straight line and meaning with other researcher that have demonstrated that green tea (*Camellia sinensis*) has antibacterial properties against resistant bacterial strains like VRE(vancomycin-resistant-enterococci), MRSA(methicillin-resistant-staphylococcus-aureus), and *P. aeruginosa* (8, 9, 10). Charctrstics of (greentea) that prevent bacterial from growing and growth are primarily due to components of polyphenolic, which include epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, which have been demonstrated to be effectiveness much more against a differents of Gram (Positive and Negative) bacteria.(12 ,11)

Although the exact process by which green tea exerts its antimicrobial properties is not entirely known, it is believed to entail the interaction of green tea's polyphenolic components with bacterial cell membranes. The structural and functional integrity of the bacterial cell membranes may be compromised as a result of this interaction (7). Additionally, it has been discovered that green tea and -lactam medicines work together to combat MRSA (13, 14). This indicates that green tea and -lactam antibiotics work better together than they do separately to prevent MRSA development. Epigallocatechin gallate, the primary tea polyphenol, has been demonstrated to reverse methicillin resistance in MRSA by preventing the manufacture of PBP2 (15). Additionally, it has been discovered that

epigallocatechin gallate increases the efficacy of inhibitors of cell wall production that are non-lactam and -lactam.(16)

Green tea's capacity to stop dangerous germs from adhering to host cell membranes is another idea for how it works (17). This may be accomplished by how green tea proanthocyanidin epigallocatechin gallate interacts with bacterial outer membranes to limit bacterial attachment to mammalian epithelial cells without affecting the cells themselves (18, 19). The capacity of green tea to influence the activity of dihydrofolate reductase, an enzyme necessary for the production of purine and pyrimidine by pathogenic bacteria, is one hypothesized mode of action for the beverage (20). Nucleic acids, which are crucial for bacterial growth and reproduction, must be synthesized in order for this enzyme to function. Green tea extract may interfere with pathogenic bacteria's capacity to synthesis nucleic acids and hinder their development and proliferation by decreasing the activity of dihydrofolate reductase.

According to the study's findings, *C. sinensis* leaf extract may one day be used in conjunction with conventional therapy to treat illnesses brought on by multidrug-resistant strains of *S. aureus* and *P. aeruginosa*. Prior to being employed as a medicinal agent, more investigation is required to ascertain the bioavailability of the active ingredients in green tea as well as the extract's toxicity and safety. The antibacterial activity of the methanol extraction method was shown to greatly outperform the other methods, and these results are consistent with other investigations (21, 22, 23, 24,25).

## 5. Conclusion

In light of the obtained results, we can concluded that The extract from green tea leaves may be helpful in the fight against new drug resistance brought on by the germs mentioned above..

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