Antimicrobial Activity of Water and Methanol Leaf Extracts of Green Tea (Camellia Sinensis) Against Some Pathogenic Bacteria

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Abstract
This study was carried out to investigate the antimicrobial activity of water and methanol leaf extracts of Camellia sinensis against some pathogenic bacteria. Susceptibilities of S. aureus (SR 1), S. aureus (SR 2), S.typhimurium, E.coli, P. aeruginosa and Proteus to different antibiotics (Ampicillin, Streptomycin, Rifampicin and Neomycin) by disc diffusion test was carried out. Results revealed that S. aureus (SR 1), S. aureus (SR 2), P. aeruginosa, and S.typhimurium were resistant to Ampicillin and Rifampicin. E. coli was resistant to Ampicillin, Rifampicin and Neomycin while Proteus was resistant to all antibiotics used in this study. Antimicrobial activity of water and methanol leaf extracts of green tea at different concentrations (22, 02, 02, 02, and 12 2 2) mg/ml against pathogenic bacteria was tested. Results showed that green tea water and methanol leaf extract showed inhibitory effect against pathogenic bacteria and methanol extract exhibited better antimicrobial effect against Staphylococcus aureus.

Keywords: Green tea, antimicrobial activity, methanol extract.

Introduction
Green tea is a type of tea made solely from the leaves of Camellia sinensis, that has undergone minimal oxidation during processing. Varieties can differ substantially due to variable growing conditions, processing and harvesting time [1]. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols. The cardinal antioxidative ingredient in the green tea extract is green tea catechins which comprise four major epicatechin derivatives; namely, epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), and epigallocatechingallate (EGCG) [2]. Other components include three kinds of flavonoids, known as kaempferol, quercetin, and myricetin. A remarkably higher content of myricetin which may have some bioactivity against pathogen is detected in tea and its extracts than in many other plants [3]. As a result of the growing problem of antibiotic resistant bacteria, studies are now being conducted regarding the antimicrobial effects of compounds found in natural foods, such as green tea [4]. Green tea has long been valued throughout the world for its therapeutic properties [5]. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catchin and polyphenol [5]. Also studies showed that moderate dialy consumption of green tea killed Staphylococcus aureus and other harmful bacteria [8]. Differences in antimicrobial activities of tea have been found to be related with the kind and degrees of fermentation of tea [4]. Studies found that extracts of tea inhibited and killed Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium, Salmonella enteritidis, Shigella dysenteriae, Shigella flexneri, and Vibrio spp; including Vibrio cholera. [7].

Materials and Methods
Plant material
The plant was bought from local market. Leaves of (Camellia sinensis) ground into fine powder using grinding machine [8].

Bacterial isolates
Bacterial isolates used in this study were supplied by Al- Mustansyria Univ., Biology Dept. Staphylococcus aureus (SR 1). Pseudomonas aeruginosa and Proteus were previously isolated from patients with wound infection. Other isolates Staphylococcus aureus (SR 2), Salmonella typhimurium and E.coli were previously isolated from patients with burn infection.

Water extracts
Twenty five grams of the leaf powder were extracted for three hrs in 252 ml of the distilled water using the soxhlet apparatus and the source of heating was water bath (72 7 2°C). The filtrate was then evaporated at 3 7 2°C using
a rotary evaporator, and the resultant crude extract was frozen at -20°C until use to prepare the required concentrations.

**Methanol Extraction**

Twenty five grams of leaf powder were extracted for 24 hours in 20 ml of 95% methanol. The filtrate was concentrated using rotary evaporator at 40°C until dryness.

**Testing susceptibility of isolates to antibiotics**

Disk diffusion test was used for testing susceptibilities of isolates to different antibiotics Amoxicillin, Streptomycin, Rifampicin and Neomycin (Oxoid). bacterial isolates were inoculated in ten ml of nutrient broth medium, the cultures were incubated at 37°C to mid log phase (1-2hrs). 1 μl of inoculated broth then transferred to Muller-Hinton agar plates. A sterile cotton swab was used in three different planes to obtain an even distribution of the inocula for inoculating triplicate plates. With sterile forceps, the selected antibiotic disks were placed on the inoculated plate and incubated the plates at 37°C for 24hrs in an inverted position. Then diameter of inhibition zone were noted and measure by a ruler in millimeters.

**Determination of the antimicrobial activity**

Extracts activities were determined against pathogenic bacteria. For dried leaves water and methanol extract, the stock solution was prepared by dissolving (8/1) g of leaves extract residue with (2/1) ml sterile distilled water then, other concentrations were prepared (1/1, 1/2, 1/3, 1/4 and 1/5) mg/ml. The extract solutions were sterilized by filtration using Millipore filter (1/1, 1/2, 1/3) μm under aseptic conditions.

The nutrient agar medium was mixed well and 2 ml was poured in Petri-dishes. The medium was swabbed with 1 ml of a suspension containing (1×10^6) cfu/ml of the pathogenic bacteria (S.aureus (SR 1), S.aureus (SR 1), P.aeruginosa, Ecoli, P.aruginosa, and Proteus).

The well-plate diffusion technique was used. Five plugs were removed from each agar plate using a sterile Pasteur pipette to produce 5mm-diameter well (1/1, 1/2). To each hole, (1/5) μl from different concentrations of each extract was added and allowed to diffuse at room temperature for 30 min to identify the intrinsic extracts. The plates were incubated aerobically at 37°C for 24 hrs. Each extract was tested against each organism in triplicate. The antimicrobial activity of the plant extracts were recorded as the mean diameter of the resulting inhibition zones of growth measured in millimeters.

**Results and Discussion**

Results shown in Table (1) indicated that the resistance to antibiotics was varied according to the nature of isolates and kind of antibiotics. E. coli was resistant to Amoxicillin, Rifampicin and Neomycin while Proteus was resistant to all antibiotics used in this study. Results showed that S. aureus (SR 1), S. aureus (SR 1), P.aeruginosa, and S.typhimurium were resistant to Amoxicillin and Rifampicin.

<table>
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<tr>
<th>Isolates</th>
<th>Antibiotics (μg/ml)</th>
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<td>S. aureus SR 1</td>
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<td>S. aureus SR 2</td>
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<td>S.typhimurium</td>
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<td>P. aeruginosa</td>
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<td>Proteus</td>
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</table>

S: Sensitive, R: Resistant.

**Table (1)**

**Susceptibility of bacterial isolates to antibiotics**

**AM: Amoxicillin, S: Streptomycin, RF: Rifampicin, N: Neomycin**

Results in Table (1) and Fig.(1) showed that high concentration of green tea water extract (1/4 and 1/5) mg/ml exhibited an inhibitory effect against pathogenic bacteria according to the zone of inhibition. Maximum inhibition diameter was 20mm against S.aureus (SR 1) isolated from skin infection and 17, 18, 17 and 16 mm, followed by 20, 20, 20, 17, 17 and 20 mm against S.aureus (SR 1), E.coli, P.aruginosa, and Proteus. 13, 13, 13, 13 and 13 mm against P.aeruginosa, and 10, 10, 10, 10 and 12 mm against S.typhimurium and 11, 11, 11, 11 and
against E.coli. This may be due to the ability of water extract to exhibit a wide spectrum inhibition effect against Gram positive and Gram negative bacteria.

Results in Table (1) Fig.(7) shown that high concentration of green tea methanolic extract (100 and 1000 mg/ml) exhibited the inhibitory effect against pathogenic bacteria according to the zone of inhibition. Maximum inhibition diameter was (27 mm) against (S.aureus (SR1)) isolated from skin infection and (10, 17, 15, and 11) mm, followed by (15, 25, 23, 19 , and 10) mm against (S.aureus (SR2)), (10, 17, 15, 13, and 11) mm against (P.aeruginosa), (10, 11, and 10) mm against (Proteus) (12, 12 and 12) mm (S.typhimurium) and (11, 11, 10, and 10) against E.coli.

Table (1)
Diameter of inhibition zone caused by Camellia sinensis leaves water extract at various concentrations on some pathogenic bacteria.

<table>
<thead>
<tr>
<th>Conc. of the extract mg/ml</th>
<th>S. aureus SR1</th>
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<th>S.typhimurium</th>
<th>E.coli</th>
<th>P. aeruginosa</th>
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Table (1) shown that high concentration of green tea methanolic extract (100 and 1000 mg/ml) exhibited the inhibitory effect against pathogenic bacteria according to the zone of inhibition. Maximum inhibition diameter was (27 mm) against (S.aureus (SR1)) isolated from skin infection and (10, 17, 15, and 11) mm, followed by (15, 25, 23, 19 , and 10) mm against (S.aureus (SR2)), (10, 17, 15, 13, and 11) mm against (P.aeruginosa), (10, 11, and 10) mm against (Proteus) (12, 12 and 12) mm (S.typhimurium) and (11, 11, 10, and 10) against E.coli.

Table (1) Diameter of inhibition zone caused by Camellia sinensis leaves methanol extract at various concentrations on some pathogenic bacteria.

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<tr>
<th>Conc. of the extract mg/ml</th>
<th>S. aureus SR1</th>
<th>S. aureus SR2</th>
<th>S.typhimurium</th>
<th>E.coli</th>
<th>P. aeruginosa</th>
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-ve = no activity.

Fig.(1) Inhibitory effect of Camellia sinensis water leaf extracts against (Staphylococcus aureus SR1, Staphylococcus aureus SR2, Salmonella typhimurium, Pseudomonas aeruginosa, E. coli and proteus) on solid media as demonstrated by the inhibition zones produced with the well-diffusion antagonosm method.
Fig. (4) Inhibitory effect of *Camellia sinensis* methanolic leaf against (*Staphylococcus aureus* SR †, *Staphylococcus aureus* SR ‡, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *E. coli* and *Proteus*) on solid media as demonstrated by the inhibition zones produced with the well-diffusion antagonosm method.

This result was agreed with [7] who reported that daily consumption of green tea can kill Gram positive *Staphylococcus aureus* and other harmful bacteria. Also it has been reported that the green tea contain catechin and polyphenols. These compounds have been found to possess antibacterial and antiviral action as well as anticarcinogenic and antimutagenic properties.

It was proven that green tea has anticancer and anti hypercholesterole activities, it has also antibacterial activity that includes inhibition of Gram positive cocci, Gram negative bacilli and resistant strains such as vancomycin-resistant enterococci and methicillin resistant *S. aureus* [9], as well as multi-drug resistance *P. aeruginosa* [10].

Various studies have shown significant suppressive effects of green tea against many microorganisms, for example *Salmonella typhimurium* [11], *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *E. coli*, *S. aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonasshigelloides*, *P. aeruginosa* and many other species of bacteria [12,13,14 and 15].

Catechin shows antibacterial activity particularly affecting the membrane fluidity in both, hydrophilic and hydrophobic regions of lipid bilayers of the microorganism. The antibacterial activities of catechins were predominantly related to the gallic acid moiety and the hydroxyl group member [16,17]. The mode of action of catechin involves including rapid leakage of small molecules entrapped in case of intraliposomal space and aggregation of the liposomes have been reported earlier. Catechins also show antibacterial activity by inhibiting the action of DNA polymerases [18].

However, several high-quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement. In addition, numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase. It has also been proposed that sophoraflavone G and (−)-epigallocatechingallate inhibit cytoplasmic membrane function, and that licochalcones A and C inhibit energy metabolism [19].

References


الخلاصة

اجريت هذه الدراسة لمعرفة الفعالية التثبيطية للمستخلص المائي والكحولي لأوراق الشاي الأخضر Camellia sinensis ضد بعض أنواع البكتريا المرضية. قدرت حساسية العزلات المستخدمة لبعض المضادات الحيوية (الأميبيسين وريفامبيسين ونيواميسين) اذ اظهرت النتائج ان S. aureus (SR1)، و S. aureus (SR2)، و P. aeruginosa S. typhimurium كانت مقاومة E. coli للايميبيسين وريفامبيسين ونيواميسين اما فكانت Proteus الامبيسين وريفامبيسين ونيواميسين اما مقاومة للمضادات الأربعة المستخدمة في الدراسة. اما فعالية المستخلص المائي والكحولي لأوراق الشاي الأخضر اظهرت النتائج ان الفعالية التثبيطية للمستخلص الكحولي ضد البكتريا المرضية كانت أفضل من المستخلص المائي.