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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 *Camillia sinensis* against some pathogenic bacteria. Susceptibilities of *S. aureus (SR١), S. aureus (SR٢), 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 (SR١), S. aureus (SR٢), 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 (٢٠,٤٠,٦٠,٨٠ and ١٠٠) 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 [١]. 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)[٢].

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 [٣]. 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 [٤]. Green tea has long been valued throughout the world for its therapeutic properties [٥]. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tanins, catchin and polyphenol [٦]**.** Also studies showed that moderate dialy consumption of green tea killed *staphylococcus aureus* and other harmful bacteria [٧]. Differences in antimicrobial activities of tea have been found to be related with the kind and degrees of fermentation of tea [٨]. 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*. [٧].

Materials and Methods

Plant material

The plant was bought from local market. Leaves of (*Camellia sinensis*) ground into fine powder using grinding machine [٩].

Bacterial isolates

Bacterial isolates used in this study were supplied by Al- Mustansyria Univ., Biology Dept. *Staphylococcus aureus* (SR١), *Pseudomonas aeruginosa and Proteus* were previously isolated from patients with wound infection. Other isolates *Staphylococcus aureus* (SR٢), *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 ٢٥٠ ml of the distilled water using the soxhlet apparatus and the source of heating was water bath (٧٠°C). The filtrate was then evaporated at ٧٠°C using

a rotary evaporator, and the resultant crude extract was frozen at -٢٠°C until use to prepare the required concentrations [١٠].

Methanol Extraction

Twenty five grams of leaf powder were extracted for ٦ hours in ٢٥٠ ml of ٩٥٪ methanol. The filterate was concentrated using rotary evaporator at ١٠٠ °C until dryness [١١].

Testing susceptibility of isolates to antibiotics

Disk diffusion test was used for testing susceptibilities of isolates to different antibiotics Ampicillin, Streptomycin, Rifampicin and Neomycin (Oxoid). bacterial isolates were inoculated in ten ml of nutrient broth medium, the cultures were incubated at ٣٧C° to mid log phase ١٨hrs. ١٠٠µl 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 ٣٧°C for ٢٤hrs in an inverted position. Then diameter of inhibition zone were noted and measure by a ruler in mm[١٢].

Determination of the antimicrobial activity

Extracts activities were determind against pathogenic bacteria. For dried leaves water and methanol extract, the stock solution was prepared by dissolving (٥) g of leaves extract residue with (٥٠) ml sterile distilled water then, other concentrations were prepared (٢٠,٤٠, ٦٠, ٨٠ and ١٠٠) mg/ml. The extract solutions were sterilized by filtration using Millipore filter (٠,٤٥) µm under aseptic conditions [١٣].

The nutrient agar medium was mixed well and ٢٠ ml was poured in Petri-dishes. The medium was swabbed with ٠,١ ml of a suspension containing (١,٥×١٠^{٨}) cfu/ml of the pathogenic bacteria (*S.aureus (SR ١),* (*S.aureus (SR ٢) S.typhimurium, 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 ٥mm-diameter well [١٤,١٥]. To each hole, (١٠٠) µl from different concentrations of each extract was added and allowed to diffuse at room temperature for ٢٠ min to identify the intrinsic extracts. The plates were incubated aerobically at ٣٧°C for ٢٤ 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 (١) indicated that the resistance to antibiotics was varied according to the nature of isolates and kind of antibiotics. *E. coli* was resistant to Ampicillin, Rifampicin and Neomycin while *Proteus* was resistant to all antibiotics used in this study. Results showed that *S. aureus (SR ١), S. aureus (SR ٢), P.aeruginosa, and S.typhimurium* were resistant to Ampicillin and Rifampicin.

Table (١)
Susceptibility of bacterial isolates to antibiotics

Isolates	*Antibiotics (µg/ml)*			
	AM ٢٥	*S ١٠*	*RF ١٠*	*N ١٠*
S. aureus SR ١	R	S	R	S
S. aureus SR ٢	R	S	R	S
S.typhimurium	S	S	R	S
E.coli	R	S	R	R
P. aeruginosa	R	S	R	S
Proteus	R	R	R	R

S: Sensitive, R: Resistant.

AM: Ampicillin, S: Streptomycin, RF: Rifampicin, N: Neomycin

Results in Table (٢) and Fig.(١) showed that high concentration of green tea water extract (٨٠ and ١٠٠) mg/ml exhibited an inhibitory effect against pathogenic bacteria according to the zone of inhibition. Maximum inhibition diameter was ٢٥mm against *S.aureus* (SR٢) isolated from skin infection and ٢٣, ١٨, ١٦ and ١٢ mm, followed by ٢٢,٢٠,١٧,١٦ and ١٣ mm against *S.aureus* (SR١,١٨,١٧,١٥,١٠ and ١٠ mm against *P.aeruginosa,* ١٨,١٧,١٥,١٢ and ١٠ mm against *Proteus,* ١٥,١٣,١١,١٠ and ١٠) mm against *S.typhimurium* and ١١,١٠,١٠,١١ and

١٠ 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.

Table (٢)
Diameter of inhibition zone caused by Camellia sinensis leaves water extract at various concentrations on some pathogenic bacteria.

Conc. of the extract mg/ml	Diameter of inhibition zone (mm)				
	٢٠	٤٠	٦٠	٨٠	١٠٠
S. aureus SR١	١٣	١٦	١٧	٢٠	٢٢
S. aureus SR ٢	١٢	١٦	١٨	٢٣	٢٥
S.typhimurium	١٠	١٠	١١	١٣	١٥
E.coli	١٠	١١	١٠	١٠	١١
P. aeruginosa	١٠	١٠	١٥	١٧	١٨
Proteus	١٠	١٢	١٥	١٧	١٨

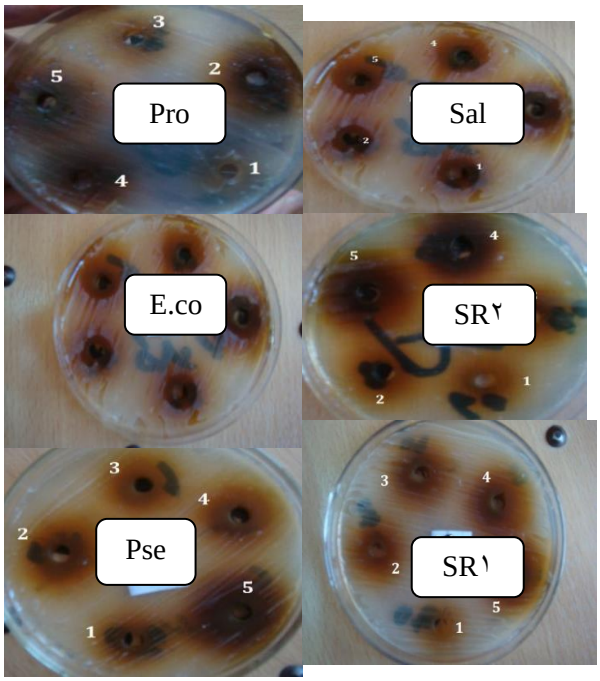

Fig.(١) Inhibitory effect of Camellia sinensis water leaf extracts against (Staphylococcu aureus SR ١, Staphylococcus aureus SR ٢, Salmonella typhimurium, Pseudomonas aeruginosa, E. coli and proteus) on solid media as demonstrated by the inhibition zones produced wilth the well-diffusion antagonosm method.

Results in Table (٣) Fig.(٢) shown that high concentration of green tea methanolic extract (٨٠ and ١٠٠) mg/ml exhibited the inhibitory effect against pathogenic bacteria according to the zone of inhibition. Maximum inhibition diameter was (٢٧mm) against (*S.aureus* (SR٢) isolated from skin infection and (٢٠,٢٠,١٨ and ١٥) mm, followed by (٢٥,٢٥,٢٣,١٩ and ١٨) mm against (*S.aureus* (SR١), (١٨,١٧,١٥,١٣ and ١١) mm against (*P.aeruginosa*), (١٢, ١١ and ١٠) mm against (*Proteus)* (١٠,١٠ and ١٠) mm (*S.typhimurium*) and (١١,١١,١٠,١٠ and ١٠) against *E.coli*.

Table (٣)
Diameter of inhibition zone caused by Camellia sinensis leaves methanol extract at various concentrations on some pathogenic bacteria.

Conc. of the extract mg/ml	Diameter of inhibition zone (mm)				
	٢٠	٤٠	٦٠	٨٠	١٠٠
S. aureus SR١	١٨	١٩	٢٣	٢٥	٢٥
S. aureus SR ٢	١٥	١٨	٢٠	٢٠	٢٧
S.typhimurium	-ve	-ve	١٠	١٠	١٠
E.coli	١٠	١٠	١٠	١١	١١
P. aeruginosa	١١	١٣	١٥	١٧	١٨
Proteus	-ve	-ve	١٠	١١	١٢

-ve = no activity.

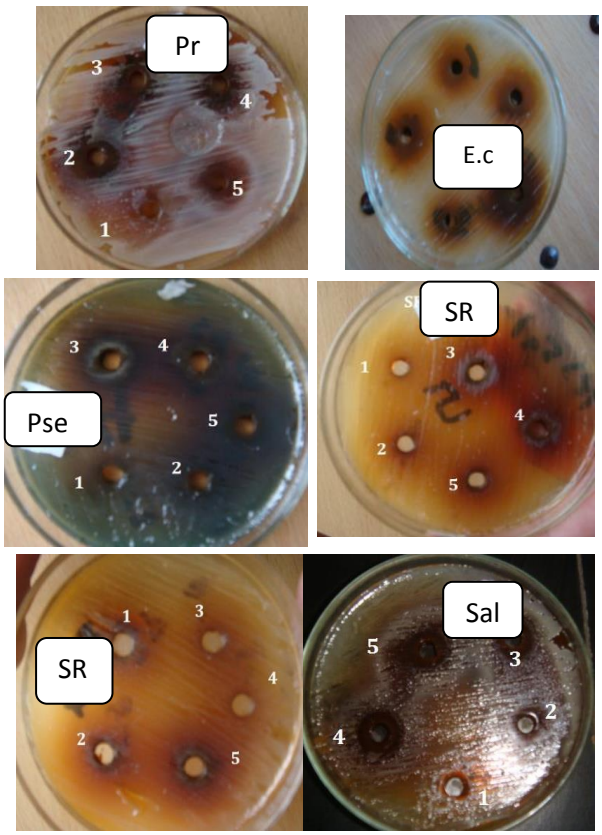

Fig.(٣) Inhibitory effect of Camellia sinensis methanolic leaf against (Staphylococcu aureus SR ١, Staphylococcus aureus SR ٢, Salmonella typhimurium, Pseudomonas aeruginosa, E. coli and Proteus) on solid media as demonstrated by the inhibition zones produced wilth the well-diffusion antagonosm method.

This result was agreed with [٧] 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 anti bacterial activity that includes inhibition of Gram positive cocci, Gram negative bacilli and resistant strains such as vancomycin-resistant enterococci and methicillin resistant *S. aureus* [١٦]., as well as multi-drug resistance *P. aeruginosa* [١٧,١٨].

Various studies have shown significant suppressive effects of green tea against many microorganisms, for example *Salmonella typhimurium* [١٩]. , *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica*, *E. coli*, *S. aureus*, *Vibrio cholerae*, *Campylobacter jejuni*, *Plesiomonasshigelloides*, *P. aeruginosa* and many other species of bacteria [٢٠,٢١,٢٢ and ٢٣].

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 [٢٤,٢٥]. 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 [٢٦].

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 [٢٧].

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الخلاصة

اجريت هذه الدراسة لمعرفة الفعالية التثبيطية للمستخلص المائي والكحولي لاوراق الشاي الاخضر *Camellia sinensis* ضد بعض انواع البكتريا الممرضة. قدرت حساسية العزلات المستخدمة لبعض المضادات الحيوية (الامبيسلين وريفامبيسين وستربتومايسين ونيومايسين) اذ اظهرت النتائج ان *S. aureus (SR١),* و *S. aureus (SR٢),* و *P.aeruginosa* و *S .typhimurium* كانت مقاومة للالامبيسلين و ريفامبيسين و E.coli كانت مقاومة الامبيسلين و ريفامبيسين و نيومايسين اما Proteus فكانت مقاومة للمضادات الاربعة المستخدمة في الدراسة. اما فعالية المستخلص المائي والكحولي لاوراق الشاي الاخضر اظهرت النتائج ان الفعالية التثبيطية للمستخلص الكحولي ضد البكتريا الممرضة كانت افضل من المستخلص المائي.