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الخلاصة تضمن هذا البحث اختبار الفعالية الحيوية للمستخلصات المائية والكحولية لأوراق الشاي الأخضر على بعض أنواع البكتريا المرضية خارج الجسم الحي تضمنت بكتريا _E.coli و P.aeruginosa و S.aureus باستخدام طريقة الحفر على وسط مولر - هنتن الصلب بينت النتائج إن جميع التراكيز المستخدمة (٥، ١٠ و ٢٠) % من المستخلص الكحولي لأوراق نبات الشاي الأخضر كان لها فعل تثبيطي على الأنواع البكتيرية المرضية المستخدمة مقارنة بفعل المستخلص المائي الذي الذي الفهر فعل تثبيط على بكتريا E.coli و ٤٠٠ فقط.

<u>Abstract</u>

This research aims to study the inhibition effect of aqueous and alcoholic extracts of green tea leaves on some pathogenic bacteria in vitro, the bacteria included *E.coli. P.aeruginosa* and *S.aureus* by using walls method on Muller-Hinton media. Results revealed that all three concentration (5,10& 20)% of alcoholic extracts to green tea showed inhibitory effects on selected organisms compared with aqueous extracts which showed inhibitory effects on *E.coli* and *S.aureus* only.

Introduction

Due to increased occurrences of bacterial resistance to synthetic pharmaceutical intibiotics, there has been a shift in medical research towards natural plants that could be used as effecting antimicrobial agents(1).

Camellia sinensis is commonly known as green tea is mainly cultivated in India and china. Green tea has many beneficial effects on the body on of them The antibacterial action (2). So Green tea has been proposed as one such natural treatment for bacteria according to many studies have concluded that green tea is an effective antimicrobial agent against many pathogenic bacteria including: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi,Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella ,flexneri*, *Shigelladysenteriae*, *Streptococcus pneumoniae* and *vibrio cholerae* (3,4,5).

The chemical composition of green tea varies with climate, season, horticultural practices and position of the leaf on the harvested shoot. The major components of interest are the Terpenes and polyphenols. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery and in traditional and alternative medicines such as aromatherapy. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene (6).

The major polyphenols in green tea are flavonoids. The four major flavonoids in green tea are the Catechins Epicatechin (EC), Epigallocatechin (EGC), Epicatechin Gallate (ECG) and Epigallocatechin Gallate (EGCG). Epigallocatechin gallate is viewed as the most significant active component. The leaf bud and first leaves are richest in EGCG. The usual concentration of total polyphenols in dried green tea leaves is about 8 to 12%. Other compound of interest in dried green tea leaves include terpenes, gallic acid, quercetin, kaempferol, myricetin, caffeic acid and chlorogenic acid (7).

The antibacterial action of tea is attributed mostly to the polyphenolic components it contains, it could potentially be exploited at all different levels: from protection of bacterial infection on an individual basis by consumption of polyphenols to the large-scale commercial use of polyphenols in order to prevent contamination of food products by pathogenic bacteria(3,7).

The purpose of the present study is to investigation the inhibitory effects of aqueous and alcoholic extracts of green tea leaves on some pathogenic bacteria, potentially providing ground for natural alternatives to pharmaceutical antibiotic medication.

Materials & Methods

Tea extraction

Dried green tea leaves were collected from a local market in Baghdad city. different concentrations 5,10& 20% of aqueous and alcoholic green tea extracts were prepared according to method described by(3) in which the suspension was held at room temperature for 3h and then centrifuged at 15000 rpm for 10 min.

Test organisms

Pathogenic bacterial isolates included *E.coli*, *S.aureus* and *P.aeruginosa* were used to tested the inhibitory effects of green tea extracts. Nutrient broth were used for growing and diluting the microorganism suspensions, in which bacterial strains were grown to exponential phase in nutrient broth at 37 C° for 18 h and adjusted to a final density of (1×10^8) cfu/ml according to Macfadin (8).

Antibacterial Susceptibility test

A modified agar diffusion methods was used to determined antibacterial activity (9).the surface of sterile Mueller- Hinton agar plates was inoculated with 0.2 ml broth culture of test organisms ,three wells of 6 mm in diameter were aseptically punched on each agar plate using a sterile cork bore .fixed volume 0.1 ml of tea extracts was carefully placed in each well. The plates were then covered and incubated at $37C^{\circ}$ for 24 h.

The zone of inhibition in each well was obtained by measuring the underside of the plate in two planes with a ruler calibrated in millimeters. The control was placed with 0.1 ml of the extracting solvent and incubated.

Results and discussion

The effects of 5,10and 20% concentration of aqueous &alcoholic green tea extracts on selected bacteria are presented in tables 1&2, from the results it can be seen that all three concentrations of alcoholic extracts of green tea showed inhibitory effects on selected organisms while aqueous extract showed inhibitory effects on *E.coli*& *S.aureus* only. This could be because it contains more active ingredients in alcoholic extracts than aqueous extracts of green tea, in which the extraction using acetone & methanol may extract more poly phenol than water alone(9,10)and as it seems in all studies the antibacterial action of tea is attributed to the polyphenolic components it contains which are believed to act as antioxidants(11,12).So it found to be extremely effective against various strains of Pathogenic bacteria that can be harmful and in some incidences, even fatal(11,13).

Among the pathogenic microorganisms *S.aureus* proved to be very sensitive to the addition of extracts to the media then *E.coli*. this fact have been noted by other researchers(3,4,9,11,13) which indicated that polyphenols as a part of catechin damages the lipid bilayer and that may be explain the greater bactericidal effect of catechin to Gram-positive bacteria than Gram negative bacteria (14,15).

Table 1: Antibacterial activity of aqueous extracts to green tea on selected organisms

Organism	Inhibition zones in mm		
	5%	10%	20%
E.coli	2	3	5
S.aureus	4	7	10
P.aeruginosa	-	-	-

 Table 2: Antibacterial activity of alcoholic extracts to green tea on selected organisms

Organism	Inhibition zones in mm		
	5%	10%	20%
E.coli	4	7	10
S.aureus	6	10	16
P.aeruginosa	2.5	4	10







Fig 2: the inhibitory effects of green tea extracts on the growth of S.aureus



Fig 3: the inhibitory effects of green tea extracts on the growth of *P.aeruginosa*

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