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## Effectiveness of Aqueous extract of Green, Black and Red Tea Leaves against some types of Gram positive and negative bacteria

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### ABSTRACT:

**Objective:** An evaluation for Antibacterial activity of aqueous extract of Green, Black, Red, and Tea Leaves against gram positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus faecalis*, *Streptococcus mutans*, *Streptococcus faecalis* and gram negative bacteria *Escherichia coli*, *Salmonella typhi*, *Moraxellacatarralis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Acinetobacter*, *Serratia spp.*

**Methods:** Three types of aquatic extract of green, black and red tea leaves were used to determine the antibacterial activity. Agar well diffusion method was used in this study.

**Results:** All three types of tea aquatic extract exhibited excellent and best antibacterial activity against both gram positive and gram negative bacteria and show good inhibition zone and better than the effect of ciprofloxacin against different types of bacteria.

**Conclusion:** aqueous extract of Green, Black, Red, and Tea Leaves show high antibacterial activity against both gram positive and gram negative bacteria, therefore most types of tea can provide protection to a certain extent against our natural enemies like bacterial pathogens.

**KEYWORDS:** White, Red and Black tea, Antibacterial, antibacterial activity. I

### INTRODUCTION:

The most popular drink world-wide "tea" is produced from *Camellia sinensis* plant[1] with four varieties; white, green, Oolong, and black, Oolong tea from partially fermented leaves; while black tea from fully fermented leaves.

From buds or very young tea leaves; white tea is produce; while green tea is made from mature unfermented leaves [2]. Red tea or Rooibos tea is naturally caffeine free making it a good choice for breastfeeding or pregnant women, red tea contain high number of antioxidants[3]. *Camellia sinensis* plant is a safe, nontoxic, cheap and available traditionally in Asian countries. These properties make it a very good alternative antimicrobial agent [4]. Due to rich content of phenolic compounds in *Camellia sinensis* leaf has the preventive potential for esophageal, colon, lung cancers, urinary infections and dental caries. These polyphenols may account for as much as 30% of the dry weight of fresh tea leaves in green tea, while black tea contains between 3 and 1 percent[5]. There are many compounds in green tea have broad spectrum of biological activities such as antifungal, and antitumor activity and antioxidant functions[5]. Four polyphenolic compounds, are present in tea extract according to the previous studies, Epigallocatechingallate (EGCG), Epicatechingallate (ECG), Epigallocatechin (EGC) and Epicatechin (EC) are significant antioxidants constituents. EGCG is the most luxuriant component in tea extract and the most potent chemical tested for biological activity[7,34]. Aim to determine antimicrobial activity of tea extracts leaves against commonly isolated pathogens (Gram positive and negative bacteria) from hospitalized patient with intestinal ailments, blood and skin infections. These bacterial isolates include Gram negative bacteria: *Salmonella typhi*, *Salmonella typhimurum*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescences*, *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Acinetobacter*, *Escherichia coli*, *Serratia spp.* and Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus agalactiae*

### MATERIALS AND METHODS:

#### Preparation of Aqueous extracts:

Tea leaves were collected from a retail food store (Al- Hilla) 2016. Then aqueous extract (Green, Black and Red Tea Leaves) was soaked in fifty gram of powder by 100 ml distilled water, allowed to stand for 72 hr and sterilized by filtration (using Millipore 0.45 filter paper). This extract was considered as fifty percent (50%) of the extract concentration[8].

#### Bacterial Isolates :

A total of 11 Gram negative, and 8 Gram positive (isolated from clinical samples) were used in this study. The bacterial isolates represented by; *S. aureus*, *S. epidermidis*, *S. pyogenes*, *E. faecalis*, *S.pneumoniae*, *P. aeruginosa*, *P. fluorescences*, *E. coli*, *S. typhi*, *E. aerogenes*, *K. pneumoniae*, *Proteus mirabilis*, *P. vulgaris*, *Acinetobacter*. These bacteria were activated and cloned three successive times in nutrient agar and stored on nutrient agar slant at 4°C. Conventional biochemical tests were used for identification of these organisms[9].

**Antibacterial activity assay:**

Agar disc diffusion method was used for determination antibacterial activity[9]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms, the bacterial inoculum was spread by sterile an L-shaped rod glass spreader. Ciprofloxacin were disk was used as a positive control and was plated in the center of agar plate. (The plates were performed in triplicates). All plate o the tested organisms were allowed for overnight incubation at 37°C. After one day (24 h)incubation, each extract was screened their inhibition zone for all isolates. The zone of inhibitions wa measured by measuring the diameters of scale in millimeter (mm).

**In vitro Antimicrobial activity testing using Agar well diffusion assay:**

Loopfull bacterial isolates growths were inoculated into nutrient broth tube and incubated at 37°C for 18 hours. Normal saline was used for preparation of bacterial suspensions and adjust th turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing  $1.5 \times 10^8$  CFU / ml. Dip cotton swab into adjustment suspension and streak the entir Mueller-Hinton agar, all plates were left for one 5 -15 minutes at room temperature to dry. Media were cut into four wells (5mm diameter) by cork borer and add 0.1ml of the extracts. The plate were incubated at 37°C for overnight. Zone of inhibition was measured from the edge of well to the edge of inhibition of growth [10, 35.36, 37].

**RESULT AND DISCUSSION:**

The inhibition zone diameter of tested bacteria were illustrated in Fig 1 -3, which reveals that the aqueous extract of (Green, Black and Red Tea Leaves) have been observed that they have been showed potential antimicrobial activity which was varied based on its effects because their activity affect by their concentration and may depending on the species of bacterial isolates. Thi activity may due to its content of alkaloids, and flavonoids [11].

Concerning aqueous extract of black tea antimicrobial activity has been reveals in figure(1);

The highest antimicrobial activity of black tea were against *Streptococcus* species; in *Streptococcus agalactia* was with inhibition zone (33)mm then *Streptococcus pneumonia*, *Streptococcus mutanus* with (30)mm inhibition zone and *Streptococcus fecalis* was (28)mm.

This may related to the high content of phenolic compounds in black tea[13].These compounds cause cell death by degrading the cell wall (disrupt the cytoplasmic membrane and interfere wit membrane-integrated enzymes) or by damaging the proteins of cell wall[14].

**Figure (1): Antibacterial activity of black tea against bacterial isolates\**

Hamdi et al's found that black tea has a preventive effects on biofilm formation of *Streptococcus mutans* bacteria [15]. Other study showed that the antibacterial activity of black tea is nc extensive [16]. Aqueous extract of black tea aqueous extract was also exhibits high activity against tested *staphylococcus* species: *Staphylococcus aureus* *Staphylococcus epidermidi* *Staphylococcus saprophyticus* with inhibition zone (28, 30, 30)mm respectively. The most sensitive bacteria was *Staphylococcus aureus* to the addition of tea extracts to the media[16]. Th aqueous extract has less activity against *P. aeruginosa* and *E. coli*. *Proteusmerabilis* *Proteus vulgaris*, *Klebsiella pneumonia*, *Enterobacter spp*, *Acinetobacter* and *Serratia spp*. These are Gram negative bacteria which typically resistant to antimicrobial agents than Gram-positive bacteria. This has been explained by the structure of the outer membrane permeability barrier in Gram negative bacteria, which limits access of the antimicrobial agents to their targets in the bacterial cells. About of 42.19% of *S. typhi* was inhibited by this extract [18];Other study reported the black extract had a good activity against *P. aeruginosa* and *E. coli*[19]. Concerning aqueous extract of red tea antimicrobial activity has been reveals in figure(2);

**Figure (2): Antibacterial activity of red tea against bacterial isolates**

The highest antibacterial activity of aqueous extract of red tea was against *Streptococcus fecalis*, *Staphylococcus saprophyticus* with (30 mm) inhibition zone; followed by *Streptococcus agalactia*, *Acinetobacter* and *Streptococcus mutanus* (28mm) inhibition zone. This finding was compatible with other study reported by (20) that Rooibos extracts possess antimicrobial activit against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus mutans* in liquid culture.

Red tea is wealthy with polyphenols and flavonoids which useful resource inside the discount of cellular damage due to loose radicals that may weaken herbal defenses and subsequently resul in ageing and the onset of sickness; Polyphenols as catechin act on a few bacterial lines belonging through producing hydrogen peroxide [21] and through changing the permeability of th microbial membrane [22]. Microbes careworn by way of publicity to polyphenols upregulate proteins related to protecting mechanisms, which protect cells whilst concurrently down regulatin diverse metabolic and biosynthetic proteins involved, for instance, in amino acid and protein synthesis in addition to phospholipid, carbon, and energy metabolism [23]. Furthermore polyphenols were interfere with bacterial quorum sensing, the production of small signal molecules by way of bacterial cells of *Escherichia coli*, *Pseudomonas putida* and *Burkholderiacepaci*. that cause the exponential increase of a bacterial population[24]. However, unclear whether or not the antimicrobial pastime of the complicated rooibos extract is due to the polyphenols alone c to a few different element of the extracts.

Many studies conducted the antimicrobial abilities of green tea; Chou *et al.*[25], proven that green tea, specifically, are capable of killing bacteria since green tea demonstrated higher overa performance against *Escherichia coli* and *Salmonella*. Inexperienced tea's effectiveness as an antimicrobial agent can in component be attributed to its low degree of fermentation. At some point of the fermentation manner, catechins, which includes EGCG are destroyed, decreasing the tea's antimicrobial activity. This may be definitely seen inside the consequences of the take a look a as the noticeably fermented black tea killed the least microorganism in almost every case[25].

**Figure (3): Antibacterial activity of green tea against bacterial isolates**

The properties of inexperienced green tea which inhibit bacterial boom are in particular associated with their polyphenolic components which include epicatechin, epicatechingallate epigallocatechin, and epigallocatechingallate against numerous Gram-positive and Gram-negative bacteria[27]. Similarly to the antimicrobial effects of mentioned catechins (damage to th bacterial mobile membrane, inhibition of fatty acid synthesis, inhibition of enzyme activity, etc.), there are some consequences that could make contributions to the full antimicrobial impact i infected individuals. These outcomes consist of inhibition of irritation (mainly infection caused by oxidative pressure, which include vascular), extra specifically, with the aid of growing th synthesis of nitric oxide [28]. Inhibiting angiotensin II and IL-6 brought on c-reactive protein expression [29]., suppression of IL-6 and RANK manufacturing in infected osteoblast-like cell [30]., inhibiting IL-8 manufacturing [31], and inhibiting hyaluronidase hobby (activated by continual irritation) by inhibition of IL-12 [32].

In the present work we also compare between the result obtained by tested extract (Green, Black, Red, Tea Leaves and Mentha) and results obtained by ciprofloxacinantibiotic (as mo: traditional treatment for infection caused by tested bacteria) concerning their antimicrobial activity against tested bacteria;

**Figure 4: effect of ciprofloxacin against different types of bacteria.**

All three types of tea aqueous extracts showed more activity as antimicrobial agent against tested bacterial isolates compare to ciprofloxacin according to inhibition zone diameter and eve antibiotic resistant strains(*Pseudomonas aeruginosa*, *Pseudomonas fluorescences*, *Klebsiella pneumoniae*) and less sensitivity bacteria to ciprofloxacin (*Enterobacter spp.*, *Proteus mirabili* *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermidis*). These bacterial strain is a mainly multi-drug resistant bacteria that are most commonly found, especially throug nosocomial infections [33].

Present study concludes that the tea extract can be controlled growth of ciprofloxacin resistance bacterial strains which involved in this study within vitro condition.

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