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PHARMACOLOGICAL EVALUATION OF COMMERCIALLY AVAILABLE BLACK TEA AND GREEN TEA FOR ANTI-BACTERIAL ACTIVITY

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ABSTRACT

Aim:- The aim of the study is to evaluate the anti-bacterial activity of commercially available black tea (*Thea sinensis)* and green tea (*Camellia sinensis*).**Materials and methods:-** Commercial green tea and Black tea were obtained from super market 10g of each were extracted separately by soaking in 100 ml of distilled water for two days. the samples (two concentration 50µg & 100µg) positive and the negative controls were added to each well aseptically by Kirby Bauer Agar well diffusion assay method. This procedure was repeated for each Petri plates then the petri plates were incubated at 37˚C for 24 hrs. After incubation the plates were observed for the zone of inhibition. **Result and discussion:-** In this study, the prepared sample was tested to identify the pharmacological activity. During this study two bacteria were isolated. The isolated bacteria were determined by gram staining. Two different bacteria identified were staphylococcus aureus, Escherichia coli. The inhibition zone was developed around the disc was measured. From the result, Black tea extracts exhibit greater antimicrobial activity in S. Aureus, while in green tea extracts exhibit its highest activity against E. coli strains. The zone of inhibitory concentration for Black tea and Green tea is effective for S.Aureus and E.coli which were found to be at higher100µl concentration when compared to50µl concentration. This study also shows that the Black tea extract gives the highest zone of inhibition against S. aureus, while green tea extract shows the highest activity against E. coli strains. **Conclusion:-**The present study concluded that black tea and green tea extracts both shows strong inhibitory effect against Gram-positive and Gram-negative pathogens. From, the result it is noticed that both the teas extract shows strong antibacterial activity and act as good therapeutic agents to fight against the infection.

Keyword: Zone of inhibition, E.coli, S.aurea, Green tea, Black tea.

INTRODUCTION

The Camellia sinensis leaves are infused to make the beverage tea. Black tea is the most consumed type of tea worldwide, with green, oolong, and white teas following suit. Black tea is primarily consumed in the United States, Europe, Africa, and India. It is made by crushing and drying fresh tea leaves to cause fermentation before final processing. Black tea's distinctive flavour and colour come from the complex theaflavins and other flavonoids that are formed during fermentation when some of the catechins combine [1].

In addition to trace amounts of proteins, carbs, amino acids, fats, vitamins, and minerals, tea has antioxidant qualities. Although it contains a wide variety of other chemical compounds as well, the aroma and health benefits of tea are primarily attributed to polyphenols. Numerous studies have documented the health benefits of green tea against various diseases, which are attributed to its polyphenol content. Green tea has far higher concentrations of these polyphenols than black or oolong tea, which explains why it has antioxidant qualities [2].

Catechins, such as (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC), are unique polyphenolic compounds found in green tea. Half to 70% of catechins are EGCG. The majority of studies done with green tea have focused on EGCG, the main catechin found in tea. Up to 200 mg of EGCG, which has been demonstrated to have chemo preventive and chemotherapeutic effects against a variety of cancer types, can be found in one cup of green tea [3]. Three to five cups of green tea should be consumed daily to achieve a minimum of 250 mg of catechins [4].

BLACK TEA

Black tea is a stronger flavour than other teas. Camellia taliensis is occasionally used all five varieties are manufactured from the leaves of the shrub (or small tree) Camellia sinensis[5]. Black tea has many antibacterialactivity and antioxidant activity. Medicinal properties of tea **include anticancer, anti-inflammatory effect,**

antioxidant, antiviral, anthelminthic and antimicrobial[6].

GREEN TEA

Green tea (Camellia sinensis) is a kind of unfermented tea that retains the natural substance in fresh leaves to a great extent.Green tea has been recommended for patients with hypertension, hyperlipidaemia, coronary heart disease, arteriosclerosis, and diabetes.Green tea has been shown in earlier research to have significant benefits for the treatment of cancer, heart disease, obesity, diabetes, bone health, and oral health.antibacterial activity catechin, ECG, EGCg and ECg components are responsible hence these constituents are mostly used to identify the antibacterial activity[7]. Compared to green tea, black tea has many more components, in part due to oxidation processes that take place during "fermentation". The chemical combination in a cup of tea becomes more complex when the dried, finished tea leaves are extracted into water and undergo additional reactions [8].

MATERIALS AND METHODS

Preparation of sample Extraction:

Commercial green tea and Black tea were obtained from super market. In a 250 ml sterile conical flask, 10g of each of the Black tea and green tea were extracted separately by soaking in 100 ml of distilled water for two days. Whatman filter paper No. 1 was used to filter the extracts. Before being used, the filtrates were kept in universal bottles and chilled at 4°C.

Microbial inoculum preparation:

The nutrient broth was prepared, then identified bacterial colonies were inoculated into the broth culture and were used for antimicrobial activity.

Kirby Bauer Agar Well Diffusion Assay:

The nutrient agar medium was prepared and sterilized by autoclaving at 121˚C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile Petri plates and allowed to solidify the Bacterial broth culture was swabbed on each petri plates using a sterile bud. Then wells were made by well cutter. The solvent distilled water used for extraction of plants, and ethanol used as a negative control, gentamicin is used as a positive control. The samples (two concentration 50µg & 100µg) positive and the negative controls were added to each well aseptically. This procedure was repeated for each Petri plates then the petri plates were incubated at 37˚C for 24 hrs. After incubation the plates were observed for the zone of inhibition.

RESULT

In this study, the prepared sample was tested to identify the pharmacological activity. During this study two bacteria were isolated. The isolated bacteria were determined by gram staining. Two different bacteria identified were staphylococcus aureus, Escherichia coli. The Antibacterial activities were checked by agar well diffusion method. The concentration of black tea and green tea aqueous, distilled water extracts used were 50µl, 100µl.

In this study gentamic in antibiotic disc were used as positive control and Ethanol were used as negative control. Two samples were prepared for the test to identify the antibacterial activity, where sample A is black tea and sample B is green tea. The selected gram-positive bacteria are

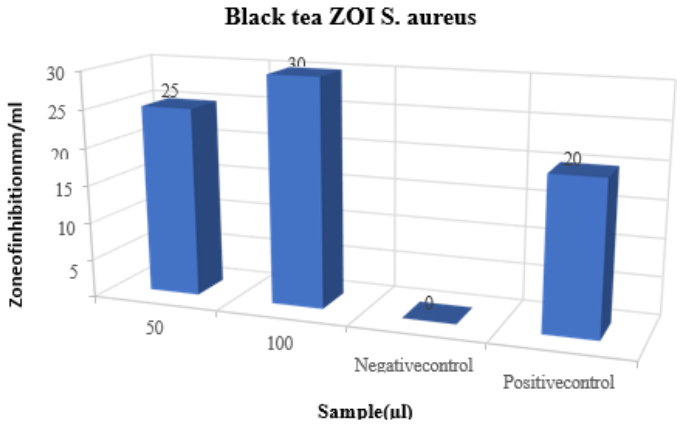
S. aureus and gram-negative bacteria is E. coli.The zone of inhibition was measured for each sample. The measurement of inhibition zones that developed around the discs served as the basis for the evaluation of antimicrobial activity. From the table 1, zone of inhibition of the given sample was observed, where the different concentration of sample (50µl,100µl) and negative control and positive control shown were against both S.Aureus and E.coli.Figure1shows the Sample A green tea with Staphylococcus aureus gram positive bacteria.

Table– 1 Zone of inhibition of sample A - Black tea

SAMPLE	Extract 100µl Added and Zone of Inhibition (mm/ml)			
	50µl	100µl	Negative Control	Positive Control
Staphylococcusaureus	25	30	0	20
E.coli	15	22	0	25

Figure-1: Sample A Black tea-Staphylococcus aureus

In black tea, the S. Aureus exhibit the highest zone of inhibition in 100µl concentration is 30mm, where in50µl concentration it is25mmand in positive control it shows20mm, but no zone of inhibition is seen in negativecontrol.Graph1shows Zone of inhibition of black tea on S.Aureu

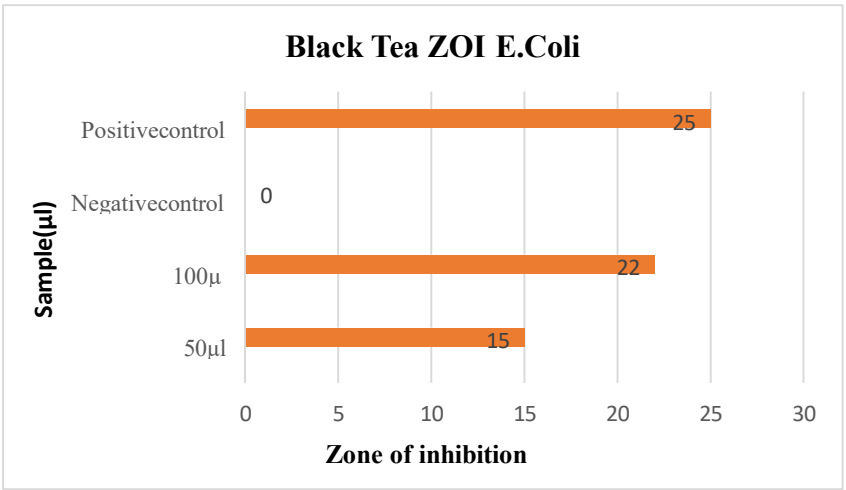


Graph:1 Zone of inhibition of black tea on S. Aureus

Figure-2: SampleA Blacktea– E.coli

Figure 2 shows sample A Black tea – E. coli gram negative bacteria. In black tea, the E. coli exhibit the highest zone of inhibition in 100µl concentration is 22mm, where in 50µl concentration it is 15mm and in positive control it shows 25mm but no zone of inhibition is seen in negative control.

The greater effective was shown in Positive control against both staphylococcus aureus and E. coli. The zone of inhibition was not developed Negative control. From this, the result shows that 100µl concentration of sample is more effective than 50µl concentration samples in the both tea extracts. Graph 2 shows Zone of inhibition of Black Tea on E.coli.



Graph 2: Zone of inhibition of black tea on E.Coli

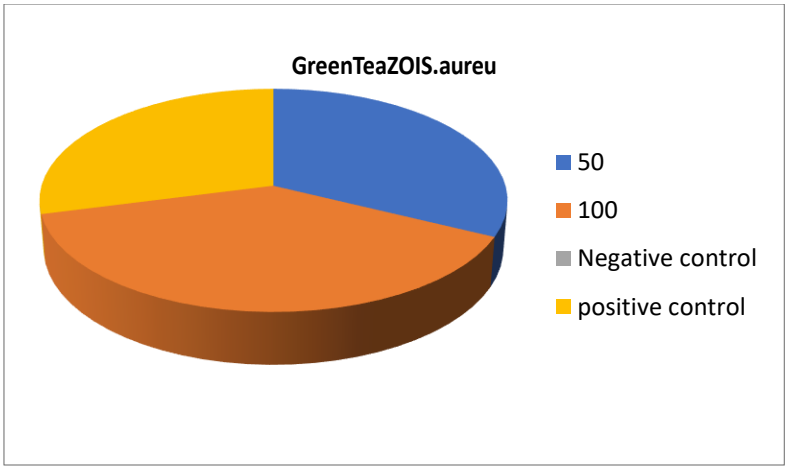
From the table 2, zone of inhibition of the sample B (Green tea) was observed, where the differentconcentrationofsample50µl,100µlandth epositivecontrolandnegativecontrolwere shown against both S. Aureus and E. coli. Figure 3 represent sample B Green tea - Staphylococcus aureus.

Table-2 Zone of inhibition of sample B (Green tea)

SAMPLE	Extract100µlAdded and Zone of Inhibition (mm/ml)			
	50µl	100µl	Negative Control	Positive Control
Staphylococcusaureus	18	24	0	20
E.coli	23	28	0	25

Figure-3: Sample B Green tea-Staphylococcus aureus

In green tea, the S. Aureus exhibit the highest zone of inhibition in 100µl concentration is 24mm,where in 50µl concentration it is18 mm and in the positive control it shows 20mm,but no zone of inhibition is seen in negative control. Graph 3 shows Zone of inhibition of Green Tea on S. Aureus.



Graph 3: Zone of inhibition of Green Tea on S.Aureus

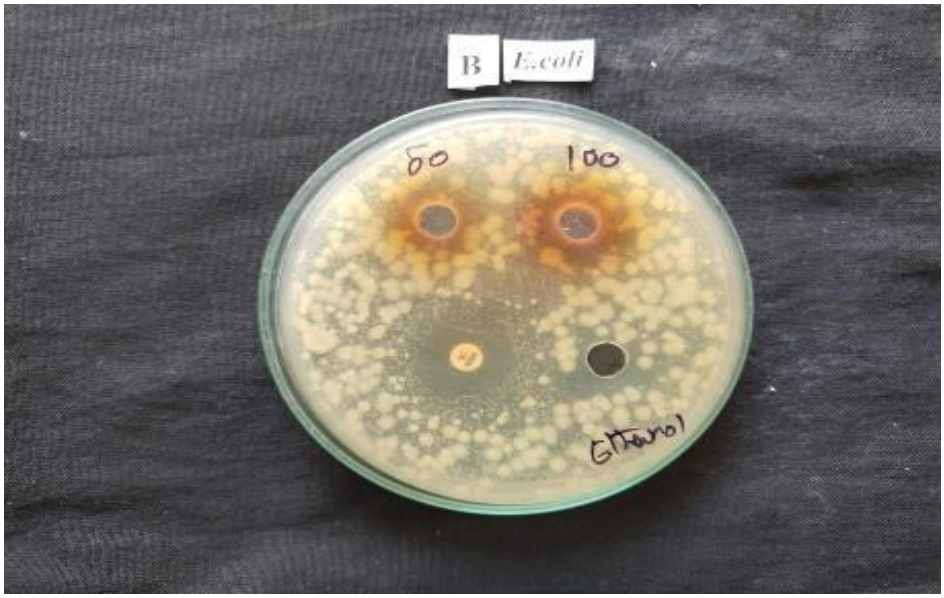
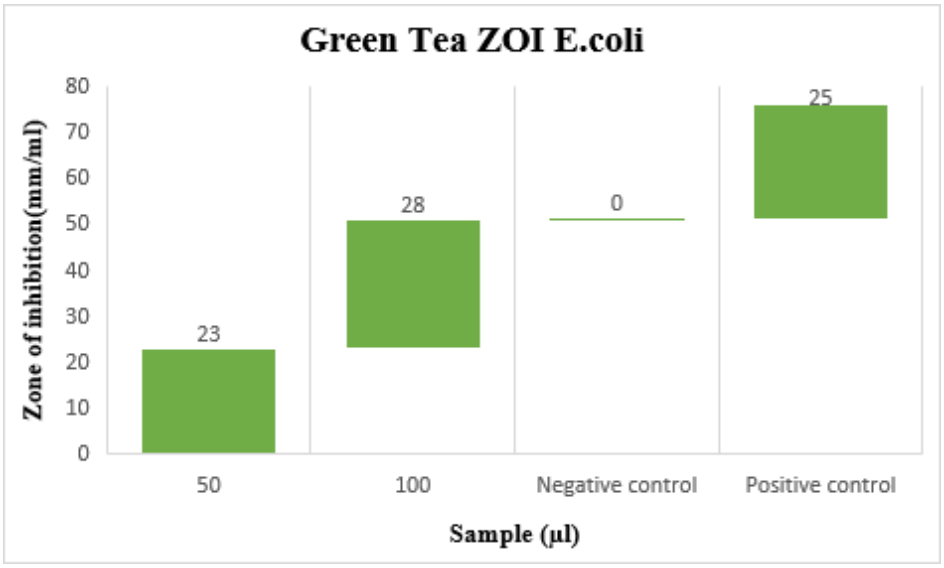


Figure-4: Sample B Green tea–E.coli

Figure 4 shows sample B Green tea–E.coli. In Green tea, the E.coli exhibit the greater zone of inhibition in 100µl concentration is 28mm, where in 50µl concentration is 23mm and the positive controlshows 25mm but no zone of inhibition in was developed in negative control. The greater effective was shown in Positive control against both staphylococcus aureus and E. coli. The zone of inhibition was not developed in Negative control. From this ,the result shows that 100µl concentration of sample is more effective than 50 µl concentration samples in the both green tea extracts. Graph 4 explains the Zone of inhibition of Green Tea on E. coli



Graph 4: Zone of inhibition of GreenTea on E.coli

The data presented that the Black tea extract gave the highest zone of inhibition against S. Aureus. While, green tea showed the highest activity against E. coli strains.

Tea is known to possess antibacterial activity against a number of bacteria. Antimicrobial property in tea is due to presence of polyphenols. Specific antioxidant polyphenols, called catechins, play an important role in green tea's inhibition of bacterial growth.

Studies have demonstrated that the antibacterial, anti-viral, anti-carcinogenic and anti- mutagenic effects of polyphenols and catechins. The inhibition of pathogens may be attributed to these compounds.

The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damages bacterial cell membrane. In this study, we haven't examine the tea constituents presences but it may be a reason to confirm the anti-microbial property of both the teas.

DISCUSSION

Many previous studies were conducted regarding different pharmacological activity such as antibacterial and antimicrobial activity of green tea and black tea. Dailey et al 2015,conducted a study regarding the green tea extracts and its activity which has shown more activity against positive gram than gram negative bacteria membranes[9].

Katsuhiro mabe et al 1999, performed a research work regarding the tea catechins have an antibacterial effect against H.pylori and may have a therapeutic effect against gastric mucosal injury caused by this organism[10]. Araghizadeh et al, 2013 summarized that the activity of aqueous green tea extract on twenty isolated gum and teeth strains from each of the Streptococcus mutans, *Aggreg actinomycetemcomitans*, Porphyromonas gingivalis and Prevotella intermedia, showed that green tea extract had a strong anti-bacterial activity on *S. mutans*.

Cho et al, 2007 reported that the growth of *E. coli* can be inhibited green tea polyphenols compounds[11]. The way of action of these compounds depends on the changing of cell membrane fatty acid composition.Hu et al., 2002 performed a studywhich shows the antimicrobial activity that increase in the inhibition zone diameters on combination of green tea extracts with penicillin G[12]. It also shows the effect of tea extracts on inhibitory activities with β-lactams antibiotics against antibiotic resistant *S. aureus*. Hamdi et al., 2008 conducted research regarding the black tea extracts exhibited high antimicrobial activity against streptococcus mutans more than green tea extracts.

Currently, the present study shows the pharmacological activity of green tea and black tea. The study was conducted by collecting the sample from the local shop. The sample was extracted In this study the zone of inhibition of green tea and black tea was determined separately under different concentration 50µl and 100µl concentration and two bacterial strains were used i.e. gram positive and gram-negative bacteria.

The inhibition zone was developed around the disc was measured. Fromthe result, Black tea extracts exhibit greater antimicrobialactivity inS. Aureus, while in greentea extracts exhibit its highest activity against E. coli strains. The zone of inhibitory concentration for Black tea and Green tea is effective for S.Aureus and E.coli which were found to be at higher100µl concentration when compared to50µl concentration. This study also shows that the Black tea extract gives the highest zone of inhibition against S. aureus, while green tea extract shows the highest activityagainst E. coli strains.

CONCLUSION

The present study concluded that black tea and green tea extracts both shows strong inhibitory effect against Gram-positive and Gram-negative pathogens. This study also suggests that both teas could have wide spectrum anti-microbial activity.

From, the result it is noticed that both the teas extract shows strong antibacterial activity and act as good therapeutic agents to fight against the infection and the zone of inhibition was checked by agar well diffusion method.

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