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Antibacterial and Synergistic Activity of Different Tea Crude Extracts against Antibiotic Resistant *S. Aureus, E. Coli* and a Clinical Isolate of *S. Typhi*

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ABSTRACT

The antibacterial and synergistic effects of black, green, white tea and other specialty teas such as purple tea extracts processed from Kenyan tea germplasm were evaluated and compared with extracts from processed teas of Chinese and Japanese cultivars using disc diffusion method. Methicillin and penicillinase resistant *S. aureus* ATCC 25923 was most susceptible to the tea extracts; showing the largest inhibition diameters. Extracts from processed black, black tea buds, green, purple coloured leaf (both aerated and non-aerated) and white teas weakly inhibited ($p>0.05$) *E. coli* ATCC 25922 and a clinical isolate of *S. typhi* at a concentration of 1mg/ml after 24 hours. Synergism was observed between all tea extracts and penicillin G against methicillin and penicillinase resistant *S. aureus* ATTC 25923. This suggests that the concomitant administration of tea and penicillin G may not impair the antibacterial activity of penicillin G.

KEYWORDS: Black tea; green tea; white tea; methicillin and penicillinase resistant *S. aureus*; *E. coli*; *S. typhi*

INTRODUCTION

Tea is the most popular drink in the world and has several polyphenolic compounds with potential for antibacterial effects. Catechins from tea extracts have been observed to be cytotoxic to microbial pathogens and therefore may be useful as antibacterial agents (Hamilton-Miller, 1995). Extracts of green tea have been established to inhibit food borne pathogens such as *Staphylococcus aureus*, *Shigella dysenteriae*, *Vibrio cholerae*, *Campylobacter jejuni* and *Listeria monocytogenes* (Negi *et al.,* 2003).

There is growing evidence that indicates that the catechin components of green tea are responsible for the observed antibacterial activity, and that Epigallocatechin , Epigallocatechin gallate and Epicatechin gallate constitute the most important antibacterial agents (Yam *et al.,* 1997). Sunphenon, a commercially available preparation of tea polyphenols, has been shown to prevent the attachment of a cariogenic *Streptococcus mutans* strain to hydroxyapatite and also to inhibit its glucosyltransferase activity (Otake *et al.,* 1991). Black tea which is a major source of phenolics, including theaflavins and thearubigins (Luczaj and Skrzydlewska, 2005) has also been shown to have antibacterial properties both *in vivo* and *in vitro* (Bandyopadhyayet *et al.,* 2005).

The synergistic effects of EGCG and β-lactams have been examined in various β-lactamase-producing clinical isolates. Extensive research has revealed that β-lactams and EGCG have synergistic effects against multidrug-resistant bacteria (Stapleton *et al.,* 2004). Despite the valuable data generated so far from green tea, not much data has been generated on the potential antibacterial and synergistic properties of black tea, white tea and purple tea products. In this study different types of tea products processed from different tea germplasm grown in Kenya were assayed for their antibacterial and synergistic properties on antibiotic resistant strains of bacteria.

2.0 MATERIALS AND METHODS

2.1 Bacteria

The test bacteria of American Type Culture Collection (ATCC) were sourced from the Kenya Medical Research Institute, Centre for Respiratory Disease Research (KEMRI-CRDR) and included methicillin and penicillinase resistant *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and a clinical isolate of *Salmonella typhi*.

2.2 Preparation of Tea Samples

The tea samples were sourced from Tea Research Foundation of Kenya (TRFK),Timbilil Estate, Kericho (latitude 0° 22'S, longitude 35° 21'E, altitude 2180 m amsl) and processed at the TRFK miniature factory as described by Karori *etal.,* (2007).

2.3 Biochemical profiling of the tea extracts based on catechins

A modified method of Zuo*et al.,* (2002) which is based on high performance liquid chromatography was used to assay for the tea catechins of as described by (Kerio *etal.,* 2012).

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2.4 Estimation of total polyphenols in the tea extracts

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols in the tea extracts according to ISO (BS ISO 14502-1: 2005(E)).

2.5 Analysis of total theaflavin content in the tea sample by flavognost method

Black, green, purple and white teas were assayed for total theaflavins (TFs) using the flavognost method of Hilton and Palmer Jones, (1973).

2.6 Spectrophotometric determination of total thearubigins in the tea samples

Total thearubigins (TRs) were determined in the tea samples using the method of Roberts and Smith, (1961).

2.7 Freeze Drying of Tea Liquors

Tea liquors derived from the processed tea samples were freeze dried according to the method described by Turkmen *et al.,* (2009).

2.8. Antimicrobial Assays

The agar disc diffusion method was used to screen for antimicrobial activities of the tea liquors according to the National Committee of Clinical and Laboratory Standards (NCCLS, 2011). To standardize the bacterial inoculums for susceptibility test, McFarland No. 0.25 was used to give a cell density of $1.5x10^8$/ml. Chloramphenical was used as a positive standard control against the test bacteria. The discs used absorbed 0.01ml of the sample hence the concentration of each sample extract was established for determination of minimum inhibitory concentration (MIC) (Esiome*et al.,* 2006).

2.8.1 Minimum inhibitory concentrations

Tea liquors that presented inhibitory properties *in vitro* in the screening activity were evaluated for their MIC using the disc diffusion test. The MIC was determined as the lowest drug concentration that inhibited growth, as recommended by the National Committee of Clinical and Laboratory Standards (NCCLS, 2011).

2.8.2 Determination of the combined activity of tea extract and antibiotics

The stock solutions of both tea extracts and antibiotics were mixed in such a way that each mixture contained from zero parts of the antibiotics and ten parts of tea to ten parts of the antibiotics and zero parts of tea. Triplicates of this process were reproduced for each of the strains of methicillin and penicillinase resistant *S. aureus*ATCC 25923, *E. coli* ATCC 25922 anda clinical isolate of *S. typhi*. After 24 hours at 4°C pre-diffusion time interval, the plates were then incubated at 37°C for 24 hours, after which the inhibition zone diameters (IZDs) surrounding the discs were measured.

2.9 Statistical analysis

Data were subjected to analysis of variance using MSTATC software. The Duncan's Multiple Range Test (DMRT) was used to separate the means.

3.0 RESULTS

3.1 Biochemical Analysis of Black, Green and White Tea Products from different germplasm

3.1.1 Total Catechin Content

The total catechin content for green, black and white tea products processed from 11 tea cultivars grown in Kenya and assayed in this study are presented in Table 1. The results revealed that the tea cultivars that produced the different tea products significantly differed ($p \leq 0.05$) in total catechin content.

There were significant differences ($p \leq 0.05$) in total catechin contents between the processed black, green and white teas. Green teas contained significantly ($p \leq 0.05$) higher amounts of total catechins than black teas. Processed green teas from Kenyan cultivars were significantly higher in total catechin content than those from Chinese and Japanese cultivars. White teas processed from the Kenyan cultivars TRFK 301/5 and AHP S15/10 had the highest levels of total catechins at 22.8% and 22.3%, respectively.

Among the Kenyan purple leaf coloured tea cultivars, the highest catechin content was recorded in non-aerated tea products from clones TRFK 73/1 (with 16.1%) and TRFK 306/3 (with 11.9%). In black tea, the highest catechin content was recorded in products from cultivar TRFK 6/8 and those from the purple leaf coloured cultivar TRFK 306/3 with 6.4%and 6.2%, respectively, while the lowest total catechin content was recorded in products from the purple leaf coloured clone TRFK K-purple with a content of 3.2% .

3.1.2 Catechin fractions

There were significant differences ($p \leq 0.05$) in all catechin fractions assayed in this study (Table 1). Results revealed that black teas had lower catechin levels for all the assayed fractions than the processed green and white teas. Green tea manufactured from the Kenyan cultivars AHP S15/10, BBK 35 and TRFK 303/577 recorded higher EGCG levels of 8.6%, 8.7% and 8.9%, respectively when compared with green teas processed from the Chinese cultivar Hanlu (7.7%) and Japanese cultivar Yabukita (5.6%). Among the different black tea products, the Kenyan cultivar BBK 35 had the highest EGCG level of 6.7%. White tea processed from the Kenyan cultivar TRFK 301/5 was significantly higher in the other gallatedcatechin, ECG, when compared to all other teas. Green tea products from the Chinese and Japanese cultivars were low in EGC. The content of EC was not significantly different between green teas processed from Kenyan cultivars and those from Chinese and Japanese cultivars.

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3.1.3 Gallic acid

Gallic acid content varied significantly (p≤0.05) among the different tea products. It was an abundant constituent of black teas processed from the buds only and white tea products. Gallic acid content in black teas ranged from 0.4% to 0.7%, while for black tea processed from the terminal buds, it ranged from 1.3% to 1.4% (Table 1). The highest gallic acid content was recorded in white teas from cultivars TRFK 301/5 and AHP S15/10. Among the green teas processed from the Kenyan germplasm, TRFK 306/3 had the highest gallic acid content of 1.1% and with the purple leaf coloured cultivar TRFK 73/1 giving the lowest content.

3.1.4 Total Tea Polyphenols

The results revealed that black, green and white tea products processed from the test germplasm differed significantly (p≤0.05) in the levels of total polyphenols (Table 2). Green teas processed from Kenyan germplasm were high in total polyphenols with their levels ranging from 20.2% to 24.4% compared to green teas processed from the Chinese and the Japanese germplasm which ranged from 16.7% to 18.5%, respectively. Cultivar TRFK 6/8, a high black tea quality Kenyan clone exhibited the highest total polyphenol content of 24.4% and 19.3% in green and black processed teas, respectively. Total polyphenols from processed aerated teas from cultivars AHP S15/10, TRFK 6/8 and TRFK 306/3 (purple) were higher than the green teas from the Japanese cultivar Yabukita. The purple leaf coloured cultivars TRFK 306/3, TRFK 73/1 and TRFK K-purple produced non-aerated teas that were not significantly different (p≤0.05) in their total polyphenol content with green teas processed from cultivars with green coloured leaf. Black teas processed only from the terminal leaf bud were significantly (p≤0.05) higher in total polyphenol content than black teas processed from the youngest two leaves and a bud. White teas processed from plucked shoots of the two cultivars AHP S15/10 and TRFK 301/5 were not significantly (p≤0.05)different in total polyphenol content from conventional green teas.

3.1.5 Total the aflavin (TFs) and total the arubigin (TRs) levels of black, green and white tea products

There was no significant difference in the total TRs levels for Kenyan, Chinese and Japanese green teas. This was also exhibited inblack tea and black tea buds from the Kenyan cultivars. Black teas had the highest levels of total TFs and total TRs which ranged from 1.1% to 1.7% and 14.6% to 17.2%, respectively (Table 2). White tea had the lowest total TFs as compared to green and black teas. TRs were particularly low in white teas processed from cultivars AHP S15/10 and TRFK 301/5. The results from the present study clearly showed that some TRs were present in green tea products and unaerated tea products from the purple leaf colouredtea clones. Further observations revealed that in green and white teas, TRs were formed in the presence of low levels of TFs unlike in black tea where the TFs levels were slightly higher (Table 2).

3.2 Antibacterial Activity

3.2.1 Methicillin and penicillinase resistant *S. aureus* ATCC 25923

Data obtained from this study, indicated that methicillin and penicillinase resistant *S. aureus* ATCC 25923 was susceptible to the tea extracts (Table 3). Black teas from clones TRFK 6/8, AHP S15/10 and BBK 35 had no significant difference in their inhibitory activity with the green teas processed from leaf of Kenyan cultivars. There was also no significant difference in the inhibitory activity between the Kenyan black tea products with tea processed as black tea products from the terminal buds and some of the Kenyan, Chinese and Japanese green teas studied. Black tea products from clones TRFK 6/8, AHP S15/10 and BBK 35 had a higher inhibitory activity against methicillin and penicillinase*S. aureus* ATCC 25923 as compared to green tea from cultivar TRFK 73/1 and green tea from the Chinese cultivars assayed in this study.

3.2.2 *E.coli* ATCC 25922

In this study,*E. coli* ATCC 25922 was inhibited weakly by black tea and black tea from the buds(Table 3). There was no significant difference (p>0.05) in the inhibitory effects of black tea and black tea buds at 1mg/ml after 24 hours. This was also exhibited by green, purple tea extracts processed from Kenyan tea cultivars, Chinese and Japanese green tea extracts. White tea extracts processed from clone TRFK 301/5 exhibited the highest inhibitory effect with a diameter zone of inhibition of 22mm. Generally, from this study all the teas processed as black, green and white tea products did not significantly differ in their inhibitory effects. Clone TRFK 73/1 processed as black tea and clone TRFK 301/5 processed as black tea from the terminal tea buds exhibited a slightly higher inhibitory effects as compared to the other black tea products.

3.2.3 Clinical Isolate *S. Typhi*

A clinical isolate of *S. typhi* used in this study showed wide differences in the MIC as indicated for tea from clone AHP S15/10. The results obtained also showed that clinical isolate of *S. typhi* used in this study was inhibited by the majority of tea extracts(Table 3). The black tea extractsdid not differ significantly (p>0.05) in the inhibitory effects with green tea extracts. Processed Kenyan black tea buds had no inhibitory effects while white tea extracts processed from clones AHP S15/10 and TRFK 301/5 had the highest inhibitory effects as compared to all the teas studied.

3.3 Minimum Inhibitory Concentrations

The Kenyan black teas, black tea buds, green teas, teas from purple leaf coloured cultivars and white teas studied had an inhibitory effect against methicillin and penicillinase resistant *S. aureus* ATCC 25923. However, the results obtained also indicated wide variations in the MIC of tea extracts from some of the tea cultivars that had a higher

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inhibitory effect. The tea cultivars that produced tea extracts with the highest inhibition zones were BBK 35, TRFK 6/8, TRFK 303/577, TRFK K- purple (unaerated) and AHP S15/10, TRFK 301/5 for green and white tea, respectively, as shown in Table 4.

3.4 Synergistic, Antagonistic and Additive effects of Tea Liquors and Antibiotics

3.4.1 *Methicillin* and *penicillinase* resistant *Staphylococcus aureus* ATCC 25923

The results of this study showed a marked increase in the inhibition zone diameters in tea extracts combined with penicillin G against methicillin and penicillinase resistant *S. aureus* ATCC 25923 except for black tea processed from clones TRFK 303/577 and TRFK 306/3 which showed a decrease thus indicating an antagonistic effect(Table 3). Moreover, an antagonistic effect was seen when tea extracts were combined with gentamicin, tetracycline and ampicillin leading to decrease in the antibacterial activity. This clearly indicates that tea extracts synergize with penicillin G against methicillin and penicillinase resistant *S. aureus* ATCC 25923. Additive effects were not observed in combinations of tea extracts with gentamicin, tetracycline or ampicillin (Table 3).

3.4.2 *Escherichia coli* ATCC 25922

Antagonism was observed when tea extracts were combined with gentamicin and tetracycline at sub-inhibitory concentrations (Table 3). A combination of black, green (Kenyan, Chinese and Japanese) tea extracts with gentamicin and tetracycline did not significantly differ ($p>0.05$) with tea extracts, gentamicin or tetracycline alone. Synergism was only observed in black tea processed from the buds clone TRFK 301/5 with penicillin G. In addition, an additive effect was also observed in TRFK 301/5 black tea buds extract, AHP S15/10 white tea extract and TRFK 6/8 green tea extract with ampicillin and penicillin G, respectively. Similarly, there was no significant difference in combination of tea extracts with penicillin G and ampicillin.

3.4.3 Clinical Isolate of *S.typhi*

There was significant difference in the means ($p\leq0.05$) of the inhibitory effects of black tea extracts combined with gentamicin as compared to black tea extracts alone (Table 3). Therefore, it was noted that black tea extracts did not synergize with gentamicin. This was also exhibited by black tea buds and green tea from the Kenyan, Chinese and Japanese cultivars except white tea. Green tea the Chinese cultivar Hanlu, exhibited an additive effect when combined with gentamicin. Tetracycline combined with black tea extracts differed significantly ($p\leq0.05$) in the inhibitory effects as compared to black tea extracts alone. Black tea processed from terminal buds had no inhibitory effects even in combination with tetracycline, penicillin G and ampicillin. Green teas also did not differ significantly ($p>0.05$) when the tea extracts were combined with tetracycline as compared to tea extracts alone. However, antagonism was observed when tetracycline was combined with all the tea extracts (Table 3).

4.0 DISCUSSION

The levels of polyphenols in the different types of tea products were determined in this study. The results of the study revealed Kenyan teas as high in the levels of their total polyphenols compared to teas processed from Chinese and Japanese germplasm which is in agreement with results from previously reported studies (Wachira and Kamunya, 2005; Karori*et al.,* 2007). The general trend among the samples assayed showed that non-aerated tea had higher total polyphenol content than aerated tea from the same sample. The variation in the polyphenolic composition of the different tea products is ascribed to the different processing methods applied particularly the leaf maceration and auto-oxidation steps during manufacturing. During black tea manufacture, the gallocatechins are first oxidized and dimerized to theaflavins and thearubigins because of their high oxidation potential and high concentration in leaves(Mahanta and Hemanta, 1992). Several other factors have been discovered that influence the polyphenol content of a tea product. These include genotype, geographical origin, soil composition, harvesting time, post harvest treatment and physical structure of the leaves (Lin *et al.,* 2003).The results obtained in this study can therefore be expected to vary with seasons and it could be important to carry out a study on the seasonal variations of the polyphenols in the tea products derived from the assayed tea germplasm. Owing to the fact that tea contains several polyphenols, it is also likely that even such a proposed study may reveal that some derivatives are more stable than others.

In general, the total catechin content in white and green tea products were significantly higher ($p\leq0.05$) than those of aerated tea products from the same clones. Individual catechincontent also varied significantly ($p\leq0.05$) among the tea products with EGCG and EGC recording higher levels while C, EC and ECG being less abundant in non-aerated tea products. The findings of this study corroborated with those of Karori*et al.,* (2007) who found that green teas had significantly higher catechin content than black teas. Black tea is obtained by a post harvest auto-oxidation reaction which is catalyzed by polyphenol oxidase while green teas are not taken through this step during processing and the leaf is initially steamed to inactivate the polyphenol oxidase enzyme. The enzymatic oxidation of catechins located in the vacuole is as a result of polymerization of flavan-3-ol monomers to form TFs and TRs which are compounds that have an influence on the quality of black tea (Owour and Obanda, 2001).In this study, aerated tea products had lower amounts of the individual catechins due to the formation of TFs and TRs.

In this study, white tea which is predominantly manufactured from the young apical hairy bud of specific varieties of tea had high levels of EGCG and EGC that are present in higher amounts in fresh young leaves. These findings were similar to those of Saijo*et al.,* (2004) who

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determined the chemical constituents of young tea leaves and the change occurring during leaf development. The decrease in the gallic acid esters of catechin such as EGCG and EGC during leaf development means that there is a slow biosynthesis of gallic acid moiety in each catechingallate compared with dry matter production.

Black tea products from this study had high levels TFs and TRs as the main fermentation products as compared to green and white tea products. Nonetheless, TFs and TRs were also detected in green and white tea products. Obanda*et al.,* (2004) and Li *et al.,* (2005) reported that theaflavins can be oxidized further to form thearubigins that are heterogeneous in nature and that contribute significantly towards taste, color and body of black tea. Wilson and Clifford, (1992) explained the factors affecting the formation and degradation of theaflavins and thearubigins in black tea and observed that maximum synthesis of theaflavins occurs when oxygen is in excess to support benzotropolone ring formation.

Because of their rich polyphenolic composition, tea products may offer a potentially rewarding route for the identification of novel antibacterial agents. However, since polyphenolcomposition of tea product can be influenced by the geographical location and growing conditions where the crop is cultivated and also by tea processing methodologies and cultivars from which raw material is sourced (Wu and Wei, 2002), then different types of tea products processed from different cultivars grown in different environments are likely to give different bioefficacy levels against target microbes.Indeed, results obtained from this study revealed that different tea products exerted significant antibacterial activity against antibiotic resistant strains and clinical isolates of bacteria. Similar types of studies have also indicated the efficacy of tea as an antibacterial agent (Peter *et al.,* 2005).

The results on the antibacterial activity indicated that the green tea products as well as purple leaf coloured (unaerated) tea and white tea (silvery tips) products processed from Kenyan tea cultivars exerted the highest antibacterial activity, while black tea and black tea processed from terminal tea buds, had lower inhibitory activity. This may indicate that the presence of catechins with the hydroxyl moieties at the 3', 4', and 5' position on the B ring contributed significantly to the inhibitory activity of both green, purple leaf colured(unaerated) tea and white tea. This is agreement with a study reported by Nance *et al.,* (2006),who concluded that antibacterial activity of catechins is predominantly as a result of gallic moiety and hydroxyl group member. Besides this,the highest antimicrobial activity recordedin this study also corresponded to the highest total polyphenols content.

Several of the individual tea polyphenol fractions in black, green and white tea products had significant antibacterial effects. These included EC, EGCG, ECG, TFs and TRs. Gramza and Korczak (2005), who studied the effects of individual catechins separately and indeed, established that EGCG and EGC had the highest antibacterial activity. Susceptibility of bacterial strains to the tea extract has been shown to be related to differences in cell wall components (Ikigai*et al.,* 1993; Hamilton-Miller, 1995). It has been hypothezised that antimicrobial activitiy of tea extracts could be due to the fact that the negatively charged EGCG binds strongly to the positively charged lipid bilayer of Gram-positive bacteria. Catechins partitioning in the lipid bilayer membrane result in loss of cell structure and function and finally led to cell death (Cox *et al.,* 2001).

Polyphenols have been also been reported to exhibit antibacterial activities through their reactivity with protein related polyamide polymers (Haslam, 1996). Further, inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes (Scalbert, 1991). Since some phenolic compounds notably proanthocyanidins are vulnerable to polymerization in air through oxidization reactions, an important factor governing their toxicity is their polymerization size. Oxidized condensation of phenols may therefore also result in the toxification of microorganisms.

Our results also indicated that the antibacterial effects of tea extracts differed depending on the concentration and type of the tea extract and also on the target micro-organism. Tagur*iet al.,* (2006) also established thatthe antibacterial potency of polyphenols is dependent upon the target bacterial species as was observed in this study. For example, in this study, the green tea extract was active against Gram-positive bacteria, *S. aureus*, but was not effective against *E. coli* and *S. typhi* or the two Gram-negative strains, at the concentrations tested here.It has been reported that the bactericidal effect of EGCG is stronger for gram-positive bacteria than for gram-negative bacteria due to the difference in the amount of EGCG absorbed by the bacterial cell (Tagur*iet al.,* 2006).

There was a marked increase in the inhibition zone diameters when tea extract was combined with penicillin G. Zhao *et al.,* (2001) and Hu *et al.,*(2002) also reported enhanced inhibitory when Japanese tea was combined with β-lactams antibiotics against methicillin resistant *S. aureus* ATCC 25923. Such synergistic inhibition by tea extracts and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract (Tiwari*et al.,* 2005). Antibacterial agents used in this study have different mechanisms of action such as protein synthesis inhibition for tetracycline and gentamicin sulfate; and cell wall synthesis inhibition for penicillin G and ampicillin.

In this study, tea extracts and pencillin G synergistically inhibited the growth of methicillin and penicillinase resistant *S. aureus* ATCC 25923 possibly because they directly or indirectly attack the same siteas the peptidoglycan of the cell wall (Zhao *et al.,* 2001). Furthermore, when ampicillin (an inhibitor of cell wall synthesis) was combined with tea extracts, an additive effect was observed which could be hypothesized as resulting from attack on same sites by both tea extract and ampicillin which is also a β-lactam antibiotic. We hypothesize that the tea extractsinduced damage of the bacterial cell wall by interfering with its biosynthesis through direct binding with peptidoglycan and hence synergizing the activity of antibiotic against methicillin resistant *S. aureus* ATCC 25923.Antagonistic effects were observed in this study when tea extracts were combined with tetracycline and gentamicin. Antagonism is thought to occur when the bacteriostatic drug reaches the site of infection before the bactericidal drug (Levinson and Jawetz, 2002).

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The results of the combination studies were also additive which indicates that the inhibitory actions of the combined agents were equivalent to the sums of the actions of the single agents (Sanders *et al.,* 1993). Since both agents (tea and antibiotics) inhibit bacteria by different mechanism of action(Sabbath, 1982), an additive or synergistic interaction is expected to occur (Jawetz*et al.,* 1952). For example, tea contains various polyphenols which have been shown to exert profound antibacterial effects against a broad spectrum of bacteria, including *S.aureus*via membrane perturbations (Esimone*et al.,* 2006). Perturbation of the cell membrane by tea results in a loss to free passage of materials in and out of cell leading to lysis of the cell, which eventually results in death. Pencillin G and ampicillin on the other hand, inhibit the third and final stage involved in the synthesis of peptidoglycan, which is a heteropolymeric component of the cell wall, which provides a rigid mechanical stability by virtue of its highly cross-linked lattice work structure (Sabbath, 1982). This double attack of both agents on different target sites of the bacteria could theoretically lead to either an additive or a synergistic effect (Jawetz*et al.,* 1952).

However, because the strains of bacteria used in this study were resistant to the drugs used, synergism was the most likelyinteraction to occur. Usually, the combination of two agents exhibit significant synergism only if the test organism is resistant to at least one of the agents. Since medicinal plants produce a variety of substances with antimicrobial properties, screening programs are expected to find out new compounds well suited to the development of new antibiotic drugs. Present findings suggest a potential antibacterial activity of tea extracts against Gram-positive and Gram-negative bacteria; however, complementary studies should be conducted to further evaluate this biological property.

5.0 CONCLUSION

From a clinical standpoint, the data presented in this study may indicate a possible use of tea extracts together with penicillin G and ampicillin to manage methicillin and penicillinase resistant *S. aureus* ATCC 25923 infected patients. The findings of this study therefore lend credence to the view that, tea is a safe beverage even for those under treatment with some antibiotics. However,tea extracts and assayed antibiotics were determined *in vitro*, but both antibiotics and polyphenolic compounds undergo metabolic processes in the body and there is less information on the interaction of the related metabolites. Thus, an *in vivo* study needs to be carried out.

Competing interests

The author(s) declare that they have no competing interests.

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TABLES

Table 1: Percent total catechins, individual catechin fractions and gallic acid (%) levels of processed teas from different germplasm grown in same environment

Tea samples		Individual catechins					
	TC%	EGCG%	EGC%	ECG%	EC%	C%	GA%
Black tea products from Kenyan germplasm							
APH S15/10	5.22	4.82	3.24	1.51	0.66	0.27	0.73
BBK 35	4.95	6.71	3.69	2.80	1.29	0.39	0.72
TRFK 303/577	5.41	4.93	2.11	1.70	0.92	0.14	0.73
TRFK 6/8	6.36	4.34	3.70	1.68	1.05	0.20	0.59
Purple coloured tea							
TRFK K-Purple	3.22	2.48	0.31	2.44	0.66	0.46	0.36
TRFK 306/3	6.18	3.51	1.15	2.08	0.64	0.44	0.73
TRFK 73/1	5.22	3.87	1.57	1.57	0.84	0.19	0.48
Mean	5.22	4.38	2.25	1.97	0.87	0.30	0.62
Black tea products from buds of Kenyan Germplasm							
AHP S15/10	9.06	5.73	4.62	1.63	0.22	0.61	1.26
TRFK 301/5	10.84	5.57	3.24	3.80	0.59	0.38	1.38
Mean	9.95	5.65	3.93	2.72	0.41	0.50	1.32
Green tea products from Kenyan germplasm							
AHP S15/10	17.46	8.58	3.52	2.35	1.05	0.43	0.85
BBK 35	19.65	8.76	3.64	3.49	1.60	0.49	0.92
TRFK 303/577	19.96	8.93	5.21	2.66	1.64	0.19	0.71
TRFK 6/8	17.63	7.58	4.61	2.42	1.53	0.30	0.64
Purple coloured tea							
TRFK K-Purple	12.34	4.58	1.23	3.68	1.07	0.68	0.57
TRFK 306/3	11.92	4.56	1.45	3.33	1.11	0.40	1.09
TRFK 73/1	16.10	7.13	3.88	2.39	1.46	0.25	0.46
Mean	16.44	7.16	3.36	2.90	1.35	0.39	0.74
Green tea products from germplasm of other sources							
Hanlu st 830 (China)	13.98	7.73	1.12	2.35	1.38	0.13	0.66
Yabukita st. 536 (Japan)	10.68	5.63	1.00	1.78	1.30	0.12	0.36
Mean		6.68	1.06	2.07	1.34	0.13	0.51
White tea products from Kenyan germplasm							
AHP S15/10	22.29	10.63	5.82	2.61	0.33	0.66	1.42
TRFK 301/5	22.79	10.12	2.86	6.02	0.97	0.48	2.05
Mean	22.79	10.38	4.34	4.31	0.65	0.57	1.74
CV%	16.16	3.15	4.20	2.35	4.74	13.92	5.46
LSD (p≤0.05)	0.76	0.37	0.35	0.17	0.22	0.35	0.22

TC, Total catechin; EGCG, Epigallocatechingallate; EGC, Epigallocatechin; ECG, Epicatechingallate; EC, Epicatechin; C, Catechin; GA, Gallic acid.

Table 2: Percent total polyphenols, total theaflavins and total thearubigins levels of processed tea products from different germplasm grown in same environment

Tea Samples	TP%	TFs%	TRs%
Black tea products from Kenyan germplasm			
Green leaf coloured cultivars			
AHP S15/10	18.8	1.1	15.5
BBK 35	17.5	1.3	16.2
TRFK 303/577	17.4	1.5	15.4
TRFK 6/8	19.3	1.7	14.6
Purple leaf coloured cultivars			
TRFK K-Purple	16.2	1.3	17.2
TRFK 306/3	18.7	1.3	15.7
TRFK 73/1	16.3	1.5	15.6
Mean	17.7	1.4	17.9
Black tea products from buds of Kenyan germplasm			
AHP S15/10	17.2	1.4	13.1
TRFK 301/5	19.0	1.1	10.4
Mean	18.1	1.4	11.7
Green tea products from Kenyan germplasm			
Green leaf coloured cultivars			
AHP S15/10	20.2	0.4	7.7
BBK 35	20.9	0.4	6.8
TRFK 303/577	22.8	0.4	8.7
TRFK 6/8	24.4	0.5	9.3
Purple leaf coloured cultivars			
TRFK K-Purple	19.7	0.6	10.2
TRFK 306/3	22.2	0.4	11.2
TRFK 73/1	21.5	0.4	8.8
Mean	21.7	0.5	8.9
Green tea products from germplasm of other sources			
Hanlust. 830 (China)	18.5	0.3	9.6
Yabukitast. 536 (Japan)	16.7	0.2	9.8
Mean	17.6	0.3	9.7
White tea products from Kenyan germplasm			
AHP S15/10	22.0	0.1	0.8
TRFK 301/5	25.2	0.1	0.9
Mean	23.6	0.1	0.9
CV%	3.8	17.6	6.6
LSD (p≤0.05)	0.7	0.5	0.8

TP- total polyphenols; TFs- total theaflavins; TRs- total thearubigins

Table 3: Antibacterial, synergistic, antagonistic and additive effects of tea liquors and antibiotics against methicillin and penicillinase resistant *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and a clinical isolate *S. typhi* determined by zones of inhibition (mm)

Tea Sample	Tea alone (1mg/ml)	Gentamicin + Tea	Tetracycline + Tea	Penicillin G + Tea	Ampicillin +Tea
Black tea products from Kenyan germplasm					
Green leaf coloured cultivars					
AHP S15/10	16.0[6.0](8.0)	12.3[6.0](7.0)	12.0[6.0](7.0)	18.7[6.0](11.3)	12.0[6.0](8.3)
BBK 35	16.0[6.0](7.0)	12.7[6.0](6.0)	13.0[6.0](7.7)	19.3[6.0](7.0)	12.7[6.0](7.0)
TRFK 303/577	14.0[6.0](9.3)	8.0[6.0](8.3)	8.0[7.0](6.3)	12.3[7.3](8.3)	12.0[7.3](8.3)
TRFK 6/8	16.3[6.0](7.7)	14.3[6.0](7.7)	16.0[6.0](8.3)	18.0[6.0](9.0)	8.7[6.0](8.0)
Purple leaf coloured cultivars					
TRFK K-Purple	13.7[6.0](6.0)	7.3[6.0](6.0)	8.0[6.0](6.0)	17.3[6.0](6.0)	9.0[6.0](6.0)
TRFK 306/3	14.0[6.0](6.0)	14.0[6.0](6.0)	14.7[6.0](6.0)	16.3[6.0](6.0)	11.3[6.0](6.0)
TRFK 73/1	14.3[7.0](6.0)	11.0[6.0](6.0)	10.0[6.0](6.0)	18.0[6.0](6.0)	8.0[6.0](6.0)
Mean	**14.9[6.2](7.1)**	**11.4[6.0](6.7)**	**11.7[6.1](6.8)**	**17.2[6.2](7.7)**	**10.5[6.2](7.1)**
Black tea products from buds of Kenyan germplasm					
AHP S15/10	13.7[6.0](6.0)	12.7[6.0](7.0)	12.3[6.0](6.0)	18.0[6.0](6.0)	11.3[6.0](6.0)
TRFK 301/5	14.3[7.3](6.0)	11.3[6.0](6.0)	14.0[7.0](6.0)	18.7[10.0](6.0)	9.7[8.3](6.0)
Mean	**14.0[6.7](6.0)**	**12.0[6.0](6.5)**	**13.2[6.5](6.0)**	**18.3[8.0](6.0)**	**10.5[7.2](6.0)**
Green tea products from Kenyan germplasm					
Green leaf coloured cultivars					
AHP S15/10	16.7[7.0](6.0)	9.3[7.0](6.0)	14.0[7.0](6.0)	18.0[8.3](6.0)	11.0[6.0](6.0)
BBK 35	22.0[8.7](8.0)	11.7[7.3](6.0)	14.0[7.0](7.0)	16.7[8.3](11.3)	9.3[7.0](7.0)
TRFK 303/577	19.0[8.0](7.0)	10.3[7.0](6.0)	12.0[8.0](7.0)	18.7[7.3](10.0)	7.7[7.3](9.0)
TRFK 6/8	21.0[8.0](7.3)	8.0[6.0](6.0)	6.0[7.0](7.0)	21.0[6.0](11.0)	8.0[8.3](10.3)
Purple leaf coloured cultivars					
TRFK K-Purple	18.0[7.7](6.0)	12.7[6.0](6.0)	8.0[6.0](6.0)	23.0[7.3](6.0)	11.0[6.0](6.0)
TRFK 306/3	17.0[7.0](7.7)	13.3[7.0](6.0)	12.0[7.0](7.0)	17.0[7.3](8.3)	11.0[7.3](7.3)
TRFK 73/1	13.3[7.0](7.0)	13.0[6.0](7.0)	13.7[7.0](7.7)	18.0[7.7](10.3)	11.7[7.0](12.3)
Mean	**18.1[7.6](7.0)**	**11.2[6.6](6.1)**	**11.4[7.0](6.8)**	**18.9[7.5](9.0)**	**9.9[7.0](8.3)**
Green tea products from germplasm of other sources					
Hanlust. 831 (China)	14.7[7.0](7.3)	16.0[6.0](8.3)	17.0[7.0](6.0)	22.0[7.0](7.3)	13.0[7.7](7.3)
Yabukitast. 536 (Japan)	16.0[7.0](8.0)	13.0[7.0](7.0)	12.7[7.0](7.0)	19.0[7.3](8.3)	12.0[7.7](7.0)
Mean	**15.3[7.0](7.7)**	**14.5[6.5](7.7)**	**14.8[7.0](6.5)**	**20.5[7.2](7.8)**	**12.5[7.7](7.2)**
White tea products from Kenyan germplasm					
AHP S15/10	18.0[7.0](25.0)	10.7[6.0](6.0)	15.0[7.3](7.3)	20.3[8.0](19.0)	9.3[7.0](8.3)
TRFK 301/5	22.0[11.0](12.3)	11.3[6.3](7.0)	13.7[7.0](7.0)	17.0[7.0](16.0)	9.0[7.0](8.0)
Mean	**20.0[9.0](18.7)**	**11.0[6.2](6.5)**	**14.3[7.2](7.2)**	**18.7[7.5](17.5)**	**9.2[7.0](8.2)**
Distilled water	6.0[6.0](6.0)	6.0[6.0](6.0)	6.0[6.0](6.0)	6.0[6.0](6.0)	6.0[6.0](6.0)
Chloramphenicol (0.60µg/ml)	32.0[20](23)				
Antibiotics alone (µg/ml)					
Gentamicin 1.96		18.0[8.0](8.0)			
Tetracycline 1.96			19.0[9.0](9.0)		
Penicillin G 1.96 [250] (125)				14.0[8.0](10.0)	
Ampicillin 1.96 [62.5] (15.64)					18.0[8.0](7.0)

CV% = 2.24 [3.27] (3.72)

LSD ($p \leq 0.05$) = 0.24 [0.16] (0.22)

Parentheses[x] - *E .coli*; brackets (x) - *S .typh*

Table 4: Minimum inhibitory concentrations (MICs) of tea liquors against methicillin and penicillinase resistant *Staphlococcusaureus* ATCC 25923 determined by zones of inhibition (mm)

Tea sample conc.(mg/ml)	1.0	0.50	0.25	0.125	0.063	0.031	0.016	MIC
Green tea products from Kenyan germplasm								
Green leaf coloured cultivars								
*BBK 35	22	16	11	8	6	6	6	125µg/ml
*TRFK 6/8	21	16	11	9	8	7	6	31.26µg/ml
*TRFK 303/577	19	16	14	11	6	6	6	125µg/ml
Purple leaf coloured cultivar								
*TRFK K-purple	18	11	8	7	6	6	6	125µg/ml
White tea products from Kenyan germplasm								
*AHP S15/10	18	14	8	7	6	6	6	125µg/ml
*TRFK 301/5	22	15	10	9	7	6	6	62.5µg/ml