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Effects of Kenyan black tea water soluble components on theaflavins interaction with antibiotics against selected pathogenic bacteria

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Abstract. *Bor A, Kenya E, Mbithi JJN, Mutai C. 2017. Effects of Kenyan black tea water soluble components on theaflavins interaction with antibiotics against selected pathogenic bacteria. Bioteknologi 14: 47-59.* This research is intended at differentiating the antibacterial activities of hot water extract of black tea having 18 µg/mL of theaflavins and 18 µg/mL of isolated theaflavins and their blend with antibiotics such as ampicillin. Their blended impacts with antibiotics were resolved by utilizing disk diffusion and modified Checkerboard method. The chi-square test was used to test the null hypothesis, which stated that water soluble components have no effect on theaflavins interaction with antibiotics. The water-soluble components of black tea extract were extracted with hot water, with the theaflavins being measured using the Flavognost method. Similar amounts of theaflavins were extracted using organic solvents and silica gel column chromatography. The concentrates of hot water extract and isolated theaflavins showed synergistic activity with selected antibiotics. However, the level of synergism differed significantly at $P<0.05$, with isolated theaflavins having a higher level. The difference in inhibitory effect between blended concentrates of hot water extract and isolated theaflavins with MIC (10.4 µg/mL) of ampicillin against *Salmonella typhi* was significant ($\chi^2 = 0.56$; $P<0.05$). The differences in inhibitory effect were also significant at ($\chi^2 = 0.699$; $P<0.05$) between the two black tea extracts blends with MIC (4.3 µg/mL) of norfloxacin against *Pseudomonas aeruginosa*. The blend of concentrates of hot water extract and isolated theaflavins with MIC (2 µg/mL) of ciprofloxacin differed significantly in level of impediment at ($\chi^2 = 1.98$; $P<0.05$) against *Staphylococcus aureus*. When the concentrates of the two black tea extracts were blended with MIC (5.25 µg/mL) of tetracycline, the inhibitory effect differed significantly at ($\chi^2 = 2.27$; $P<0.05$) against *Enterobacter aeruginosa*. It was also significant at ($\chi^2 = 0.4$; $P<0.05$) when concentrates of the two black tea extracts were blended with MIC (12 µg/mL) of chloramphenicol against *Escherichia coli*. The differences in inhibitory effect were attributed to the interactions within the tea infusion between water soluble components and theaflavins. Theaflavins in black tea infusions are being partially counteracted by one or more chemical components in it and it lowers the overall activity. However, the pattern of activity of isolated theaflavins and hot water extract of black tea were alike. This suggests that the theaflavins are the principal bioactive compounds in black tea infusions, despite the existence of interaction. Isolated theaflavins and hot water extracts of black tea restored the activity of lower concentrations of antibiotics below MIC to vulnerable breakpoints. The two black tea extracts together with antibiotics can be used in the treatment and prevention of bacterial infections.

Keywords: Antibiotics, Kenyan black tea, pathogenic bacteria, theaflavins, water soluble components

INTRODUCTION

Diseases caused by microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. While the development of resistant strains is inevitable, the slack ways of administering and using antibiotics in human, veterinary medicine and in agriculture has greatly exacerbated the process (Kenneth 2008). Wound infections, gonorrhea, tuberculosis, pneumonia, septicemia, childhood ear infections and staphylococcal infections are just a few of the diseases that have become hard to treat with antibiotics (Amy 2008; Kenneth 2008).

Traditional methods of antibiotic discovery have failed to keep pace with the evolution of bacterial resistance, which suggests that new strategies to combat bacterial infections may be required (Peter and Floyd 2007). Microbial development of resistance, as well as economic incentives, have resulted in research and development in the search for new antibiotics to always maintain a pool of effective drugs (Kenneth 2008). Current research has focused on strengthening the antibacterial action of the existing antibiotics through blended therapy (Esimone et al. 2003; Nwafor et al. 2003). The advantages of blended antibiotic therapy are broadened spectrums of antimicrobial activity, occurrence of synergistic activity and prevention of bacterial resistance development (Aurer and Planeak 2004).

Sometimes, antibiotics are willfully or inadvertently consumed along with herbs or beverages. This shows the potential interaction between drugs and herbs, which can be beneficial or harmful. One of the herbs that are widely consumed concomitantly with most drugs is tea (Esimone et al. 2003). Research on tea has shown that it has some medicinal properties, including antimicrobial effect against a wide range of bacteria, fungi, and viruses (Sakanata et al. 1989; Toda et al. 1991).

The tea plant is member of *Camellia sinensis*. The two main varieties are *Camellia sinensis var sinensis* and

Camellia sinensis var. assamica. Tea is an infusion of the leaves of *Camellia sinensis* plant and is one of the most widely consumed beverages in the world (Higdon 2007). Tea is composed of several bioactive chemicals. The existence of alkaloids, saponins, tannins, catechin and other polyphenols in tea is revealed by the phytochemical screening. Recent research mainly focused on the potential health benefits of a class of compounds in tea known as flavonoids (Lai et al. 2001).

Flavonoids in fresh tea leaves are catechins which are a group of natural polyphenols (Lai et al. 2001). Another group of polyphenol pigments are theaflavins which are in black tea. Theaflavins are structured from polymerization of catechins due to oxidation by polyphenol oxidase at the fermentation stage during the manufacture of black tea. Theaflavins contribute to the characteristic bright orange-red color of black tea, accounting for approximate 2g/100g of the dried water extract of black tea (Higdon 2007). Catechins and theaflavins are believed to have a wide range of pharmaceutical benefits such as antibacterial, antihypertensive, antioxidative, hypolipidemic, antiviral and antifungal properties (Hara et al. 1991).

Current research on Japanese Sencha tea (green tea) and Indian Lipton brand black tea have shown that tea extracts have had effect on antibiotics effectiveness. Most of the research has been carried out on green tea extracts, rather than black tea extracts. Research using Indian Lipton brand black tea extracts showed synergistic activity with chloramphenicol and other antibiotics like gentamycin, methicillin and nalidixic acid against *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia enterocolitica* and *Escherichia coli* (Tiwari et al. 2005). Gallic acid extract from black tea showed a synergistic effect with amikacin and sulfamethoxazole tested in a dose-dependent manner against *E. coli* (Tirang et al. 2007).

Research was done using hot water extract of Sencha (Japanese Green Tea) and methicillin to find out the blend effect. It was found that the extract of Sencha tea is not only capable of impeding methicillin resistant *Staphylococcus aureus* (MRSA), but also reestablishing the activity of methicillin against MRSA. Hara et al. (1991) also examined that the extract of tea acts synergistically with methicillin against MRSA. The synergistic effect was attributed to cathecins. The Sencha group of teas have high levels of ascorbic acid (Vitamin C), tannin and most of the catechins (Goto et al. 1996). Other research has indicated that Kenyan black teas have remarkable levels of the unoxidized flavan-3-ols (theaflavins and thearubigins) associated with human health (Owuor and Obanda, 1995).

The objectives of this research was (i) To determine antibacterial activities of hot water extract of Kenyan black tea on selected clinical isolates and standard bacteria (ii) To determine antibacterial activities of theaflavins in Kenyan black tea on selected clinical isolates and standard bacteria; (iii) To determine the effect of hot water extract of Kenyan black tea on the efficacy of blended theaflavins with selected antibiotics. (iv) To determine synergism between theaflavins and common antibiotics.

MATERIALS AND METHODS

Collection of sample materials

The commercial black tea which was processed and packed by Kenya Tea Packers Limited (KETEPA) was used as sample material and it was purchased in Nairobi

Preparation of hot water extract of black tea

The method of Yam et al. (1998) as described by Mbata et al. (2006) was used to extract the black tea with the aid of hot water. Into 100 mL of boiling water, two grams of tea was poured and left for 12 minutes and then it was sifted to make solution containing 2g/100 mL. The extract was freeze dried to powder form and stored at-4^0 C in refrigerator until it was needed.

Measurement of total theaflavins content of black tea

The Flavognost method (Hilton, 1973) was used to measure the total theaflavins in hot water. Briefly, 175 mL of boiling water was used to infuse black tea (9g). The infusion temperature was kept close to boiling point for 10 minutes. A vacuum flask with continuous mechanical shaking was used to carry out the infusion. When it was finished, that hot liquor was sifted and quickly cooled in cold water. Then, 10 mL aliquot of the filtered infusion was shaken for 10 minutes with the addition of 10 mL of isobutyl methyl ketone and, at last, the two layers were allowed to separate. Next, a mechanical shaker mixed 2 mL of the aliquot of the upper layer, 4 mL of ethanol and 2 mL of flavognost reagent (2% w/v diphenylboric acid-2-aminoethyl ester in ethanol) well. The mixture was left for 15 minutes at room temperature and the absorbance was read in spectrophotometer at 625nm. A mixture of isobutyl methyl ketone and ethanol (1:1, v/v) was used as blank. The content of theaflavins in black tea was calculated with the following formula:

$$\text{Theaflavins } (\mu \text{ mol/g}) = E_{625} \times 47{,}900/\text{ DM}$$

Where E_{625} was optical density, 47,900 was a constant and DM was the dried material of tea sample.

Extraction of theaflavins

Lai et al. (2001) method was used to extract theaflavins. 250g of black tea was weighed and extracted three times using 1.875l of 70% ethanol and then sifted. After the removal of ethanol in a rotary evaporator, the remaining water solution was extracted subsequently using chloroform (0.375l), ethyl acetate (0.25l) and butanol (0.25l). The ethyl acetate extract was implemented onto a silica gel column 80 (0.80 to 1.65cm i.d; silica gel 60M, 230-240 mesh). The total TF fraction was obtained when the column was purified with a mixture of chloroform and ethyl acetate 1:1 (v/v) followed by increasing the ratio of chloroform to ethyl acetate to 4:1 (v/v). The total theaflavin fraction was stored at -4^0C.

The purified fractions from the silica gel column were spotted on chromatographic plates with a thin layer of silica gel. They were then subjected to a mobile phase (solvent mixture) having ethyl acetate-acetic acid-water at

a ratio of 10:2:3. After being sprayed, the dissimilar compounds showed up as distinct spots at a distance from where they were spotted on the plates. The spots (compounds) that have the same relative mobility front (Rf) were merged. The absorbance of the fractions was measured at 380 and 460 nm using spectrophotometer. Theaflavins are known to have maximum absorbance at 380 nm which is especially related to their benzotropolone rings. The chemical (electron) shift around the benzotropolone ring that develops the bright red color of the theaflavins, is accountable for the absorbance maximum at 460 nm. The theaflavins fractions were confirmed by reacting 2 mL of theaflavins solution, 4 mL of ethanol and 2 mL of flavognost reagent (2% w/v diphenylboric acid-2-aminoethyl ester in ethanol). Diphenyl boric acid ethanolamine (Flavognost reagent) responded to the benzotropolone nucleus to form a green chromophore with a broad absorption maximum at 625 nm.

Preparation of tea extracts stock and working solutions

Two grams of black tea yielded 0.90 g when the water extract was freeze dried. Two-fold dilutions were produced to gain 100%, 50%, 25%, and 12.5% concentrations. 2 grams of the same black tea yielded 36µg or µmol of theaflavins. A doubling dilution of isolated theaflavins was produced to gain 100%, 50%, 25%, and 12.5% concentrations. The concentrations were stored at -4^{0}C until they were needed.

Preparation of antibiotic stock and working solutions

The antibiotics were taken off from storage (-20ºC) and allowed to come to room temperature. Each (250 mg) of the antibiotics were weighed and liquefied in suitable solvents and diluted in appropriate diluents (Appendix IV) to make a final 100 mL solution. The following formula was used to gain stock solutions:

$$W = \frac{1000 \times V\ (\text{mL}) \times C\ (\mu\text{g mL}^{-1})}{P\ (\mu\text{g mL}^{-1})}$$

Where:

P = potency given by manufacturer in relation to base C = final concentration of the solution

W = weight of antibiotic in mg to be liquefied in V = volume required in ml (20 mL)

The solutions supply of chloramphenicol, tetracycline, norfloxacin, ciprofloxacin and ampicillin were kept at-20 ^{0}C until they were needed.

Determination of MIC of antibiotics

Doubling dilutions of solution supply was produced to gain minimum inhibitory concentration (MIC) of each antibiotic against respective test organism using modified Bauer-Kirby method reported in the National Committee for Clinical Laboratory Standards (NCCLS 2002) report. Incubation was carried out at 37ºC for 24 hours and impediment zones were compared with those recommended by NCCLS (2002).

Preparation of blended concentrates of black tea extracts and antibiotics

A series of two-fold dilutions (1-⅛) were established using MIC of each antibiotic as the starting concentration. These concentrations were then blended andd merged with concentrations of hot water extract of black tea and isolated theaflavins (100%, 50%, 25% and 12.5%). Sterile discs were soaked in blended concentrations, air dried at room temperature and kept at-20ºC until they were needed.

Test organisms

Control and clinical isolates of *Escherichia coli, Enterobacter aeruginosa, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 were from the National Public Health Laboratories, Nairobi, Kenya.

Identification of bacterial strains

Viability tests were done by picking the organisms from the stock using a sterile loop and inoculating into 9 mL of peptone water media. They were then incubated at 37ºC for 3 hours, followed by then sub-cultured in sterile agar plates having Mueller Hinton Agar (MHA) and incubated at 37ºC for 24 hours. Well isolated colonies were picked and used for identification.

Biochemical typing

Organisms from well separated colonies of akin appearance were taken from each of the plating media for further testing and an identification mark was put on the bottom of the Petri dish corresponding to the colonies. Five non-lactose fermenting (NLF) colonies were selected and put into separate tubes of Triple Sugar Iron agar (TSI), Simmon citrate and Urea agar. TSI was seeded by stabbing the butt and then streaking the slant with a zigzag configuration. Simmon citrate was also seeded by streaking the surface of the slant with a zigzag configuration. Urea agar was seeded by stabbing into medium four times. The test tubes with loose caps were incubated at 37ºC for 24 hours. The biochemical reactions were deciphered according to biochemical reaction chart for *Enterobacteriaceae, Aeromonas* and *Plesiomonas.*

Preparation of inoculants

One colony of each bacterial culture was selected and seeded into nutrient broth and incubated at 37ºC for 24 hours. The density of a bacterial suspension in nutrient broth was adjusted to match the turbidity of the 0.5 McFarland standard which was equivalent to 1.5 x 10^5 colony forming units (CFU)/mL.

Sensitivity test

An inoculum having 1.5 x 10^5 CFU/mL was taken off using a sterile cotton wrap and spread on Mueller Hinton agar plates. Impregnated discs were aseptically placed evenly on the surface of agar and pressed firmly. The plates were left for 3 hours to allow the antibiotics to diffuse. The inoculated plates were incubated overnight at 35ºC. The zones of impediment were measured using a transparent

ruler on the following morning after 18 hours incubation.

Chi-square test

The chi-square test was used to test the null hypothesis.

The formula for calculating chi-square (χ^2) is: $(\chi^2) = (O-E)^2/E$

Where: O is the examined result (mean impediment zone diameter of isolated theaflavins), and E is the expected result (mean impediment zone diameter of hot water extract of black tea).

RESULTS AND DISCUSSION

Comparison of inhibitory effect of isolated theaflavins and hot water extract of Kenyan black tea on *Salmonella typhi*

The inhibitory effect of hot water extract of black tea and isolated theaflavins are shown in Table 1. Isolated theaflavins showed stronger inhibitory effect as evidenced by large impediment zones. The 100%, 50%, and 25% concentrates of the two tea extracts impeded *S. typhi*. However, it resisted the 12.5% concentration of both extracts. Both 100% and 50% concentrations showed stronger inhibitory effect than minimum inhibitory concentration of ampicillin (10.4 µg/mL).

There was a statistically remarkable difference in the inhibitory effect between isolated theaflavins and hot water extract of black tea based on the diameters of zones of impediment at (χ^2 = 0.94; P<0.05). However, there was comparability in pattern of activity as the inhibitory effect increased, with increasing concentration of both tea extracts.

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Pseudomonas aeruginosa*

Hot water extract of black tea showed lower inhibitory effect (smaller impediment zone diameter) on *P. aeruginosa* as compared to isolated theaflavins. Both 100% and 50% of the hot water extract of tea impeded *P. aeruginosa*, while 25% and 12.5% concentrates did not (Table 2). Only 12.5% concentrate of isolated theaflavins failed to inhibit *P. aeruginosa*. The 100%, 50% and 25% of isolated theaflavins and 100% and 50% of hot water extract showed stronger inhibitory effect than minimum inhibitory concentration of norfloxacin (6.4 µg/mL). The difference in inhibitory effect between isolated theaflavins and hot water extract of black tea was significant at (χ^2 = 1.02; P<0.05). There was comparability in pattern of activity.

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Pseudomonas aeruginosa* standard (ATCC 27853)

All the concentrates of both hot water extract and isolated theaflavins, except the 12.5% dilution, impeded *P. aeruginosa* standard (ATCC 27853) (Table 3). Isolated theaflavins had a stronger impediment than hot water extract of black tea as examined in larger impediment zones. The 100%, 50% of both hot water extract and isolated theaflavins and 25% concentrates of isolated theaflavins also showed stronger inhibitory effect than minimum inhibitory concentration of norfloxacin (4.3 µg/mL).

There was a significant difference between inhibitory effect of concentrates of hot water extract and isolated theaflavins of black tea at (χ^2 = 0.56; P<0.05). The pattern of inhibitory effect was however akin. *P. aeruginosa* standard (ATCC 27853) was more susceptible to both tea extracts (Table 3) than *P. aeruginosa* (Table 2). *P. aeruginosa* resistance to hot water extract of black tea was high, showing a high difference in inhibitory effect with isolated theaflavins at (χ^2 = 1.02; P<0.05). The difference in inhibitory effect between the tea extracts on *P. aeruginosa* standard (ATCC 27853) was significantly lower at (χ^2 = 0.56; P<0.05).

Comparison of inhibitory effect on hot water extract and isolated theaflavins of Kenyan black tea on *Staphylococcus aureus* standard (ATCC 25923)

Isolated theaflavins effectively impeded *S. aureus* standard (ATCC 25923), as compared to hot water extract of black tea, as shown by larger impediment zone (Table 4). All the concentrates 100%, 50%, 25% and 12.5% of isolated theaflavins had stronger activity than minimum inhibitory concentration (1.2 µg/mL) of ciprofloxacin.

Only 12.5% concentration of hot water extract of black tea had a lower activity than the minimum inhibitory concentration (1.2 µg/mL) of ciprofloxacin. There was a statistical difference in inhibitory effect between isolated theaflavins and hot water extract of black tea on *S. aureus* standard (ATCC 25923) at (χ^2 = 4.42; P<0.05).

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Staphylococcus aureus*

Hot water extract of black tea had lower inhibitory activity on *S. aureus* than isolated theaflavins (Table 5). Isolated theaflavins effectively impeded with large zones of impediment. All the concentrates 100%, 50%, 25% and 12.5% of isolated theaflavins showed stronger inhibitory effect than the minimum inhibitory concentration (2 µg/mL) of ciprofloxacin. Only 12.5% concentrate of hot water extract showed lower inhibitory effect than minimum inhibitory concentration (2 µg/mL) of ciprofloxacin. The inhibitory effect between isolated theaflavins and hot water extract of black tea was significant at (χ^2 = 1.01; P<0.05).

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Enterobacter aeruginosa*

The 12.5% concentrates of hot water extract and isolated theaflavins did not inhibit *E. aeruginosa*. However, isolated theaflavins showed stronger inhibitory effect than hot water extract of black tea (Table 6). Only 25% concentration of hot water extract of tea showed lower inhibitory effect than minimum inhibitory concentration of (5.25 µg/mL) of tetracycline. The 100%, 50% and 25% of

isolated theaflavins showed stronger inhibitory effect than minimum inhibitory concentration (5.25 µg/mL) of tetracycline. The difference in level of inhibitory effect between isolated theaflavins and hot water extract of black tea on *E. aeruginosa* was significant at ($\chi^2 = 3.04$; $P<0.05$).

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Escherichia coli*

The 100%, 50%, 25% and 12.5% concentrations of both black tea extracts effectively impeded *E. coli*. However, isolated theaflavins showed stronger activity with larger zones of impediment (Table 7). All the concentrates of hot water extract and isolated theaflavins showed better inhibitory effect than the minimum inhibitory concentration of chloramphenicol. The difference in inhibitory effect between concentrates of isolated theaflavins and hot water extracts of black tea was significant at ($\chi^2 = 1.62$; $P<0.05$). The pattern of inhibitory effect was akin as it increased with increasing concentration of both tea extracts.

Comparison of inhibitory effect of hot water extract and isolated theaflavins of Kenyan black tea on *Escherichia coli* standard (ATCC 25922)

Escherichia coli standard (ATCC 25922) was effectively impeded by hot water extract and isolated theaflavins of black tea (Table 8). However, hot water extract of black tea showed lower inhibitory effect as shown by smaller zones of impediment than that of isolated theaflavins. Both tea extracts have a strong impediment to *E. coli* standard (ATCC 25922) than the minimum inhibitory concentration (8.4 µg/mL) of chloramphenicol. The inhibitory effect between concentrates of isolated theaflavins and hot water extracts was significant at ($\chi^2 = 1.74$; $P<0.05$). This difference was slightly higher than that of *E. coli* ($\chi^2 = 1.62$; $P<0.05$).

The effect of hot water extract of Kenyan black tea on the efficacy of antibiotics

The 100%, 50% and 25% concentrates of hot water extract of black tea with minimum inhibitory concentration (10.4 µg/mL) of ampicillin acted synergistically against *S. typhi* as shown in Figure 1.A. The examination of the sum of activity of concentrates of hot water extract of black tea with MIC of ampicillin as presented in Table 1 did not exceed that presented in Figure 1.A. The doubling dilutions of the MIC (5.2, 2.6 and 1.3 µg/mL) of ampicillin had no activities of their own. Their activities were restored when they were blended with 100%, 50% and 25% concentrates of hot water extract of black tea. Those were also seen as the activities of the blended hot water concentrates with 5.2, 2.6 and 1.3 µg/mL of ampicillin (Figure 1.A), which were exceeded that of the concentrates of tea extract only as presented in Table 1. The restoring of activity of ampicillin clearly demonstrated synergism with hot water extract of black tea.

Synergism between MIC (4.3 µg/mL) of norfloxacin, a fluoroquinolone and the 100% and 50% concentrates of hot water extract of black tea against *P. aeruginosa* was also examined (Figure 1.B). That compared with that of the sum of activity of individual concentrate of hot water extract of black tea and MIC of norfloxacin is presented in Table 2. The two concentrates of tea extract were also capable of restoring the activity of the doubling dilutions (2.15 and 1.075 µg/mL) of MIC of norfloxacin against *P. aeruginosa*.

The synergism of hot water extract of black tea with MIC of norfloxacin was further examined against *P. aeruginosa* (ATCC 27853). The bacteria was less resistant to the hot water extract of black tea than *P. aeruginosa*, resisting only the 12.5% concentrate (Figure 1.C). Therefore, the impediment zones of blended concentrates of hot water extract with MIC of norfloxacin against *P. aeruginosa* (ATCC 27853) were larger than those in *P. aeruginosa* cultures. This difference was also examined in activity of blended hot water extract of black tea with doubling dilutions of MIC of norfloxacin. The activities of doubling dilutions of MIC of norfloxacin were also restored.

Synergistic effect of hot water extract of black tea with fluoroquinolone was also examined with MIC of ciprofloxacin against *S. aureus*. The *S. aureus* was very susceptible to the blended concentrates (Figure 1.D). The hot water extract of black tea also restored the activity of doubling dilutions of MIC of ciprofloxacin (1, 0.5 and 0.25 µg/mL).

Synergism of hot water extract of black tea with MIC of ciprofloxacin was also examined when *S. aureus* (ATCC 25923) was used as test bacteria (Figure 1.E). The susceptibility of *S. aureus* (ATCC 25923) to blended concentrates of hot water extracts with MIC and its doubling dilutions of ciprofloxacin was high as compared to that of *S. aureus* (Figure 1.D).

Tetracycline with hot water extract of black tea acted synergistically against *E. aeruginosa*. Synergism was examined when 100%, 50% and 25% concentrates of hot water extract of black tea were blended with MIC (5.25 µg/mL) of tetracycline. Hot water extract was also capable of restoring the activity of doubling dilutions (2.625, 1.3125 and 0.656 µg/mL) of tetracycline (Figure 1.F).

The blend of MIC (12 µg/mL) of chloramphenicol with 100%, 50%, 25% and 12.5% concentrates of hot water extract of black tea also showed synergistic effect against *E. coli* (Figure 1.G). *E. coli* was very susceptible to the blended concentrates of hot water extract of black tea with MIC of chloramphenicol. This was examined in large impediment zones around the discs having these blends. The concentrates of hot water extract of black tea restored the activity of doubling dilutions (6, 3 and 1.5 µg/mL) of MIC of chloramphenicol.

Synergism of MIC of chloramphenicol with concentrates of hot water extract of black tea was also examined when *E. coli* (ATCC 25922) was used as test bacteria (Figure 1.H). The MIC of chloramphenicol against *E. coli* (ATCC 25922) was 8.4 µg/mL compared to 12 µg/mL of *E. coli*. *E. coli* (ATCC 25922) was therefore more susceptible to the blended MIC of chloramphenicol with concentrates of hot water extract of black tea as examined in large impediment zones (Figure 1.H). That was also examined in blended activity of doubling dilutions and 1.05 µg/mL) of MIC (8.4 µg/mL) of chloramphenicol (Figure 1.H).

Table 1. Antibacterial activity of hot water extract and isolated theaflavins of Kenyan black tea and MIC of ampicillin against Salmonella typhi.

Concentrates	N	Mean impediment zone diameter in mm		
		Ampicillin	Hot water extract	Isolated theaflavins
100%	3		14.3	16
50%	3		10.5	13.4
25%	3		7.2	8.9
12.5%	3		NI	NI
MIC of amp (10.4 µg/mL)		9.4		

Note: NI: no impediment, amp: ampicillin, N: number of replicates

Table 2. Antibacterial activity of hot water extract and isolated theaflavins of Kenyan black tea and MIC of norfloxacin against *Pseudomonas aeruginosa.*

Concentrates	N	Mean impediment zone diameter in mm		
		Norfloxacin	Hot water extract	Isolated theaflavins
100%	3		11	13
50%	3		8.34	10.7
25%	3		NI	9.2
12.5%	3		NI	NI
MIC of norf (6.4 µg/mL)		6.89		

Note: NI: no impediment, norf: norfloxacin, N: number of replicates

Table 3. Antibacterial activities of varying concentrations of isolated theaflavins and hot water extract of Kenyan black tea and MIC of norfloxacin against *Pseudomonas aeruginosa* standard (ATCC 27853).

Concentrates	N	Mean impediment zone diameter in mm		
		Norfloxacin	Hot water extract	Isolated theaflavins
100%	3		14	15.68
50%	3		11.96	13.59
25%	3		8.3	9.4
12.5%	3		NI	NI
MIC of norf (4.3 µg/mL)		8.5		

Note: NI: no impediment, norf: norfloxacin, N: number of replicates

Table 4. Antibacterial activity of varying concentrations of isolated theaflavins and hot water extract of Kenyan black tea and MIC of ciprofloxacin against *Staphylococcus aureus* (ATCC 25923).

Concentrates	N	Mean impediment zone diameter in mm		
		Ciprofloxacin	Hot water extract	Isolated theaflavins
100%	3		17	20
50%	3		15.4	17.96
25%	3		12.9	14.5
12.5%	3		10	12.2
MIC of Cipro (1.2 µg/mL)		11		

Note: Cipro: ciprofloxacin, N: number of replicates

Table 5. Antibacterial activities of varying concentrations of isolated theaflavins and hot water extract of Kenyan black tea and MIC of ciprofloxacin against *Staphylococcus aureus*.

Concentrates	N	Mean impediment zone diameter in mm		
		Ciproflox acin	Hot water extract	Isolated theaflavins
100%	3		15	19
50%	3		13.7	16.8
25%	3		11.8	14.15
12.5%	3		9.2	12.6
MIC of Cipro (2 µg/mL)		9.8		

Note: Cipro: ciprofloxacin, N: number of replicates

Table 6. Antibacterial activities of varying concentrations of isolated theaflavins and hot water extract of Kenyan black tea and MIC of tetracycline against *Enterobacter aeruginosa.*

Concentrates	N	Mean impediment zone diameter in mm		
		Tetracycline	Hot water extract	Isolated theaflavins
100%	3		14.2	17
50%	3		11	14
25%	3		6.9	10.3
12.5%	3		NI	NI
MIC of tet (5.25 µg/mL)		7.8		

Note: NI: no impediment, tet: tetracycline, N: number of replicates

Table 7. Antibacterial activities of varying concentrations of isolated theaflavins hot water extract of Kenyan black tea and MIC of chloramphenicol against *Escherichia coli.*

Concentrates	N	Mean impediment zone diameter in mm		
		Chloramphenicol	Hot water extract	Isolated theaflavins
100%	3		13	16
50%	3		11.6	14.4
25%	3		9.8	10.56
12.5%	3		7.2	8.4
MIC of chlo (12 µg/mL)		6.8		

Note: Chlo: chloramphenicol, N: number of replicates

Table 8. Antibacterial activities of varying concentrations of isolated theaflavins and hot water extract of Kenyan black tea and MIC of chloramphenicol against *Escherichia coli* standard (ATCC 25922).

Concentrates	N	Mean impediment zone diameter in mm	
100%	3	15	18
50%	3	12.8	15.2
25%	3	10.4	12.56
12.5%	3	8.35	9.4
MIC of tet (8.4 µg/mL)		8	

Note: Chlo: chloramphenicol, N: number of replicates

Synergism of isolated theaflavins of Kenyan black tea with antibiotics

Synergistic antibacterial activities of isolated theaflavins and ampicillin

A synergistic antibacterial activity of isolated theaflavins of black tea and ampicillin against *S. typhi* is presented in Figure 2.A. Isolated theaflavins acted synergistically with minimum inhibitory concentration (10.4 µg/mL) of ampicillin. Isolated theaflavins like hot water extract of black tea, restored the activity of doubling dilutions (5.2, 2.6 and 1.3 µg/mL) of MIC of ampicillin. Otherwise, the blended activity of isolated theaflavins and doubling dilutions of MIC of ampicillin would have been equal to that of concentrates of isolated theaflavins alone (Table 1) if activity was not restored.

Synergistic antibacterial activity of isolated theaflavins and norfloxacin

Isolated theaflavins showed synergistic activity with norfloxacin, a fluoroquinolone against *P. aeruginosa* (Figure 2.B). Synergism was examined when 100%, 50% and 25% concentrates of isolated theaflavins were blended with MIC (6.4 µg/mL) of norfloxacin. The concentrates of isolated theaflavins also restored the activity of doubling dilutions (2.5 and 1.075 µg/mL) of norfloxacin.

The synergistic antibacterial activity of isolated theaflavins and norfloxacin was also examined when *P. aeruginosa* (ATCC 27853) was used instead of *P. aeruginosa*. However, the level of synergism and restored activity differed. *P. aeruginosa* (ATCC 27853) was less resistant (Figure 2.C) with the MIC (4.3 µg/mL) of norfloxacin against it.

Synergistic antibacterial activity of isolated theaflavins and ciprofloxacin

The synergistic effects between 100%, 50%, 25% and 12.5% concentrates of isolated theaflavins and MIC (2 µg/mL) of ciprofloxacin against *S. aureus* was examined (Figure 2.D).These blends effectively impeded *S. aureus* as shown by large impediment zones in the cultures. Isolated theaflavins also restored the activity of doubling dilutions (1, 0.5 and 0.25 µg/mL) of ciprofloxacin.

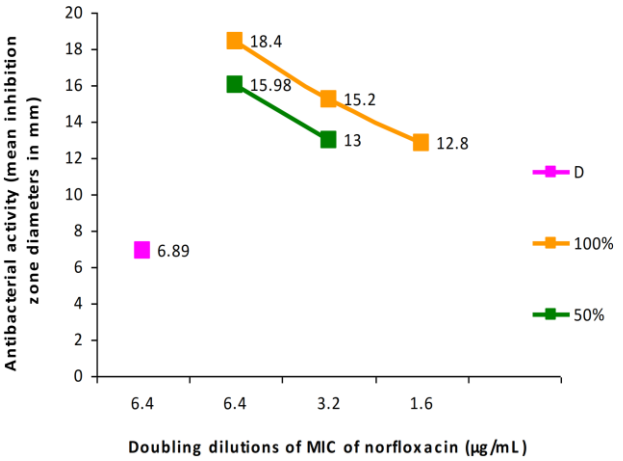

Figure 1.B. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (6.4 µg/mL) of norfloxacin against *Pseudomonas aeruginosa*. D-Impediment zone diameter of minimum inhibitory concentration (6.4 µg/mL) of norfloxacin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, N = 3.

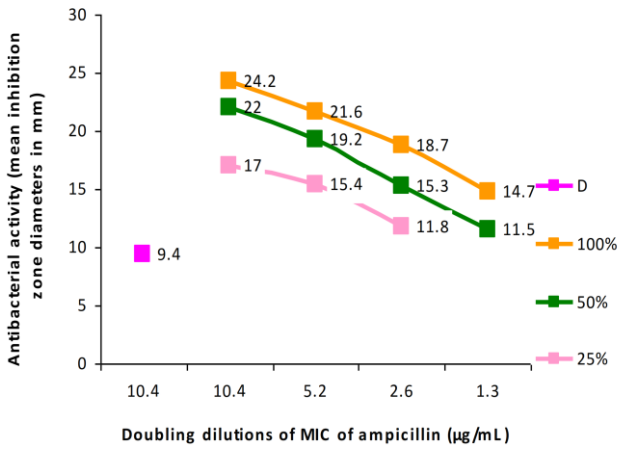

Figure 1.A. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC of ampicillin against *Salmonella typhi*. D-Impediment zone diameter of minimum inhibitory concentration (10.4 µg/mL) of ampicillin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, 12.5%-dilution of 100% to ⅛ of its original concentration, N = 3.

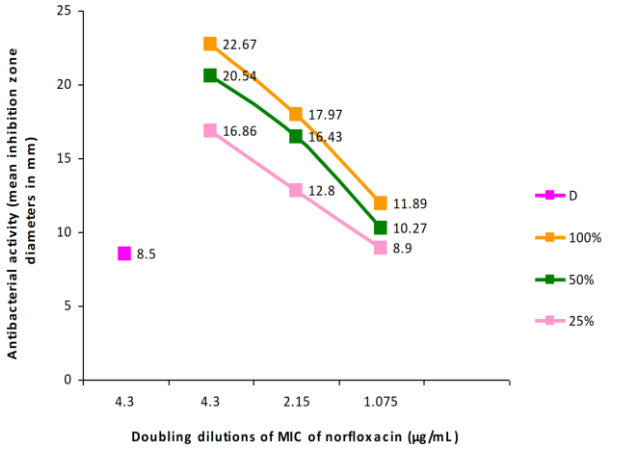

Figure 1.C. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (4.3 µg/mL) of norfloxacin against *Pseudomonas aeruginosa (*ATCC 27853*)*. D-Impediment zone diameter of minimum inhibitory concentration (4.3 µg/mL) of norfloxacin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

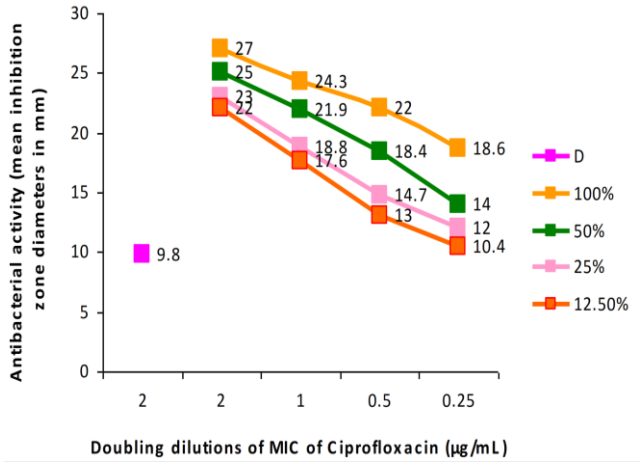

Figure 1.D. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (2 µg/mL) of ciprofloxacin against *Staphylococcus aureus*. D-Impediment zone diameter of minimum inhibitory concentration (2 µg/mL) of ciprofloxacin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, 12.5%-dilution of 100% to ⅛ of its original concentration, N = 3.

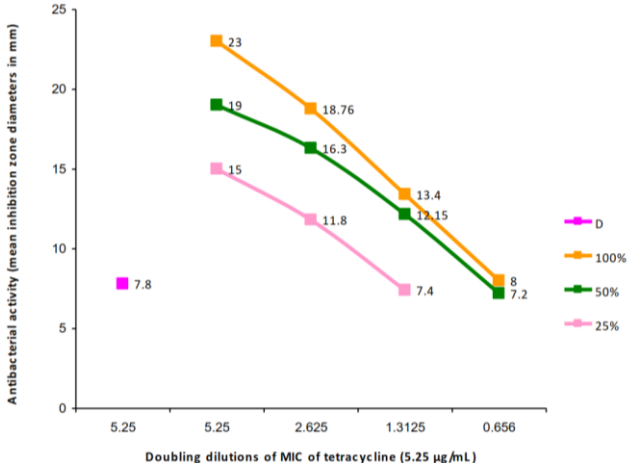

Figure 1.F. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (5.25 µg/mL) of tetracycline against *Enterobacter aeruginosa*. D-Impediment zone diameter of minimum inhibitory concentration (5.25 µg/mL) of tetracycline, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

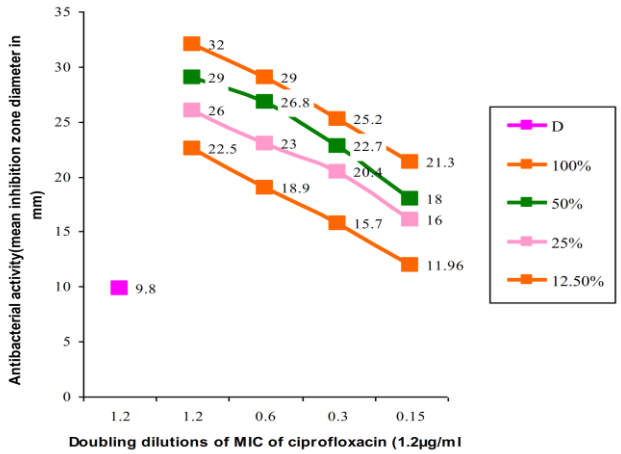

Figure 1.E. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (1.2 µg/mL) of ciprofloxacin against *Staphylococcus aureus* (ATCC 25923). D-Impediment zone diameter of minimum inhibitory concentration (1.2 µg/mL) of ciprofloxacin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, 12.5%-dilution of 100% to ⅛ of its original concentration, N = 3.

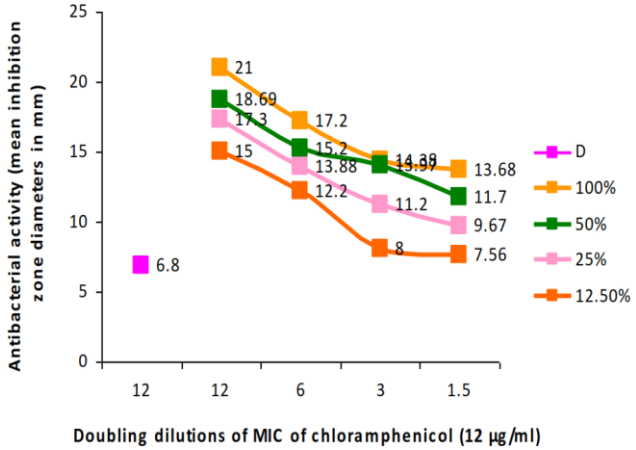

Figure 1.G. Antibacterial activity of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (12 µg/mL) of chloramphenicol against *Escherichia coli*. D-Impediment zone diameter of minimum inhibitory concentration (12 µg/mL) of chloramphenicol, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

The synergistic activity between MIC of ciprofloxacin and concentrates of isolated theaflavins was clearly demonstrated when *S. aureus* (ATCC 25923) was used instead of *S. aureus* (Figure 2.E). That was examined in larger impediment zones in *S. aureus* (ATCC 25923) cultures. Also, the activity of doubling dilutions (0.6, 0.3 and 0.15 µg/mL) of ciprofloxacin was restored by isolated theaflavins.

Synergistic antibacterial activity of isolated theaflavins and tetracycline

The blend of isolated theaflavins concentrates (100%, 50% and 25%) and minimum inhibitory concentration (5.25 µg/mL) of tetracycline showed synergistic effect. That was examined when activity was tested on *E. aeruginosa* (Figure 2.F). Isolated theaflavins concentrates restored the activity of tetracycline when blended with its doubling dilutions (2.625, 1.3125 and 0.656 µg/mL). *E. aeruginosa* has developed resistance to antibiotics. The blended formulation of tetracycline and isolated theaflavins effectively impeded.

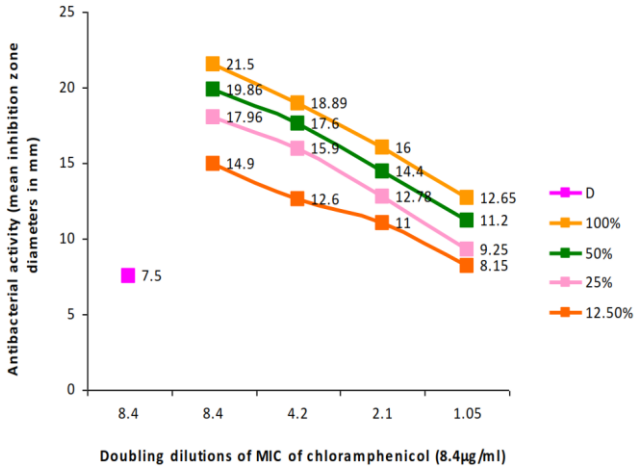

Figure 1.H. Antibacterial activities of blended concentrates of hot water extracts of black tea with doubling dilutions of MIC (8.4 µg/mL) of chloramphenicol against *Escherichia coli* (ATCC 25922). D-Impediment zone diameter of minimum inhibitory concentration (8.4 µg/mL) of chloramphenicol, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

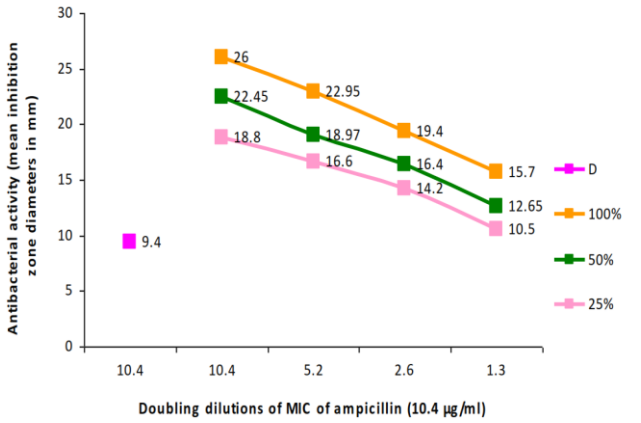

Figure 2.A. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC of ampicillin against *Salmonella typhi.* D-Impediment zone diameter of minimum inhibitory concentration (10.4 µg/mL) of ampicillin, 100%-undiluted isolated theaflavins, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

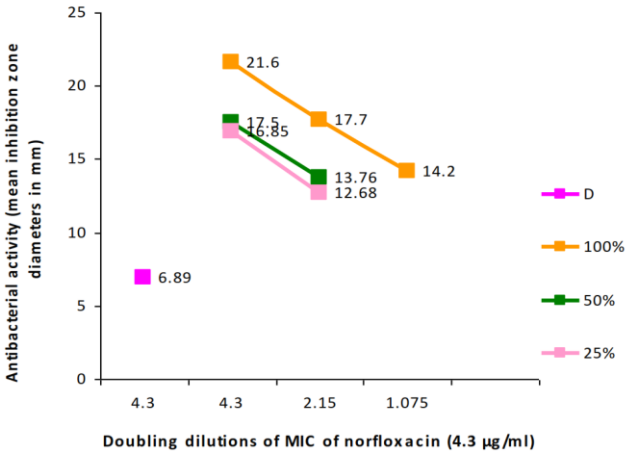

Figure 2.B. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC (4.3 µg/mL) of norfloxacin against *Pseudomonas aeruginosa*. D-Impediment zone diameter of minimum inhibitory concentration (4.3 µg/mL) of norfloxacin, 100%-undiluted isolated, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

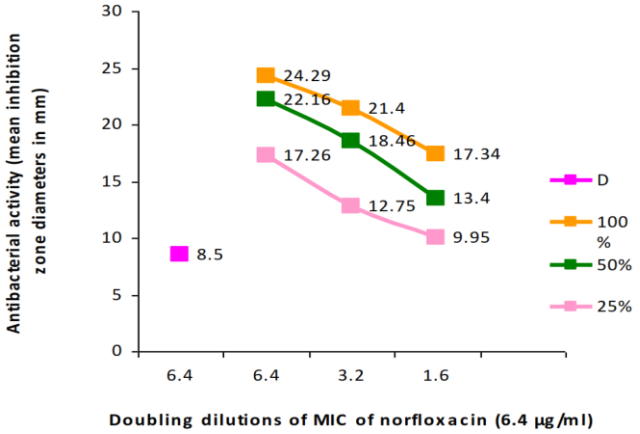

Figure 2.C. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC of norfloxacin against *Pseudomonas aeruginosa (*ATCC 27853*).* D-Impediment zone diameter of minimum inhibitory concentration (6.4 µg/mL) of norfloxacin, 100%-undiluted isolated theaflavins, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

Synergistic antibacterial activity of isolated theaflavins and chloramphenicol

Isolated theaflavins and chloramphenicol showed strong synergistic effect against *E. coli*. This was examined when minimum inhibitory concentration (12 µg/mL) of chloramphenicol was blended with 100%, 50%, 25% and 12.5% concentrates of isolated theaflavins (Figure 2.G). The blend effectively impeded *E. coli*, as shown by large impediment zones. The activity of chloramphenicol was also restored when doubling dilutions (6, 3 and 1.5 µg/mL) of its MIC were blended with isolated theaflavins concentrates. The doubling dilutions have no activity against *E. coli* on their own. The resulting activity after blend with isolated theaflavins concentrates exceeded that of the concentrates only. That indicated doubling dilutions of MIC contributed to the overall activity. Synergism was also examined when *E. coli* (ATCC 25922) was used as test bacteria (Figure 2.H).

The 100%, 50%, 25% and 12.5% concentrates of isolated theaflavins were blended with MIC (8.4 µg/mL) of chloramphenicol against *E. coli* (ATCC 25922). The differences in impediment in *E. coli* and *E. coli* (ATCC 25922) cultures was due to the differences in susceptibility.

The effect of other chemical components in black tea infusion on theaflavins interaction with antibiotics

Isolated theaflavins showed stronger inhibitory effect against the bacterial species tested. The differences in inhibitory effect was significant at (P<0.05). The difference in inhibitory effect between blended concentrates of hot water extract and isolated theaflavins with MIC (10.4 µg/mL) of ampicillin against *S. typhi* was significant at (χ^2 = 0.56; P<0.05). There was akin observation when dissimilar bacteria species and dissimilar antibiotics were used. The differences in inhibitory effect was significant at (χ^2 = 0.699; P<0.05) between the two black tea extracts blends with MIC (4.3 µg/mL) of norfloxacin against *P. aeruginosa*. It was also significant (χ^2 = 0.425; P<0.05) when *P. aeruginosa* (ATCC 27853) was used as test bacteria instead of *P. aeruginosa*. The blend of concentrates of hot water extract and isolated theaflavins with MIC (2 µg/mL) of ciprofloxacin differed significantly in level of impediment at (χ^2 = 1.98; P<0.05) against *S. aureus*. The difference in inhibitory effect was significant (χ^2 = 0.67; P<0.05) when *S. aureus* (ATCC 25923) was used instead. When the concentrates of the two black tea extracts were blended with MIC (5.25 µg/mL) of tetracycline, the inhibitory effect differed remarkably at (χ^2 = 2.27; P<0.05) against *E. aeruginosa*. Akin observation was also remarkable at (χ^2 = 0.4; P<0.05) when concentrates of the two black tea extracts were blended with MIC (12 µg/mL) of chloramphenicol against *E. coli*. While the difference in inhibitory effect was highly significant at (χ^2 = 1.039; P<0.05) when *E. coli* (ATCC 25922) was used instead.

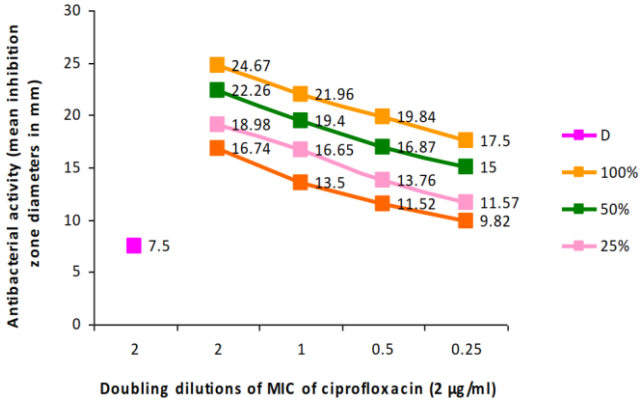

Figure 2.D. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC of ciprofloxacin against *Staphylococcus aureus.* D-Impediment zone diameter of minimum inhibitory concentration (2 µg/mL) of ciprofloxacin, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, 12.5%-dilution of 100% to ⅛ of its original concentration, N = 3.

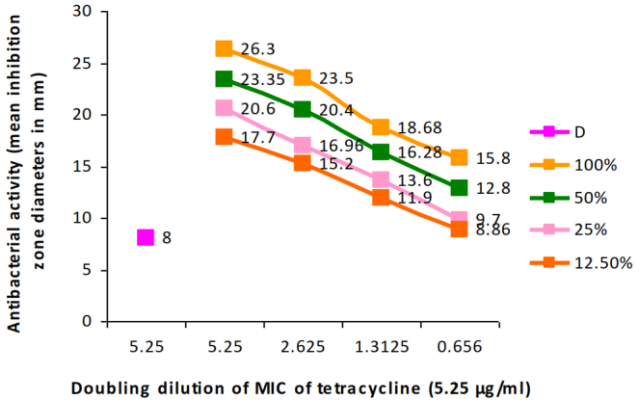

Figure 2.F. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC (5.25 µg/mL) of tetracycline against *Enterobacter aeruginosa.* D-Impediment zone diameter of minimum inhibitory concentration (5.25 µg/mL) of tetracycline, 100%-undiluted hot water extract, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

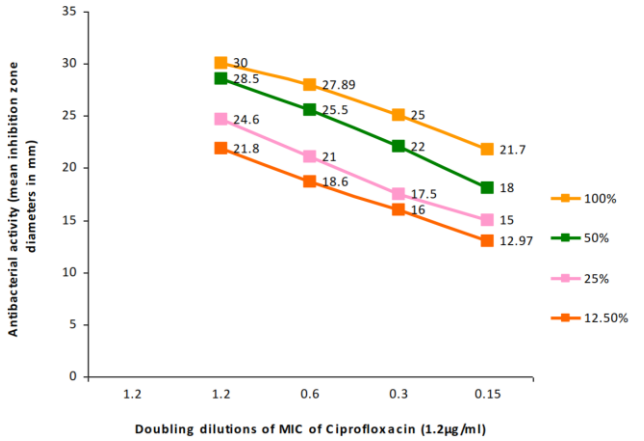

Figure 2.E. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC (1.2 µg/mL) of ciprofloxacin against *Staphylococcus aureus* (ATCC 25923). D-Impediment zone diameter of minimum inhibitory concentration (1.2 µg/mL) of ciprofloxacin, 100%-undiluted isolated theaflavins, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, 12.5%-dilution of 100% to ⅛ of its original concentration, N = 3.

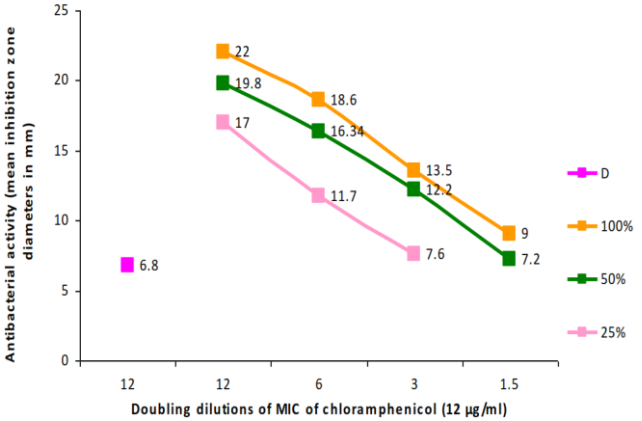

Figure 2.G. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC (12 µg/mL) of chloramphenicol against *Escherichia coli*. D-Impediment zone diameter of minimum inhibitory concentration (12 µg/mL) of chloramphenicol, 100%-undiluted isolated theaflavins, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

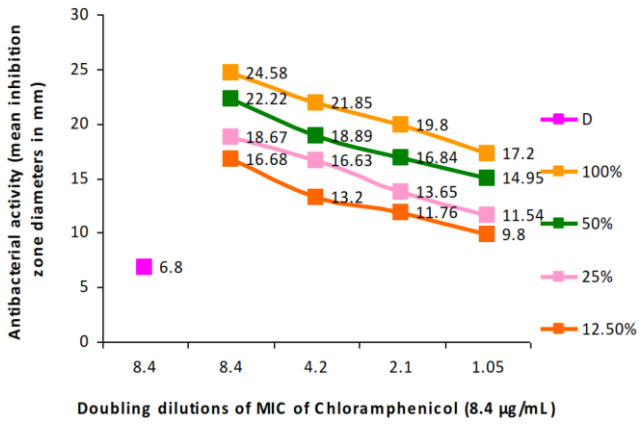

Figure 2.H. Antibacterial activities of blended concentrates of isolated theaflavins of black tea with doubling dilutions of MIC (8.4 µg/mL) of chloramphenicol against *Escherichia coli* (ATCC 25922). D-Impediment zone diameter of minimum inhibitory concentration (8.4 µg/mL) of chloramphenicol, 100%-undiluted isolated theaflavins, 50%-a half dilution of 100% concentrate, 25%-dilution of 100% to ¼ of its original concentration, N = 3.

Discussion

The present *in vitro* research clearly demonstrated that hot water extract of Kenyan black tea and theaflavins isolated from the same tea exhibit synergism with ampicillin, tetracycline, chloramphenicol, ciprofloxacin and norfloxacin. The synergistic activity was examined when minimum inhibitory concentration (MIC) of each antibiotic was blended with varying concentrations of isolated theaflavins and hot water extract of black tea. The two black tea extracts also restored the activity of lower concentrations (doubling dilutions of MIC) of antibiotics to susceptible breakpoints. The low concentrations of antibiotics had no activity of their own. The restoration of activity of antibiotics confirmed synergism between antibiotics and the tea extracts. The synergism examined in Kenyan black tea concurred with other research on dissimilar types of tea. However, most of the research has looked at green tea extracts, compared to black tea extracts.

Hot water extract of Indian Lipton brand black tea showed synergistic activity with chloramphenicol, gentamicin, methillicin and nalidixic acid against enteropathogens. Growth impediment of *S. dysenteriae* at low concentration of chloramphenicol (2.5 µg/mL) and tea extract (5.09 mg/mL) as compared to MIC of individual agent (chloramphenicol 5 µg/mL or black tea extract 9.09 mg/mL) further confirmed the synergistic activity (Tiwari et al. 2005).

Synergistic microbial growth impediment by Indian Lipton brand black tea extract and antibiotics was attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract. The results agreed with the marked reduction in MIC of oxacillin and other beta-lactams antibiotics as reported in presence of epicatechin gallate in methicillin-resistant *Staphylococcus aureus* (Tiwari et al. 2005).

The enhanced effect of Japanese green tea on inhibitory activities of antibiotics against MRSA strains have also been examined. The synergistic activity of hot water extract of Sencha (Japanese green tea) with methillicin against methicillin-resistant *Staphylococcus aureus* demonstrated possible benefits of tea extracts (Hara et al. 1991). The extract of Sencha tea was not only capable of inhibiting methillicin-resistant *Staphylococcus aureus*, but also restoring the activity of methillicin. The antibacterial activity of green tea can be explained by its content of (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG) and (-)-epicatechin-3-gallate (ECG) (Saroj et al. 1997).

The varying concentrations of hot water extract and isolated theaflavins of Kenyan black tea showed antibacterial activity against *E. coli*, *E. aeruginosa*, *S. typhi*, *P. aeruginosa*, *S. aureus*, *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923). This preliminary research of the antibacterial activities of hot water extract of processed Kenyan black tea and Nigerian Lipton tea showed inhibitory effect against *V. cholerea*, *E. coli*, *Salmonella* species, *P. aeruginosa*, *Proteus* species and *S. aureus*. The Kenyan tea showed more inhibitory actions on most of the organisms tested. The zones of impediment produced by Kenyan tea on test organisms were generally larger than those produced by the Nigerian Lipton tea. This could be because it contains more active ingredients (phytochemical substances) than the Nigerian tea, which resulted in a stronger inhibitory effect on the test organism (Mbata 2006).

Theaflavins have also been reported to have antibacterial activities against *T. mentagophytes*, *T. rubrum*, *C. albicans* and *Cryp. neoformans* (Okubo et al. 1991). Kenyan tea clones generally produce black tea with high levels of total theaflavins (Owuor and Obanda, 1995).

Hot water extract and isolated theaflavins of Kenyan black tea differed in strength of antibacterial activities. Akin difference was examined when the two black tea extracts were blended with antibiotics. The antibacterial activities and synergistic activity with antibiotics was lower for hot water extract, as compared to that of isolated theaflavins. That was even though hot water extract tested had the same amount 36 µmol (18 µmol /g) of theaflavins as isolated theaflavins (36 µmol/g). The differences in inhibitory effect examined were attributed to interactions within the tea infusion between water soluble components and theaflavins that were not isolated. Theaflavins in black tea infusion are being partially antagonized by one or more chemical components in it lowering the overall activity. The differences in inhibitory effect was significant ($P<0.05$). However, the pattern of activity of isolated theaflavins and hot water extract (infusion) of black tea were akin. This suggested that the theaflavins that were not isolated were the principal bioactive compounds in black infusion despite the existence of interactions. This inference agrees with research by Okubo et al. (1991) that showed theaflavins as the major antibacterial compound in tea. Further, Apostolides and Weisberger (1995) remarkably showed that theaflavins as the principal quality components in black tea are beneficial to human health. In addition to theaflavins, other antibacterial compounds in hot water extract of black tea are catechins, fluoride,

kaempferol, quercitin and myericitin (Higdon 2007). However, the blend of these in the hot water extract in the present research does not boost the antibacterial activity of theaflavins that were not isolated to exceed that of isolated theaflavins.

These compounds isolated from other plants have been found to have antibacterial properties. Kaempferol (3,4',5,7-tetrahydroxyflavone) and quercetin (3,3',4',5,7-pentahydroxyflavone) showed the lowest minimum inhibitory concentrations (MICs) against the clinical MRSA (Lin et al. 2008). Tryptanthrin and kaempferol isolated from the indigo plant (*Polygonum tinctorium lour*) remarkably decreased the numbers of *H. pylori* colonies in a dose-dependent manner (Maria et al. 2005). Querticin and kaempferol from other plants have been shown to have blended effects with antibiotics. Blends of rifampicin and either with kaempferol or quercetin acted synergistic ally or partially synergistic ally against the clinical MRSA. Rifampicin blended with kaempferol, or quercetin exhibited good beta-lactamase inhibitory effects (57.8 % and 75.8 %, respectively) against a representative isolate (Lin et al. 2008).

The development of bacterial resistance to antibiotics can be prevented by hot water extract and isolated theaflavins of Kenyan black tea. Synergism and restoration of activities of lower concentrates of ampicillin, chloramphenicol, tetracycline, norfloxacin and ciprofloxacin by the tea extracts as is shown in this research, a pointer to the value of the blend in combating bacterial resistance.

Black tea and its two polyphenols (theaflavins and thearubigins) have significant antimutagenic effects against *Salmonella* strains (Gupta et al. 2002). Chunxia and Yongquan (2006) also showed that theaflavins have considerable antimutagenic effects against bacterial mutagens such as sodium azide, 4-nitro-o-phenylenediamine, cumine hydro-eroxide, 2-amino-fluorene and danthron. Development of bacterial resistance has been attributed to mutation. Errors in deoxyribonucleic acid (DNA) synthesis during replication and occasional failures in the DNA repair systems result in a spontaneous mutation (Grace 2008). The ability to prevent development of antibiotic resistance is owned by certain antimutagenic agents. These include green tea catechins and other antioxidants. In many cases, these agents can exert these effects at doses which by themselves produce no visible effect on growth. These effects are exerted against resistance to antibiotics such as tetracyclines, fluoroquinolones, macrolides, beta-lactams, and aminoglycosides (Pillai et al. 2001).

Blended use of tea and antibiotics could be useful in erasing the problem of emerging drug resistance especially among enteropathogens. Multi-drug resistance by *S. typhi* was examined. In this research, all 56 isolates of *S. typhi* were sensitive to amoxicillin/clavulanate, gentamicin, cefixime, cefotaxime and ceftazidime. Multidrug resistance (MDR, resistance to three drugs) was found in 22 cases (39%) while resistance to five drugs was found in 12 cases (21%). Only two isolates were resistant to chloramphenicol (3%). All *S. paratyphi* A. isolates were sensitive to ampicillin and chloramphenicol, and resistant to nalidixic acid. Treatment of enteric fever in children based on current trends of antimicrobial susceptibility of *Salmonella enterica serovar typhi* and *paratyphi A* used ampicillin as a chosen drug. MIC distribution data for chloramphenicol revealed elevated MIC, but still in susceptible range. Therefore, recommended an urgent need for further clinical research to evaluate response to chloramphenicol in such cases (Manchanda et al. 2006).

Tetracycline resistance now occurs in an increasing number of pathogenic, opportunistic, and commensal bacteria. The use of these agents in treatment of disease is limited by the presence of tetracycline-resistant pathogens. Tetracycline resistance is often caused by the acquisition of new genes, which code for energy-dependent efflux of tetracyclines or for a protein that protects bacterial ribosomes from the action of tetracyclines (Ian and Marilyn 2001).

Two genetically distinct classes of norfloxacin-resistant *Pseudomonas aeruginosa* PAO4009 mutants were isolated spontaneously. Two norfloxacin resistance genes, nfxA and nfxB, were mapped hex-9001 and leu-9005 and between pro-9031 and ilv-9023, respectively, on the *P. aeruginosa* PAO chromosome. These findings suggested that the norfloxacin resistance mechanism in the nfxB mutant might be an alteration in outer membrane permeability to norfloxacin (Hirai et al. 1987). *P. aeruginosa* has been shown to inactivate anti-methicillin resistant *S. aureus* antibiotics as indirect pathogen (Ramphal 2007).

The blend of hot water extract and isolated theaflavins with antibiotics in this research were found to be useful *in vitro*. However, before utilizing these findings *in vivo*, there are other factors to consider. Black tea has 177-303 mg/l of caffeine. Several drugs can impair the metabolism of caffeine, increasing the potential for adverse effects from caffeine. They include cimetidine (Tagamet), disulfiram (antabuse), estrogens, fluoroquinolones, antibiotics (ciprofloxacin, enoxacin and norfloxacin), fluconazole (diflucan), fluroxamine (luvox), mexilitrine (mextil), riluzol (rilutek), terbinafine (lamisil) and verapamil (calan). High caffeine intakes may increase the risk of toxicity of some drugs, including albuterol (alupent), cloxapine (clozaril), ephedrine, epinephrine, monoamine oxidase inhibitors, phenypropanolamine and theophylline.

Results from this research showed that ciprofloxacin and norfloxacin act synergistically with both tea extracts *in vitro*. This benefit cannot be fully utilized *in vivo* because of the presence of caffeine in hot water extract of black tea. These antibiotics can be blended with decaffeinated black tea and isolated theaflavins. The black tea industry will have to produce another patented tea, in which caffeine has been removed, akin to that of green tea industry.

Flavonoids in tea which includes theaflavins have been reported to bind non-heme iron, inhibiting its intestinal absorption. Non-heme iron is the principal form of iron in plant foods, dairy products, and iron supplements. The consumption of one cup of tea with a meal has been found to decrease the absorption of non-heme iron in that meal by about 70%. To maximize iron absorption from a meal or

iron supplements, tea should not be consumed at the same time (Higdon 2007).

In conclusion**, w**ater soluble components in black tea reduces theaflavins activity but does not remarkably diminish their overall activity. Theaflavins and hot water extracts of black tea can be used to impede growing bacterial resistance despite the interactions. The blended formulations with antibiotics will be suitable for prevention and treatment. Both black tea extracts can restore the activities of antibiotics.

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