Antimicrobial, synergistic and antioxidant activities of tea polyphenols

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Polyphenols are secondary metabolites produced by tea plant, which play multiple essential roles in plant physiology and have potential health properties on human health, mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive and antimicrobial agents. Microbial resistance has become an increasing global problem; there is a need to find out novel potent antimicrobial agents with alternative modes of action as accessories to antibiotic therapy. This study investigated the antimicrobial, synergistic and antioxidant properties of tea polyphenols. The synergistic effect of tea polyphenols in combination with conventional antimicrobial agents against clinical multidrug-resistant microorganisms has been investigated and valuable data generated on the potential synergistic properties of tea polyphenols.

Keywords Polyphenols; antimicrobial; synergistic; antioxidant properties

1. Introduction

Tea (*Camellia sinensis*, family Theaceae) is the most widely consumed plant-based beverage in the world [5]. The secondary metabolites found in the tea plant and the unique combinations of these secondary metabolites are responsible for the popularity of this crop as source of consumed soft beverage. The pharmacological significance associated with tea's popularity is linked to its important phytochemicals which include polyphenols which are pharmacologically active and with potential to promote human health. Polyphenols are either flavonoids or non-flavonoids but biochemicals found in tea are mostly flavonoids [52]. A number of flavonoids are present but dietary flavonoids are usually categorized into six major groups namely flavanols, flavonols, anthocyanidins, flavones, flavonones and isoflavonoids [59]. These phytochemicals not only contribute to tea quality but also the scientific community and the food industry are motivated to extend the quality of life, and in particular treating and managing diseases especially through the use of nutraceuticals.

There are three main categories of processed tea, black tea (earated or oxidized), green tea (non-aerated) and oolong tea (semi-aerated or partially oxidized). These tea products differ in method of post-harvest manufacture. White tea which is a rare specialty tea gets its name from a specific tea plant variety, as well as a particular post-harvest processing method that contains a higher proportion of the buds that are covered with fine "silvery" hairs (trichomes) that impart a light white colour to the tea. Due to this difference in post-harvest processing, several catechins are present majorly in green and white teas in significant quantities and they include epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate, [60]. In black tea, they are oxidized and dimerized during auto-oxidation to the yellow-orange pigments, theaflavins, or polymerized to the red pigments called thearubigins [36, 29].

There are many health benefits that have been ascribed to consumption of the tea beverage, including, the effects on reduction of cholesterol, protection against cardio-vascular [61], antibacterial [58], anti-diabetic [6], anti-inflammatory [23] and antiviral [50]. Consumption of tea has also been associated with reduced risk of major diseases, including coronary heart disease and stroke [26]. Beneficial effects of tea have been attributed to the strong antioxidative activity of the tea phenolic compounds [24, 32]. Tea catechins, theaflavins and thearubigins possess strong antioxidant properties, which protect the body from damage caused by free radical-induced oxidative stress [41]. In addition, a report has presented data on the antimicrobial activity of different types of tea extracts on various pathogenic microorganisms [1].

There is growing evidence that indicates that the catechin components of green tea are responsible for the observed antibacterial activity, and that EGC, EGCG and ECG constitute the most important antibacterial agents [58]. Black tea which is a major source of theaflavins and thearubigins has also been shown to have antibacterial properties both *in vivo* and *in vitro* [3]. Theaflavin-3, 3'-digillate has been reported to have antifungal activity against *Candida albicans* and *Cryptococcus neoformans* in a dose and contact time-dependent manner [37].

Apart from its antimicrobial properties, green tea has been established to exhibit synergistic activity with some antibiotics against some enteric pathogens [54]. Tea polyphenols have particularly proven to synergistically enhance the antimicrobial activity of antimicrobial agents used against methicillin resistant *Staphylococcus aureus* [20]. Despite the valuable data generated so far from green tea, little data has been generated from black tea. In this study different types of tea products processed from different tea germplasm grown in Kenya were assayed for their antimicrobial, synergistic and antioxidant properties on antibiotic resistant strains of bacteria and fungi.

1.1. Biochemical Analysis

1.1.1. Total polyphenols

The levels of polyphenols in the different types of tea products were determined in this study (Table 1). The results of the study reported the superiority of Kenyan tea in the level of its total polyphenols compared to teas from the test Chinese and Japanese germplasm which is in agreement with results from previous studies [55]. The general trend among the samples assayed showed that non-aerated tea had higher polyphenol content than aerated tea from the same sample. A comparison between the Kenyan tea cultivars with the Chinese and the Japanese cultivars revealed that Kenyan cultivars irrespective of type of tea had higher polyphenol content than the non-aerated teas from the Japanese cultivar. In his study, Karori *et al.,* [24] also established that products derived from Kenya tea cultivars were rich in total polyphenols when compared to those from Japanese and Chinese cultivars. The comparison of green, white, and black tea products revealed that the green and white teas had higher levels of total polyphenols. The extent of variation in total polyphenol content between different types of tea products is as a result of degradation of polyphenols during the oxidation process to form theaflavins and thaerubigins which are not detectable at the 765nm wavelength used for polyphenol analysis.

Table 1 Percent total polyphenols, total theaflavins, total thearubigins and antioxidant activity of processed tea products from different germplasm grown in same environment

Tea Samples	TP%	TFs%	TRs%	AA%
Black tea products from Kenyan germplasm				
Green leaf coloured cultivars				
AHP S15/10	18.8	1.1	15.5	72.7
BBK 35	17.5	1.3	16.2	73.4
TRFK 303/577	17.4	1.5	15.4	72.4
TRFK 6/8	19.3	1.7	14.6	73.3
Purple leaf coloured cultivars				
TRFK K-Purple	16.2	1.3	17.2	72.3
TRFK 306/3	18.7	1.3	15.7	73.7
TRFK 73/1	16.3	1.5	15.6	73.3
Mean	17.7	1.4	17.9	73.0
Black tea products from buds of Kenyan germplasm				
AHP S15/10	17.2	1.4	13.1	73.8
TRFK 301/5	19.0	1.1	10.4	73.7
Mean	18.1	1.4	11.7	73.8
Green tea products from Kenyan germplasm				
Green leaf coloured cultivars				
AHP S15/10	20.2	0.4	7.7	73.5
BBK 35	20.9	0.4	6.8	73.3
TRFK 303/577	22.8	0.4	8.7	74.0
TRFK 6/8	24.4	0.5	9.3	74.2
Purple leaf coloured cultivars				
TRFK K-Purple	19.7	0.6	10.2	74.1
TRFK 306/3	22.2	0.4	11.2	74.5
TRFK 73/1	21.5	0.4	8.8	73.9
Mean	21.7	0.5	8.9	73.9
Green tea products from germplasm of other sources				
Hanlu st. 830 (China)	18.5	0.3	9.6	73.3
Yabukita st. 536 (Japan)	16.7	0.2	9.8	72.8
Mean	17.6	0.3	9.7	73.0
White tea products from Kenyan germplasm				

AHP S15/10	22.0	0.1	0.8	74.1
TRFK 301/5	25.2	0.1	0.9	74.1
Mean	23.6	0.1	0.9	74.1
CV%	3.8	17.6	6.6	0.9
LSD ($p \leq 0.05$)	0.7	0.5	0.8	0.5

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

The rolling and cutting of the tea shoots in non-orthodox manufacture causes a release of polyphenol oxidase which interacts with phenolic compounds, one simple catechin and one gallocatechin, to produce theaflavins and thearubigins [31]. 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. At the same time several factors have been known to influence the polyphenol content. These include genotype, geographical origin, soil composition, harvesting time, post-harvest treatment and physical structure of the leaves [30]. The results obtained in this study may therefore vary with seasons and it could be important to carry out a study to establish the seasonal variations of the total polyphenols in the tea products derived from the assayed clones. However, owing to the fact that tea contains several polyphenols, it is likely that even such a proposed study may reveal that some derivatives were more stable than others. In this study, the major polyphenolic compounds of tea; the catechins were assayed in teas derived from the test cultivars.

1.1.2. Total Catechins

In general, the total catechins content in white and non-aerated tea products were significantly higher than in aerated tea products from the same clones (Table 2). Individual catechin content also varied significantly among the tea products with EGCG and EGC recording higher levels while C, EC and ECG were less abundant in non-aerated tea products. The findings of this study corroborated with a similar study by Karori *et al.,* [24] who found that green tea had significantly higher catechin content than black tea used in his study. Black tea which is obtained by a post-harvest auto-oxidation reaction is catalyzed by the polyphenol oxidase enzyme while green teas do not undergo the oxidation process since they are initially steamed to inactivate the enzyme. The enzymatic oxidation of catechins located in the cell vacuole of the tea leaf 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 [38]. Aerated tea products had lower amounts of the individual catechins due to the formation of TFs and TRs. Theaflavin formation requires a trihydroxy flavan-3-ol and dihydroxy flavan-3-ol whose correct balance is necessary to ensure maximum formation of theaflavins [57].

White tea which is predominantly manufactured from the young apical hairy bud showed high levels of EGCG and EGC that are present in higher amounts in fresh young leaves. These findings were similar with the results by Saijo *et al.,* [43] who 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 catechin gallate compared with dry matter production.

Table 2 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	**12.33**	**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 ($p \leq 0.05$)	0.76	0.37	0.35	0.17	0.22	0.35	0.22

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

1.1.3. Gallic acid

Gallic acid remained unchanged in the black (aerated) and green (non-aerated) tea products as well as in tea products from the purple leaf coloured tea cultivars since gallic acid is not oxidized during oxidation (Table 2). In fresh leaf, gallic acid is present in trace amounts but accumulated during fermentation through the breakdown of galloyl esters from the catechins and/or theaflavin gallate [47]. The increase of gallic acid levels in some products from the tea cultivars is due to high levels of gallated catechins leading to formation of and subsequent breakdown of the TFs. Gallic acid is not a substrate for polyphenol oxidase, however during auto-oxidation process, theaflavic acids are formed by redox equilibrium and this is made possible by electron carrying capabilities of catechin gallates such as epicatechin gallate. The theaflavic acids are thought to oxidize the gallocatechins namely EGC and EGCG and due to their higher concentration in fresh green leaf, but it is until the later stages of auto-oxidation step that gallic acid is released. Therefore the levels of gallic acid in the teas depend on the extent to which auto-oxidation is carried out [7].

1.1.4. Total theaflavins (TFs) and total thearubigins (TRs)

Catechins are the major components of green tea leaves. In black tea, they are oxidized and dimerized during fermentation to the yellow-orange pigments, theaflavins, or polymerized to the red pigments called thearubigins. Black tea products from this study had high levels of TFs and TRs as the main auto-oxidation products as compared to green and white tea products (Table 1). These results corroborated those of Obanda *et al.,* [36] and Li *et al.,* [29] who reported that theaflavins are oxidized further to form thearubigins that are heterogeneous in nature and contribute significantly towards taste, color and body of tea. Wilson and Clifford [56] 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. This may suggest that theaflavins are not the only source of thearubigins. However, under a limiting oxygen concentration, polyphenol oxidase, which has a high affinity for the substrate, has a preferential demand for oxygen and theaflavins formation is suppressed at the expense of catechin quinone formation.

The competition for oxygen is particularly noticeable during the early stages of auto-oxidation when the concentration of the catechins is at its highest and enzyme turnover is unimpeded by substrate availability. This occurs during green tea manufacture since the enzyme is active before deactivation through steaming. For this reason, high enzyme activity in an already low oxygen concentration creates almost total anaerobiosis which suppresses benzotropolone ring formation. Consequently, as a result of this, thearubigins are formed, mainly from gallocatechins since the simple catechins are unable to react in benzotropolone ring formation. Moreover, it might be possible to minimize thearubigins formation by deactivating the enzyme immediately after plucking through a steaming procedure

although this is hardly achievable during commercial tea processing. Further research is desirable to explain in details the existence of thearubigins in green tea and the importance of steaming during tea processing.

1.2. Antioxidant Activity

In this study, there was high radical scavenging activity on the DPPH radical by both the black (aerated) and green (non-aerated) teas (Table 1). The antioxidant activity of the ordinary green teas is mainly attributed to the presence of high levels of bioactive catechins that have the ability to donate hydrogen ions to stabilize the free radicals. The high antioxidative effect of polyphenols in both white and green Kenyan teas is due to the presence of phenolic hydroxyl groups in their structures that make them potent free radical scavengers [2]. This hydroxylation confers a higher degree of stability on the catechin phenoxyl radical by participating in electron delocalisation that is an important feature of the anti-radical potential. This explains why radical scavenging is high in the gallocatechins including EGCG and EGC that are potent antioxidants [41].

The most potent antioxidant polyphenols of green and white tea namely EGC and EGCG, were found in minute amounts in black tea. These results were similar to those of Zuo *et al.,* [62] who reported that black tea products had low levels of EGCG and EGC. It is probable that the antioxidant effects of black tea, along with its antioxidant capacity, are lowered during the process of auto-oxidation. However, theaflavins contained in black tea also possess antioxidative properties and have been shown in some studies to have higher antioxidative activity than EGCG, which is the strongest antioxidant among all catechins and a precursor of theaflavins [27]. Theaflavins have more hydroxyl (OH) groups, which are considered to be necessary for exerting radical scavenging activity than do catechins, since theaflavins are dimers of catechins. The antioxidant activity of TRs in black tea can be explained by the presence of 3-OH groups, which are more or less esterified by gallic acid in the TRs structure. However, this is a highly speculative hypothesis since to date there is no definite data on TRs structures [29]. Therefore, the high percentage inhibition exhibited by black tea in this study shows that the conversion of tea catechins to TFs and TRs does not affect the radical scavenging activity of the highly dimerized products. These findings are in agreement with those of Leung *et al.,* [27].

The most effective radical scavengers in green tea are the catechins 3', 4' and 5' -trihydroxylated substitution patterns on the B-ring and/or hydroxyl groups at the C-3 position of the catechins therefore are an important feature not their antiradical potential since it confers a high degree of stability of catechin phenoxyl radical by participating in electron delocalization. This is the reason behind the high radical scavenging activities exhibited by ECGC and EGC, which have been proven to be potent antioxidants [41]. The black teas analyzed in this study also exhibited antioxidant activities with high percentages of inhibition of the DPPH radicals. The aerated and non-aerated teas processed from the purple coloured leaf tea cultivars exhibited high scavenging activity which can be ascribed to anthocyanins. These results were similar to that reported by Kerio *et al.,* [25]. The antioxidant activity of the anthocyanins can be related to several parameters like the number of hydroxyl groups in the molecule, the catechol moiety in the B-ring, the oxonium ion structure in the C-ring, the hydroxylation and the methylation patterns and to the acylation by phenolic acids [45]. Many studies have also demonstrated that both catechins and theaflavins have strong free radical-scavenging activity both *in vitro* and *in vivo*.

1.3. Antimicrobial Activity

The results obtained from this study revealed that different tea products exerted significant antimicrobial activity against antibiotic resistant strains and clinical isolates of bacteria (Table 3) and fungi (Figure 1). Similar types of studies have also indicated the efficacy of tea as an antimicrobial agent [40]. Stapleton *et al.,* [51] examined the anti-MRSA properties of a range of naturally occurring and semi-synthetic catechins and catechin gallates and found that they possessed either weak activity or no discernable activity. Such weak or discernable activity would preclude these biomolecules from systemic use as conventional antibacterial agents, but indicate that they may be suitable as topical agents for the treatment of superficial bacterial infections. This may also be able to reduce the carriage on body surfaces of opportunistic multi-drug resistant pathogens such as MRSA, where the use of antibiotics could be contra-indicated. This therefore indicates that the different tea products assayed in this study were found to possess either strong or weak activity and they can be used as conventional or topical agents respectively.

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					
Hanlu st. 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)
Yabukita st. 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 .typhi*

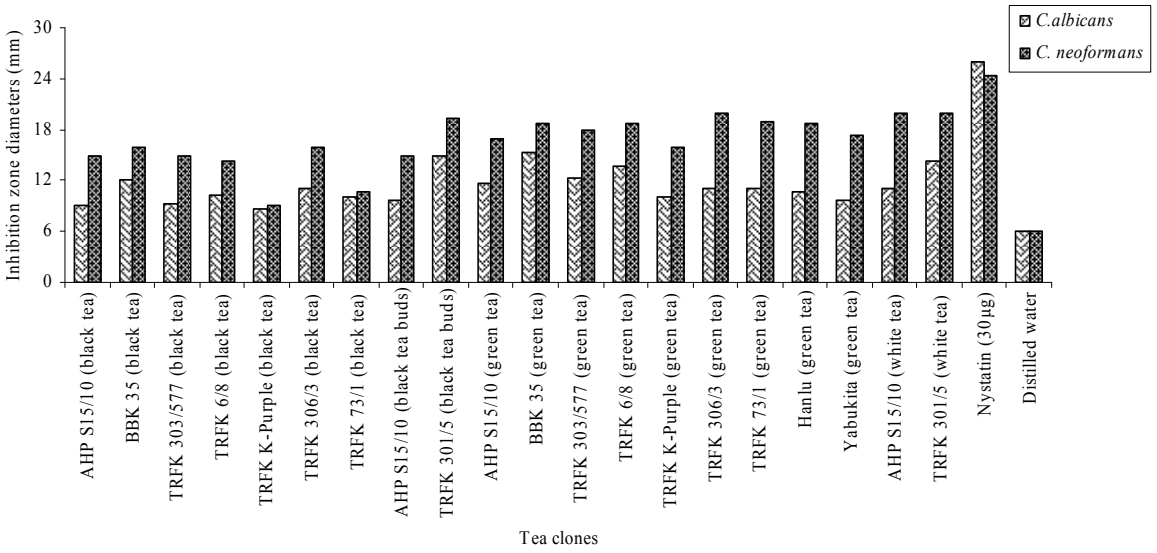

Fig. 1 Variation in antifungal activity among different types of tea extracts

The results on the antibacterial and antifungal activity also indicated that the green tea products as well as production from the purple leaf coloured (unaerated) tea and white tea (silvery tips) products processed from Kenyan tea cultivars exerted the highest antimicrobial activities, while black tea and black tea processed from terminal tea buds, had lower inhibitory activity. This may indicate that the presence of the hydroxyl moieties at 3', 4', and 5' on the B ring in the catechin and epicatechin molecules is a major contributing factor that contributed to inhibitory activity of both green, unaerated tea from the purple leaf coloured clone and white tea. This is in agreement with a study reported by Nance *et al.,* [35] who concluded that antimicrobial activity of catechins is predominantly as a result of the gallic moiety and hydroxyl group member. The highest antimicrobial activity also corresponded to the highest total polyphenols content and to antioxidant activity.

Several tea polyphenols of black, green and white tea products have significant antimicrobial effects. These included EC, EGCG, ECG, TFs and TRs. Results from this study generally revealed that the inhibition zones were significantly and positively correlated to the catechins (EGCG and EGC), total TFs and total TRs. The results obtained in this study are therefore in agreement with those of Gramza and Korczak [15], who studied the effects ofindividual catechins separately and found that EGCG and EGC had the highest antioxidant and antimicrobial activity. Susceptibility of bacterial strains to the tea extract has been shown to be related to differences in cell wall components [16]. They 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 result in cell death [12].

Furthermore, polyphenols have been reported to exhibit antimicrobial activities by binding with proteins related polyamide polymers [17]. The inhibition of microorganisms by phenolic compounds may also be due to iron deprivation or hydrogen binding with vital proteins such as microbial enzymes [46]. Phenolic compounds notably proanthocyanidins are vulnerable to polymerization in air through oxidization reactions [8]. Therefore, an important factor governing their toxicity is their polymerization size. Oxidized condensation of phenols may result in the toxification of microorganisms. On the other hand, polymerization can result in the detoxification of phenols [14]. These supports the fact polyphenols are responsible for the antimicrobial activities of the tea extracts.

The findings of this study also indicated that the antimicrobial effects of assayed tea extracts differed depending on the concentration and type of the extract; from black, green, purple leaf coloured and white tea and also the type of test organism; bacteria or fungi. The conclusion by Taguri *et al.,* [53] that the antimicrobial potency of polyphenols is dependent upon bacterial species is consistent with the findings of this study, which showed that, while the tea extract was active against the Gram-positive bacteria, methicillin and penicillinase resistant *S. aureus* ATCC 25923, it did not affect the activity of *E. coli* ATCC 25922 and the clinical isolate of *S. typhi*.

Results from this study revealed that the tea extracts were bioactive against the test fungi, *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans*. Hirasawa and Takada [18] and Sitheeque *et al.,* [48] showed antifungal activity of both green and black tea catechins against *C. albicans*. Based on these results, it is possible to conclude that tea has strong and broader spectrum of antifungal activity against *C. albicans* and *C. neoformans*. The antifungal activity shown by tea extracts is probably due mainly to the catechin EGCG and perhaps EGC in green and white tea and theaflavins and thearubigins in black tea. The contributions of the other catechins might be limited by the fact that only small amounts are presented.

Moreover, 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 [53]. EGCG is thought to carry a net negative charge in aqueous solution with pH 6-7 and furthermore, it is believed to bind to the membrane component of bacteria as a result of electrostatic interaction, which leads to membrane damage [53]. A possible interaction between tea constituents and the antibiotics may in part explain this observation. However, the inhibition of bacterial growth after 24 hours of incubation with tea extracts and also the attenuation of antibiotics by tea extracts at certain concentrations strengthen the possibility of selective drug-tea interactions.

In certain circumstances, some bacterial genes may be induced such as transcriptional dual regulator (OxyR) in *E. coli* that strengthens bacterial antioxidant defense mechanisms, which may overcome polyphenols inhibitory effects [49]. These bacterial defense mechanisms may be elicited in tea extract in a dose-dependent manner. Another possible explanation for these observations may be the presence of some interference between tea constituents at certain concentrations, which may result in lowered antibacterial activity. It is likely that microbiologic effects of black tea, along with its antioxidant capacity, are influenced by the process of 'fermentation'. It has been suggested that even the manufacturing season may affect the antimicrobial activity of tea [9]. Though this study has demonstrated the efficacy of different types of tea products on antibiotic resistant bacteria and even fungi, it has some limitations since this investigation was *in vitro*, yet both antibiotics and polyphenolic compounds undergo metabolic processes in the body upon consumption and there is less information on the interaction of the related metabolites. Moreover, tissue distribution of ingested polyphenols is poorly understood. Some of the polyphenols may confer their beneficial effects to specific parts of the body in which they are concentrated [6]. This can further be complicated by the immune system interactions with both polyphenolic compounds [19] and the invasive pathogen. Nevertheless, the *in vitro* bacterial

sensitivity test carried out in this study is still a routine procedure for clinical purposes, based on which proper antibiotics are selected to treat the infection [33].

The results of this study indicated that Gram-negative bacteria were more resistant to polyphenols than gram positive bacteria and fungi. This is perhaps due to the different cell wall compositions. One of the mechanisms of low susceptibility of Gram-negative bacteria to tea extracts may be attributed to presence of negatively charged lipopolysacharides (LPS) in the membrane [21]. The cell wall in Gram-positive bacteria is composed of 30 to 50 sheets of peptidoglycan which is external to the cell membrane and plays an essential role not only in osmotic protection but also in cell division, as well as serving as a primer for its biosynthesis [34]. In Gram-negative bacteria, however, the peptidoglycan layer is thin and is overlaid by an outer membrane composed mainly of lipopolysaccharides [61]. The structural differences between Gram-positive and Gram-negative bacteria and the low affinity between tea extracts and lipopolysaccharides may be the main factor for the different susceptibilities to tea extracts and to tea extracts-drug combination.

1.4. Synergistic, antagonistic and additive activity

The antibacterial results of this study showed a marked increase in the inhibition zone diameters on combination of tea extract with penicillin G. This is in agreement with results by [20] who reported enhanced effect of Japanese tea on inhibitory activities with β-lactams antibiotics against methicillin resistant *S. aureus* ATCC 25923. Synergistic inhibition by tea extracts and the antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract [54]. 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 [39]. The tea extracts and penicillin G synergistically inhibited the growth of methicillin and penicillinase resistant *S. aureus* ATCC 25923 possibly because they directly or indirectly attack the same site which is the peptidoglycan on the cell wall [61]. Furthermore, when ampicillin an inhibitor of cell wall synthesis, was combined with tea extracts, an additive effect was observed which could be hypothesized that ampicillin which is a β-lactam antibiotic also directly or indirectly attacks the same site. The tea extracts-induced damage of the bacterial cell wall and the possible interference with its biosynthesis through direct binding with peptidoglycan may be the major reasons for the synergism against methicillin resistant *S. aureus* ATCC 25923.

The antagonistic effects rarely occur in clinical antibacterial therapy [4]. Such effects can however occur more when a bacteriostatic drug inhibiting protein synthesis is given with a bactericidal drug. However, this was also observed when tea extracts were combined with tetracycline and gentamicin. Antagonism occurs mainly if the bacteriostatic drug reaches the site of infection before the bactericidal drug [28]. 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 lend credence to the view that tea is a safe beverage even for those under treatment with antibiotics. However, antagonistic interactions between tea extracts and antibiotics such as tetracycline and gentamicin were observed.

The results of the combination studies were also additive which shows that the inhibitory actions of the combined agents were equivalent to the sums of the actions of the single agents [44]. Since both agents tea and antibiotics inhibit bacteria by different mechanisms [13], an additive or synergistic interaction is expected to occur [22]. Tea contains various polyphenols such as epicatechin, epigallocatechin, which have been shown to exert profound antibacterial effects against a broad spectrum of bacteria, including *S. aureus* via membrane perturbations [13]. Perturbation of the cell membrane by tea results in free passage of materials in and out of bacterila cell leading to lysis of the cell, which eventually results in death. Penicillin 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. This cross linking is accomplished by a transpeptidation reaction that occurs outside the cell membrane [42]. This double attack of both agents on different target sites of the bacteria could theoretically lead to either an additive or a synergistic effect [22].

However, because the strains of bacteria used in this study were resistant to the drugs used, synergism is likely to occur. Usually, the combination of two agents exhibit significant potentiation or synergism only if the test organism is resistant to at least one of the agents. Perturbation of the cell membrane by tea results in free passage of materials in and out of bacterial cell leading to lysis of the cell, which eventually results in death. Penicillin 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.

Unlike antibiotics and chemotherapeutic agents, there are few reports concerning the possible mechanism of action of plant-derived products. Such compounds may act on the intermediary metabolism by activating enzymes, altering the action of inhibitors which affect nutrients in the medium, interfering with enzymatic processes at the level of the nucleus or ribosome, causing changes in membrane or even interfering with secondary metabolism in the target organism [11]. 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.

2. Conclusions

Different tea products have different biochemical profiles; green and white tea products are rich in catechins while black tea products are rich in TFs and TRs. Despite the above differences, the black tea products are potent in their *in vitro* antioxidant properties. Therefore, it is concluded that teas are a great source of antioxidants. Methicillin and penicillinase resistant *S. aureus* ATCC 25923 was more susceptible to all tea extracts. Even though black tea, black tea from terminal buds, some green tea products and those from purple leaf coloured cultivar did not significantly affect the zones of inhibition of a clinical isolate of *S. typhi* and the antibiotic resistant *E. coli* ATCC 25922, it is not necessarily ineffective as an antibacterial. There are many improvements and adjustments that can be made in future experiments to truly determine the effectiveness of tea products on these microbes. Tea extracts can be used in management of fungal infections caused by *C. albicans* and *C. noeformans*. The concomitant administration of tea extracts and antibiotics may not impair with the antibacterial activity of these antibiotics. The tea beverage is therefore safe even for those under treatment with penicillin G and ampicillin.

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