

ANTIOXIDANT, ANTIMICROBIAL AND SYNERGISTIC ACTIVITIES OF TEA POLYPHENOLS

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ABSTRACT

Microbial resistance to antibiotics has become an increasing global problem and 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 antioxidant, antimicrobial and synergistic properties of tea polyphenols. The tea germplasm from Kenya, China and Japan that are grown in Kenya were characterised for their biochemical profiles. The total phenolic content, theaflavins and thearubigins content of different tea products used in this study were determined spectrophotometrically according to Folin-Ciocalteu and flavognost methods, respectively. The individual catechin contents were characterised by high performance liquid chromatography (HPLC) and identified according to their HPLC retention times, elution order and comparison with authentic standards. The antioxidant activity of tea polyphenols was determined spectrophotometrically on its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The agar disc diffusion method was used to screen for antimicrobial and synergistic activities of the tea liquors. Black, green, purple coloured leaf and white (silvery tips) tea products differed significantly in the levels of total polyphenols, total catechins, catechins fractions, theaflavins and thearubigins ($P < 0.05$). Green, purple coloured leaf (aerated) and black tea from terminal buds and white tea products analysed in this study exhibited slightly higher antioxidant activity than black tea. The different types of tea products exhibited significant influence on the inhibition zone diameters against bacteria and fungi exposed to the tea extracts. Methicillin and penicillinase resistant *S. aureus* ATCC 25923, *C. albicans* ATCC 90028 and a clinical isolate of *C. neoformans* were more susceptible to all tea extracts than *E. coli* and *S. typhi*. There was synergism between most tea extracts and penicillin G against methicillin and penicillinase resistant *S. aureus* ATCC 25923.

Key Words: Catechins, methicillin, penicillinase, theaflavins

RÉSUMÉ

La résistance microbienne aux antibiotiques est devenue un problème épineux au niveau global et il est nécessaire d'identifier d'autres agents antimicrobiens efficaces avec des modes alternatifs d'action pour appuyer la thérapie antibiotique. Cette étude était menée sur les antioxydants et les propriétés antimicrobiennes et synergétiques des polyphénols du thé. Les germoplasmes de thé en provenance du Kenya, de la Chine et du Japon cultivés au Kenya ont fait l'objet d'une caractérisation de leurs profils biochimiques. La teneur phénolique totale ainsi que les théaflavines et les théarubigines dans les différents produits de thé utilisés dans cette étude étaient déterminées par spectrophotométrie respectivement sur base des méthodes dites de Folin-Ciocalteu et flavognost. Les teneurs individuelles en catéchine étaient caractérisées par chromatographie liquide à haute performance (HPLC) et identifiées sur base de leurs temps de rétention de HPLC, de l'ordre d'éluion en comparaison avec les

standards normaux. L'activité antioxydante des polyphénols de thé était déterminée par spectrophotométrie sur base de sa capacité à capter le radical 2,2-diphényl-1-picrylhydrazyl (DPPH). La méthode de diffusion sur disque d'agar était utilisée pour tester les activités microbiennes et synergétiques des extraits du thé. Les feuilles de thé de couleurs noir, verte, pourpre et les extraits du thé blanc différaient significativement du point de vue niveaux de polyphénols totaux, des catéchines totales, des fractions de catéchines, des théaflavines et des théarubigines ($P < 0.05$). Les feuilles de couleurs verte et pourpre ainsi que les thé noirs issues des bourgeons terminaux et extraits du thé blanc analysés dans cette étude ont montré une activité antioxydante légèrement supérieure à celle du thé noir. Les différents types d'extraits de thé ont montré une influence significative sur les diamètres des zones de contamination une fois exposés à des bactéries ou des champignons. *Staphylococcus aureus* ATCC 25923, *C. albicans* ATCC 90028 et un isolant clinique de *C. Neoformans* résistants à la méthicilline et à la pénicilline étaient les plus susceptibles à tous les extraits de thé en comparaison avec *E. coli* et *S. typhi*. Il y avait un synergisme entre la plupart d'extraits de thé et la pénicilline G contre *S. aureus* ATCC 25923 résistant à la méthicilline et à la pénicillinase.

Mots Clés: Catechines, méthicilline, pénicillinase, théaflavines

INTRODUCTION

Black tea (*Camellia sinensis*) which is a major source of theaflavins and thearubigins has been shown to have antibacterial properties both *in vivo* and *in vitro* (Bandyopadhyay *et al.*, 2005). Theaflavin-3, 3'-digillate has antifungal activity against *Candida albicans* and *Cryptococcus neoformans* in a dose and contact time-dependent manner (Okubo *et al.*, 1991). There is also growing evidence that indicates that catechin components of green tea have antibacterial activity (Yam *et al.*, 1997).

Apart from its antimicrobial properties, green tea is known to exhibit synergistic activity with antibiotics against some enteric pathogens (Tiwari *et al.*, 2005). Tea polyphenols synergistically enhance the antimicrobial activity of antimicrobial agents used against methicillin resistant *Staphylococcus aureus* (Hu *et al.*, 2002).

Despite the valuable data generated so far from green tea, little information has been generated from black tea. The objective of this study was to investigate the relationship between biochemical profiles of different types of tea products processed from different tea germplasm grown in Kenya and antioxidant, antimicrobial and synergistic properties on bacteria and fungi resistant to antibiotics.

MATERIALS AND METHODS

Test microorganisms and tea samples. The test bacteria of American Type Culture Collection (ATCC) were sourced from the Kenya Medical

Research Institute Centre for Respiratory Research (KEMRI-CRDR) at Nairobi and included methicillin and penicillinase resistant *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 90028 and clinical isolates of *Salmonella typhi* and *Cryptococcus neoformans*.

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

Estimation of total polyphenols and biochemical profiling of the tea extracts. The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols in the tea extracts, according to ISO (BS ISO 14502-1:2005(E)). A modified high performance liquid chromatography procedure was used to assay for the tea catechins (Zuo *et al.*, 2002).

Analysis of total theaflavins and thearubigins content. Black, green, purple and white teas were also assayed for total theaflavins (TF) using the flavognost method of Hilton and Palmer-Jones (1973).

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

Antioxidant activity of tea and freeze drying of tea liquors. The stable 2,2-diphényl-1-picrylhydrazyl radical (DPPH) was used for

determination of free radical scavenging of tea extracts using a modified method of Brand-Williams *et al.* (1995). Tea liquors derived from the processed tea samples were freeze dried according to the method described by Turkmen *et al.* (2009).

Antimicrobial assays. The agar disc diffusion method was used to screen for antimicrobial activities of the tea liquors, according to the National Committee of Clinical and Laboratory Standards (NCLSI, 2012).

Statistical analysis. Data were subjected to analysis of variance using SAS software Version 9.1. The Least Significant Difference (LSD) procedure was used to separate differences among the treatment means.

RESULTS

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

Total catechin content. The total catechins data of green, black, white tea products processed from 11 tea cultivars are presented in Table 1.

The data revealed that the tea cultivars that produced the different tea products significantly differed in total catechin content. There were significant differences ($P < 0.05$) in total catechin levels between processed black teas, green teas, white and purple leaf coloured teas. Non-aerated (green) teas contained significantly higher amounts of total catechins than aerated (black) teas. Green teas from Kenyan cultivars were significantly higher in total catechin content than those from Chinese and Japanese cultivars. White teas processed from the Kenyan cultivars, TRFK 301/5 and AHP S15/10, had the highest levels of total catechins at 22.8 and 22.3%, respectively. Among the Kenyan purple coloured leaf tea cultivars, the highest catechin content was recorded in the non-aerated tea products from clones TRFK 73/1 with 16.1% and TRFK 306/3 with 11.9%.

Total theaflavins (TFs) and total thearubigins (TRs) levels. There was no significant difference ($P < 0.05$) in total TRs levels for the Kenyan, Chinese and Japanese green teas. This was also exhibited by Kenyan black tea and black tea buds. Black tea had the highest levels of total TFs and total TRs, which ranged from 1.1 to 1.7% and 14.6 to 17.2%, respectively (Table 1). Black tea from the popular high black tea quality clone TRFK 6/8 had the highest TFs and the lowest content of TRs among the black tea products. White tea had the lowest TFs compared to green, black and aerated and unaerated teas from the purple coloured leaf clones. TRs were particularly low in white teas processed from cultivars AHP S15/10 and TRFK 301/5.

Antioxidant activity. There was no significant difference ($P > 0.05$) in the antioxidant activity among the different types of tea products (Table 1). However, antioxidant activity was marginally higher among the green teas processed from Kenyan germplasm, as well as the white teas from Kenyan clones. There was no significant difference in the antioxidant capacity between Kenyan black teas and green teas processed from cultivars Hanlu and Yabukita, from China and Japan, respectively. Purple coloured leaf (unaerated) manufactured from clone TRFK 306/3, which is rich in anthocyanins and clone TRFK

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

Tea samples	TP%	TC%	TFs%	TRs%	AA%
Black tea products from Kenyan germplasm					
Green leaf coloured cultivars					
AHP S15/10	18.8	5.22	1.1	15.5	72.7
BBK 35	17.5	4.95	1.3	16.2	73.4
TRFK 303/577	17.4	5.41	1.5	15.4	72.4
TRFK 6/8	19.3	6.36	1.7	14.6	73.3
Purple leaf coloured cultivars					
TRFK K-Purple	16.2	3.22	1.3	17.2	72.3
TRFK 306/3	18.7	6.18	1.3	15.7	73.7
TRFK 73/1	16.3	5.22	1.5	15.6	73.3
Mean	17.7	5.22	1.4	17.9	73.0
Black tea products from buds of Kenyan germplasm					
AHP S15/10	17.2	9.06	1.4	13.1	73.8
TRFK 301/5	19.0	10.84	1.1	10.4	73.7
Mean	18.1	9.95	1.4	11.7	73.8
Green tea products from Kenyan germplasm					
Green leaf coloured cultivars					
AHP S15/10	20.2	17.46	0.4	7.7	73.5
BBK 35	20.9	19.65	0.4	6.8	73.3
TRFK 303/577	22.8	19.96	0.4	8.7	74.0
TRFK 6/8	24.4	17.63	0.5	9.3	74.2
Purple leaf coloured cultivars					
TRFK K-Purple	19.7	12.34	0.6	10.2	74.1
TRFK 306/3	22.2	11.92	0.4	11.2	74.5
TRFK 73/1	21.5	16.10	0.4	8.8	73.9
Mean	21.7	16.44	0.5	8.9	73.9
Green tea products from germplasm of other sources					
Hanlu st. 830 (China)	18.5	13.98	0.3	9.6	73.3
Yabukita st. 536 (Japan)	16.7	10.68	0.2	9.8	72.8
Mean	17.6	12.33	0.3	9.7	73.0
White tea products from Kenyan germplasm					
AHP S15/10	22.0	22.29	0.1	0.8	74.1
TRFK 301/5	25.2	22.79	0.1	0.9	74.1
C V %	3.8	16.16	17.6	6.6	0.9
LSD (P<0.05)	0.7	0.76	0.5	0.8	0.5

TP = total polyphenols; TC = totalcatechins; TFs = total theaflavins; TRs = total thearubigins

6/8 processed as green tea had a high DPPH radical scavenging activity, with a mean value of 74.5 and 74.2%, respectively; followed by white teas from clones AHP S15/10 and TRFK 301/5 with 74.3 and 74.1%, respectively.

Antimicrobial activity. Methicillin and penicillinase resistant *S. aureus* ATCC 25923 was susceptible to the tea extracts (Table 2). Black teas from clones TRFK 6/8, AHP S15/10 and BBK 35 had no significant difference in inhibitory activity with the green teas processed from leaf of Kenyan cultivars. There was also no significant difference in inhibitory activity between the Kenyan black tea products with tea processed as black tea products from the terminal buds and some of the Kenyan, Chinese and Japanese green teas studied.

Escherichia coli ATCC 25922 was inhibited weakly by black tea and black tea from the buds (Table 2). There was no significant difference ($P>0.05$) in inhibitory effect of black tea and black tea buds. This was also exhibited by green and purple tea extracts processed from Kenyan tea cultivars, Chinese and Japanese green tea extracts. White tea extracts processed from clone TRFK 301/5 exhibited the highest inhibitory effect with a zone of inhibition.

The clinical isolate of *S. typhi* was inhibited by the majority of tea extracts (Table 2). Black tea extracts did not differ significantly ($P>0.05$) in the inhibitory effects with green tea extracts. Processed Kenyan black tea buds had no inhibitory effects while white tea extracts processed from clones AHP S15/10 and TRFK 301/5 had the highest inhibitory effects compared to all the teas studied.

There was no significant difference ($P>0.05$) in the antifungal activity of Kenyan black tea and purple coloured leaf (aerated) tea extracts with the Chinese and Japanese green tea extracts against *C. albicans* ATCC 90028 (Fig. 1). Un-aerated tea from purple leaf coloured and white tea extracts did not differ significantly in antifungal activity with black tea extracts against *C. albicans* ATCC 90028. Generally, different tea extracts had antifungal activity against *C. albicans* ATCC 90028.

A clinical isolate of *C. neoformans* was inhibited by all the different types of tea extracts (Fig. 1). White tea extracts from the Kenyan tea cultivars exhibited the highest antifungal activity against *C. neoformans* compared with black tea, black tea buds, green tea and aerated and un-aerated tea extracts from the purple leaf

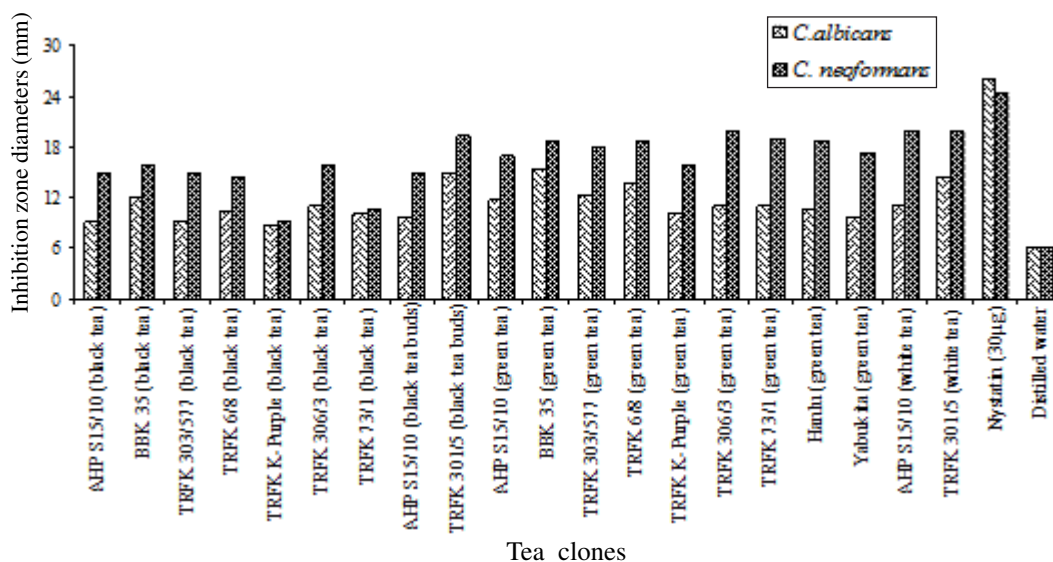


Figure 1. Variation in antifungal activity among different types of Kenya tea extracts.

TABLE 2. 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 (1 mg ml ⁻¹)	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)	12.76.0	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	13.76.0	7.36.0	8.06.0	17.36.0	9.06.0
TRFK 306/3	14.06.0	14.06.0	14.76.0	16.36.0	11.36.0
TRFK 73/1	14.3[7.0](6.0)	11.06.0	10.06.0	18.06.0	8.06.0
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	13.76.0	12.7[6.0](7.0)	12.36.0	18.06.0	11.36.0
TRFK 301/5	14.3[7.3](6.0)	11.36.0	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)	11.06.0
BBK 35	22.0[8.7](8.0)	11.7[7.3](6.0)	14.07.0	16.7[8.3](11.3)	9.37.0
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)	8.06.0	6.07.0	21.0[6.0](11.0)	8.0[8.3](10.3)

TABLE 2. Contd.

Tea sample	Tea alone (1 mg ml ⁻¹)	Gentamicin + tea	Tetracycline + tea	Penicillin G + tea	Ampicillin + tea
Purple leaf coloured cultivars					
TRFK K-Purple	18.0[7.7](6.0)	12.76.0	8.06.0	23.0[7.3](6.0)	11.06.0
TRFK 306/3	17.0[7.0](7.7)	13.3[7.0](6.0)	12.07.0	17.0[7.3](8.3)	11.07.3
TRFK 73/1	13.37.0	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)	13.07.0	12.77.0	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)	10.76.0	15.07.3	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)	13.77.0	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)	14.37.2	18.7[7.5](17.5)	9.2[7.0](8.2)
Distilled water	6.06.0	6.06.0	6.06.0	6.06.0	6.06.0
Chloramphenicol (0.60µg/ml)	32.0[20](23)				
Antibiotics alone (µg ml⁻¹)					
Gentamicin 1.96		18.08.0			
Tetracycline 1.96			19.09.0		
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<0.05) = 0.24 [0.16] (0.22); Parentheses [x] = *E. coli*; brackets (x) = *S. typhi*

Antioxidant, antimicrobial and synergistic activities of tea

coloured clone. Black tea extracts gave the lowest inhibitory activity.

Synergistic effects of tea liquors and antibiotics

Methicillin and penicillinase resistant *Staphylococcus aureus* ATCC 25923. There was a marked increase in the inhibition zone diameters in tea extracts combined with penicillin G against methicillin and penicillinase resistant *S. aureus* ATCC 25923, except for black tea processed from clones TRFK 303/577 and TRFK 306/3 (Table 2).

***Escherichia coli* ATCC 25922.** A combination of black, green (Kenyan, Chinese or Japanese) tea extracts with gentamicin and tetracycline did not significantly differ ($P>0.05$) with tea extracts, gentamicin or tetracycline alone (Table 2). Synergism was only observed in black tea processed from the buds of clone TRFK 301/5 with penicillin G. Similarly, there was no significant difference in combinations of tea extracts with penicillin G and ampicillin.

Clinical isolate of *S. typhi*. There was a significant difference in the inhibitory effects of black tea extracts combined with gentamicin, compared with black tea extracts alone (Table 2). Thus, black tea extracts did not synergise with gentamicin. This was also exhibited by black tea buds and green tea from the Kenyan, Chinese and Japanese cultivars, except white tea. Black tea processed from terminal buds had no inhibitory effects even in combination with tetracycline, penicillin G and ampicillin. Green teas also did not differ significantly ($P>0.05$) when the tea extracts were combined with tetracycline compared to tea extracts alone.

DISCUSSION

Kenyan teas were rich in total polyphenols comparable with the teas processed from Chinese and Japanese germplasm. This is in agreement with results from previously reported studies by Wachira and Kamunya (2005) and Karori *et al.* (2007). The general trend among the samples assayed showed that non-aerated tea had higher total polyphenol content than aerated tea from the same sample.

The variation in the polyphenolic composition of the different tea products is ascribed to the different process methods applied particularly the leaf maceration and auto-oxidation steps during manufacturing. During black tea manufacture, the galliccatechins are first oxidised and dimerised to theaflavins and thearubigins because of their high oxidation potential and high concentration in leaves (Mahanta and Hemanta, 1992).

Several other factors have been discovered to influence the polyphenol content of a tea product. These include genotype, geographical origin, soil composition, harvesting time, post-harvest treatment and physical structure of the leaves (Lin *et al.*, 2003).

The total catechins content in white and green tea products were significantly higher than those of aerated tea products from the same clones. The findings of this study corroborated with those of Karori *et al.* (2007), who found that green teas had significantly higher catechin content than black teas. The enzymatic oxidation of catechins located in the vacuole is as a result of polymerisation of flavan-3-ol monomers to form TFs and TRs, which are compounds that have an influence on the quality of black tea (Owuor and Obanda, 2001). In this study, aerated tea products had lower amounts of individual catechins due to the formation of TFs and TRs.

There was high radical scavenging activity on DPPH by both the black and green teas. 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 stabilise 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 (Amie *et al.*, 2003). This explains why radical scavenging was high in the galliccatechins, including epigallocatechins gallate and epigallocatechin (Zhu *et al.*, 2001).

The results on the antibacterial and antifungal activity indicated that the green tea products, as well as tea from the purple leaf coloured (unaerated) cultivar, and white tea products processed from Kenyan tea cultivars, expressed 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 factor that contributed to inhibitory activity of both green, unfermented tea from the purple leaf coloured clone and white tea. This is in agreement with a study reported by Nance *et al.* (2006) 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.

The findings of this study also indicate 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 teas and also the type of test organism; bacteria or fungi. The conclusion by Taguri *et al.* (2006) that 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*.

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 of other researchers (Zhao *et al.*, 2001; Hu *et al.*, 2002) 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 (Tiwari *et al.*, 2005).

The tea extracts and penicillin G synergistically inhibited the growth of methicillin and penicillinase resistant *S. aureus* ATCC 25923 possibly because they attack the same site which is the peptidoglycan on the cell wall (Zhao *et al.*, 2001). 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.

CONCLUSION

Green and white Kenyan 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. Tea extracts can be used in management of bacterial and fungal infections caused by methicillin and penicillinase resistant *S. aureus* ATCC 25923, *C. albicans* and *C. neoformans*, respectively. The concomitant administration of tea extracts and antibiotics may not impair antibacterial activity of penicillin G.

ACKNOWLEDGEMENT

The Tea Research Foundation of Kenya (TRFK) funded this work. The Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) facilitated the publication of this paper.

REFERENCES

- Amie, D., Amie D.D., Beslo, D. and Trinajstie, N. 2003. Structure-radical scavenging activity relationships of flavonoids. *Croat Sica Chemica Acta* 76:55-61.
- Bandyopadhyay, D., Chatterjee, T.K., Dasgupta, A., Lourduraja, J. and Dastidar, S.G. 2005. *In vitro* and *in vivo* antimicrobial action of tea: The commonest beverage of Asia. *Biological and Pharmaceutical Bulletin* 28:2125-2127.
- Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie* 28:25-30.
- Hilton, P.J. and Palmer-Jones, R. 1973. Relationship between the flavanol composition of fresh tea shoots and theaflavin content of manufactured tea. *Journal of the Science of Food and Agriculture* 24:813-818.

- Hu, Z.Q., Zhao, W.H., Asano, N., Yoda, Y., Hara, Y. and Shimamura, T. 2002. Epigallocatechin gallate synergistically enhances the activity of carbapenems against methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 46:558-560.
- International Standard (ISO). 2005. Determination of substances characteristic of green and black tea - Part 1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent. 14502-1.
- Karori, S.M., Wachira, F.N., Wanyoko, J.K. and Ngunjiri, R.M. 2007. Antioxidant capacity of different types of tea products. *African Journal of Biotechnology* 6:2287-2296.
- Leung, L.K., Yakun, S., Chen, R., Zhang, Z., Hang, Y.U. and Chen, Z.Y. 2001. Theaflavins in black tea and catechins in green tea are equally effective in antioxidant activity. *Journal of Nutrition* 131:2248-2251.
- Lin, Y.S., Tsai, Y.J., Tsay, J.S. and Lin, J.K. 2003. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. *Journal of Agricultural and Food Chemistry* 51:1864-1873.
- Mahanta, P.K. and Hemanta, B.K. 1992. Theaflavins pigment formation and polyphenol oxidase activity as a criterion of fermentation in orthodox and CTC teas. *Journal of Agricultural and Food Chemistry* 40:860-863.
- McKay, D.L. and Blumberg, J.B. 2002. The role of tea in human health: an update. *Journal of the American College of Nutrition* 21(1):1-13.
- NCLSI. 2012. National Clinical and Laboratory Standards Institute. M100-S21, Performance standards for antimicrobial susceptibility testing; Twenty-First informational supplement Wayne, PA.
- Okubo, S., Toda, M., Hara, Y. and Shimamura, T. 1991. Antifungal and fungicidal activities of tea extract and catechin against *Trichophyton*. *Nippon Saikingaku Zasshi* 46:509-514.
- Owuor, P.O. and Obanda, M. 2001. The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential. *Food Chemistry* 100:873-884.
- Roberts, E.A.H. and Smith, R.F. 1961. Spectrophotometric measurements of theaflavins and thearubigins in black tea liquors in assessments of quality in teas. *Analyst (London)* 86:94-98.
- Taguri, T., Tanaka, T. and Kouno, I. 2006. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biological and Pharmaceutical Bulletin* 29:2226-2235.
- Tiwari, R.P., Bharti, S.K., Kaur, R.P., Dikshit, R.P. and Hoondal, G.S. 2005. Synergistic antimicrobial activity of tea and antibiotics. *Indian Journal of Medical Research* 122:80-84.
- Turkmen, E.N., Sari, F., Polat, G. and Velioglu, Y.S. 2009. Antioxidant and antibacterial activities of various extracts and fractions of fresh tea leaves and green tea. *Tarim Bilimleri Dergisi* 15:371-378.
- Wachira, F.N. and Kamunya, S.M. 2005. Kenyan teas are rich in antioxidants. *Tea* 26:81-89.
- Yam, T.S., Shah, S. and Hamilton-Miller, J.M.T. 1997. Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components. *FEMS Microbiology Letters* 152:169-174.
- Zhao, W.H., Hu, Z.Q., Okubo, S., Hara, Y. and Shimamura T. 2001. Mechanism of synergy between epigallocatechin gallate and β -lactams against methicillin resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 45:1737-1742.
- Zhu, N., Wang, M., Wei, G.N., Lin, J., Yang, S.C. and Ho, I. 2001. Identification of reaction products of epigallocatechin, epigallocatechin gallate and pyrogallol with 2,2-diphenyl-1-picrylhydrazyl radical. *Food Chemistry* 73:345-349.
- Zuo, Y., Chen, H. and Deng, Y. 2002. Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and puerh teas using HPLC with a photodiode array detector. *Talanta* 57:307-316.