Determination of Epigallocatechin Gallate (EGCG) and Antibacterial Activities of Commercially Available Tanzanian Green Tea (*Camellia sinensis*)

Raphael J Shedafa[*1,3], **Joseph Sempombe**[1], **Ramadhan SO Nondo**[2], **Eliangiringa Kaale**[1,3], **Mary Temu**[4] **and Peter Imming**[5]

[1]*Department of Medicinal Chemistry, Muhimbili University of Health and Allied Sciences, Tanzania P.O. Box 65001, Dar es Salaam, Tanzania.*

[2]*Department of Biological and Pre-Clinical Studies, Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.*

[3]*Pharm R&D Laboratory, School of Pharmacy, Muhimbili University of Health and Allied Sciences P.O. Box 65001, Dar es Salaam, Tanzania.*

[4]*Department of Pharmaceutics, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.*

[5]*Martin Luther University Halle-Wittenberg, Kurt-Mothes-Strasse 3, 06120 Halle (Saale), Germany.*

*Corresponding author, e-mail: raphael.shedafa@gmail.com

Abstract

This paper presents the results of the antibacterial activity of green tea extract (*Camellia sinensis*) against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Klebsiella pneumoniae* (ATCC 708903). Quantitative and qualitative measurements of epigallocatechin gallate (EGCG) have also been reported. The analysis by High Performance Thin Layer Chromatography (HPTLC) revealed the presence of epigallocatechin gallate (EGCG) in the green tea extracts ranging from 13.16 to 18.64 mg/g. Crude extracts GT 01–GT 03 inhibited a greater number of microorganisms and presented the lowest values of MIC against *Staphylococcus aureus* (0.625 mg/mL), *Escherichia coli* (0.625 mg/mL), and *Klebsiella pneumoniae* (1.25 mg/mL). Crude extract GT 04 presented the highest values of MIC against *Staphylococcus aureus* (1.25 mg/mL), *Escherichia coli* (1.25 mg/mL), and *Klebsiella pneumoniae* (2.5 mg/mL). This study demonstrates that the popular use of green tea can be an effective and sustainable alternative for the prevention and treatment of various bacterial infections.

Keywords: *Camellia sinensis*, Antibacterial, Epigallocatechin gallate, HPTLC.

Introduction

Infectious diseases are leading causes of death worldwide and heavy burdens on developing countries (Boutayeb and Boutayeb 2005, Boutayeb 2006, Michaud 2009). Despite novel technologies and the existence of a wide range of antimicrobial agents, these diseases remain a public health challenge in many countries. Contributing to this is the emergence of multidrug-resistant pathogens for a variety of reasons, including poor healthcare facilities, abuse, and availability of antimicrobial drugs over the counter (Vincent et al. 2009). In response, the

413

World Health Organization (WHO) has accelerated its search for new antibacterial compounds that could be developed into new drugs to address this problem, and one avenue for finding these compounds is natural sources (Pandey and Kumar 2013).

Plant derived medicines are effective against multidrug-resistant pathogens, are relatively safer than conventional medicines, and offer profound therapeutic effects and cheaper treatments (Subramani et al. 2017). Plant extracts, including those derived from green tea, have been shown to exhibit inhibitory effects on the growth of pathogenic bacteria and thus can be considered for the development of new effective antibacterial agents (Ndhlala et al. 2011, Chanda et al. 2016).

Botanically, there are two major types of tea plants, Camellia sinensis; the Assam variety (*Camellia sinensis* var. *assamica*) and the China variety (*Camellia sinensis* var. *sinensis* (Wachira et al. 2013). However, teas made from these plants are classified mainly by the processing method. For example, green tea, oolong tea, and black tea are three major types of teas named based on the extent of fermentation (Chaturvedula and Prakash 2011). The tea manufacturing process involves three basic steps called withering, fixing, and rolling. During the withering process, the moisture content of the tea leaves decreases and the aroma components develop. Fixation is the process by which the enzymatic browning of dead leaves is controlled by the application of heat. In the production of green tea, the effect of heat prevents the tea leaves from fermenting. With no fermentation, green tea leaves retain their green colour and almost all their original polyphenol content. White tea is minimally processed and is extracted from the unopened bud or first bud of the plant. Oolong tea is semi-fermented and black tea is fully fermented (Liu et al. 2021). The different processes of manufacturing give the various teas their characteristic colours and flavours.

Green tea extract from *C. sinensis* leaves has been shown to have broad antibacterial activities due to its high content of catechins, especially epigallocatechin gallate (EGCG)

(Song and Seong 2007). Several studies have found that it inhibits various pathogens (Nikoo et al. 2018, Reygaert 2018). In addition, green tea has been shown to have protective effects against cancer and cardiovascular diseases (Zaveri et al. 2006, Cross et al. 2011). EGCG has been shown to have multiple effects on human pathological and physiological processes, with different mechanisms in cancer, vascularity, bone regeneration, and the nervous system (Chu et al. 2017).

However, in Tanzania, information on the amounts of EGCG, the most abundant catechins, and the antibacterial effects of green tea are limited. Therefore, this is the first study aimed at evaluating the antibacterial activities of crude extract of Tanzanian green tea *C. sinensis* against *Staphylococcus aureus, Escherichia coli*, and *Klebsiella pneumoniae*. The study also reports the qualitative and quantitative determination of Epigallocatechin gallate (EGCG) in *C. sinensis* green tea.

Materials and Methods
Chemicals and reagents

The following chemicals were used; Epigallocatechin gallate (EGCG) (purity ≥98%) from Loba Chemie PVT Ltd, Mumbai, Maharashtra, India. HPTLC reagents, methanol, formic acid, sulphuric acid, acetone, and toluene (Carlo Erba, Sabadell, Barcelona, Spain). Anisaldehyde was purchased from Sigma Chemical Co. Fairfield, OH, USA and 2-aminoethyl diphenylborinate was obtained from Merck, Darmstadt Germany. Mueller Hinton broth (MHB) (Sigma-Aldrich (Merck, Darmstadt, Germany), Nutrient agar (Techno Pharmchem, New Delhi, India), Para-Iodonitrotetrazolium (INT) chloride salt (Merck, Darmstadt, Germany) and sterile distilled water were used in all analyses.

Sample collection and extraction

Four commercial brands of green tea were purchased from a supermarket in Dar es Salaam City, Tanzania. Ten grams (10 g) portions of each sample were soaked in 100 mL (1:10 w/v) of hot water at about 100 ℃

for 20 minutes. The filtrates were evaporated into a water bath to a solid mass and air-dried. These were stored in clean labelled containers and kept in the refrigerator at 4 ℃ until use. The extracts were reconstituted using sterile distilled water for antibacterial assays.

Preparation of standard solutions and derivatizing reagents
Derivatizing reagent
2-aminoethyl diphenyborinate-(Natural Products (NP)) spraying reagent was prepared by dissolving 1 g of 2-aminoethyl diphenylborinate in 100 mL of methanol. Ninhydrin solution (0.2 %) was prepared by dissolving 0.2 g of the Ninhydrin into 100 mL of methanol.

Standard calibration curve
The standard curve for epigallocatechin gallate (EGCG) was obtained by using the peak areas of five different concentrations; 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, and 25 mg/mL obtained by dissolving EGCG reference standard in methanol-water (70:30 v/v).

Preparation of test solutions
Tea solutions were prepared by placing 10 g of powdered green tea leaves in a 100 mL flask and soaking them in 100 mL in hot distilled water at 100 °C for 15, 20, 30, 45, and 60 minutes intervals. The mixture was sonicated for 10 minutes, where 2 µL of the clear solution was applied to the HPTLC plate pre-coated with silica gel 60_{F254} (20 cm × 10 cm). Application positions were set at 20 mm from the sides and 8 mm from the bottom of the plates, the distance between the tracks was set at 19.4 mm and the band length was set at 8 mm.

HPTLC analysis
HPTLC analysis was performed using the method described by Reich et al. (2006) with minor modifications. All samples were analyzed on a CAMAG HPTLC system equipped with Linomat V for sample applicator ions and digital visualizer, a CAMAG TLC scanner 4, and Vision Cats software ver 2.5 for data acquisition CAMAG (Muttenz, Switzerland). HPTLC (Silica 60 F_{254}) was used as a stationary phase while detection was performed at visible light, UV 280 nm before and after derivatization. The mobile phase consisted of toluene-acetone-formic acid 45:45:10 (v/v/v), and images of the HPTLC densitograms were transformed into the dataset by using Vision Cats software.

Test microorganisms
Staphylococcus aureus (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Klebsiella pneumoniae* (ATCC 708903) strains from the Department of Pharmaceutical Microbiology at Muhimbili University of Health and Allied Sciences (MUHAS) were used for this study.

Determination of the minimum inhibitory concentrations
The minimum inhibitory concentrations (MICs) were determined by broth microdilution assay as described in the National Committee for Clinical Laboratory Standards (NCCLS) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines (Kiehlbauch et al. 2000, Kahlmeter et al. 2006).

Mueller Hinton broth (MHB) was used as the bacterial growth medium in the extracts and test controls. Ciprofloxacin at a concentration range of 0.0025 to 1 mg/mL was used as positive control drug (Al-Wahaibi et al. 2021, Chalkley and Koornhof 1985, Colombo et al. 2002, Garipov et al. 2017). The extracts were tested across a concentration range of 0.098 to 2.5 mg/mL and the standard EGCG ranged from 0.098 to 1.25 mg/mL (Anand et al. 2015, Parvez et al. 2019). Before setting for MICs determination, the test bacteria were reactivated by growing them for 24 hours on a fresh nutrient agar medium.

For the determination of MIC, 100 µL of autoclave-sterilized MHB was added into all wells of microtitre plates using a multi-channel micropipette. Subsequently, 100 µL of crude extracts at the concentration of 5.0 mg/mL were added to the first wells of the

plates and mixed to make total volumes of 200 µL in each well. From such wells, 100 µL were drawn after mixing thoroughly and added to the wells in the next row. The process continued down to the wells in the last row to constitute the 2-fold serial micro dilutions whereby the final 100 µL were discarded. About 0.5 McFarland-equivalent (approximately 1×10^8 CFU/mL) suspensions of the respective bacteria in normal saline were prepared by adjustments of turbidity to that of the 0.5 McFarland turbidity standard. The resulting suspensions were diluted to approximately 1×10^6 CFU/mL by mixing 0.1 mL of the bacterial suspensions with 9.9 mL of MHB. About 100 µL of the final suspensions were then added to each test and control well of the microtitre plates to give a final volume of 200 µL in each well.

The plates were then incubated at 37 ℃ for 24 hours after which they were observed for inhibition of bacterial growth. Detection of growth inhibition was by observing the colour changes after the addition of a solution of para-iodonitrotetrazolium (INT) chloride salt indicator, whereby 30 µL of 0.4 mg/mL INT were added into the wells followed by re-incubation at 37 ℃ for 30 minutes. The formation of purple or pink colour signified the presence of actively growing bacteria as opposed to inhibited growth in which the indicator remained colourless. The lowest extracts' concentrations showing complete inhibition of bacterial growth (no colour change) were taken as the MICs.

Data analysis and statistical analysis

All experiments were performed in triplicate. The EGCG contents and the MIC values presented in the results were expressed as the mean and standard deviation (mean ± SD). Statistically significant difference among groups was determined by using a One-Way Analysis of Variance (ANOVA).

Results

Antibacterial activities of *C. sinensis* (green tea) crude extract

This study investigated the inhibitory activity of aqueous extract of green tea against the growth of a variety of common human pathogenic bacteria. The antibacterial activity was evaluated at concentrations of 0.098 to 2.5 mg/mL by the microdilution method. Results showed that all bacteria tested were sensitive to green tea extract. The GT 01, GT 02, and GT 03 crude extracts were the most active against *S. aureus* and *E. coli* with a MIC value of 0.625 mg/mL. Among the extracts tested, GT 04 was the least active against all bacterial strains with MIC values of 1.25 mg/mL for *S. aureus* and *E. coli* and MIC value of 2.5 mg/mL against *K. pneumoniae*. In addition, all four crude extracts exhibited weak activities against *K. pneumoniae* with a MIC value of 1.25 mg/mL and above (Table 1). Except for *K. pneumoniae*, the results showed that both gram-negative and gram-positive bacteria were equally sensitive to tea extract.

Overall results showed that the crude extract was less active compared to the pure EGCG compound and the standard drug ciprofloxacin. The EGCG inhibited the growth of *S. aureus*, *E. coli,* and *K. pneumoniae* with MIC values of 0.3125 mg/mL, while ciprofloxacin exhibited MIC values of 0.0039 mg/mL, 0.0078 mg/mL, and 0.0625 mg/mL against *K. pneumoniae, E. coli,* and *S. aureus,* respectively (Table 1).

Table 1: Antibacterial effects of green tea *(C. sinensis)* crude extracts

	MIC (mg/mL)		
Extract	*S. aureus* ATCC 29213	*E.coli* A ATCC 25922	*K. Pnemoniae* ATCC 708903
GT 01	0.625	0.625	1.25
GT 02	0.625	0.625	1.25
GT 03	0.625	0.625	1.25
GT 04	1.25	1.25	2.5
Ciprofloxacin (standard drug)	0.0625	0.0078	0.0039
EGCG	0.3125	0.3125	0.3125

MIC = Minimum inhibitory concentration, GT 01 = Kilimanjaro green tea, GT 02 = Chai bora green tea, GT 03 = Kazi yetu green tea, GT 04 = Tropical Usambara green tea.

HPTLC analysis
Qualitative and quantitative evaluation of epigallocatechin gallate (EGCG) by HPTLC

The amount of EGCG in green tea was quantified by using HPTLC at a wavelength of 280 nm and the results are presented in Table 2. It was observed that there was no significant difference ($p > 0.05$) in the concentrations of EGCG among the tested teas at a given time interval (Table 2). Qualitatively EGCG was detected under white light as a brown quenching band at Rf 0.45 from all the studied green teas (Figure 1). Figure 2 shows the densitogram comparisons of the samples with that of the reference standard.

In this study, the hot water extraction method was applied since it is a simple way of brewing tea. As depicted in Table 1, the extraction of green tea using hot water gave a similar amount of EGCG for all the tested time points.
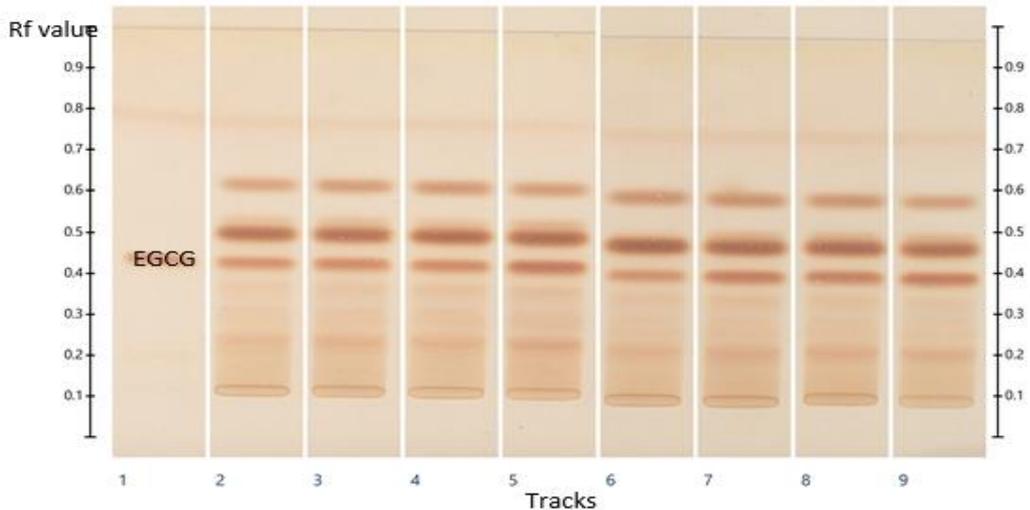

Figure 1: HPTLC Fingerprints of EGCG in green teas at 280 nm for 1-Reference, 2 -GT 01, 3-GT 02, 4-GT 03, 5 -GT 04, 6-GT 01, 7-GT 02, 8-GT 03 and 9-GT 04.
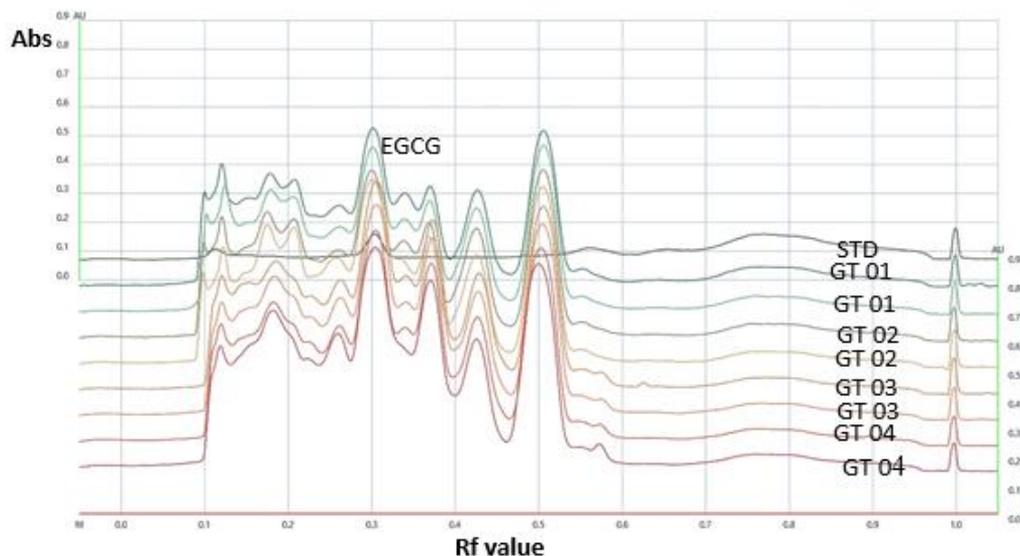
Figure 2: HPTLC densintogram showing the Rf of EGCG in all green tea samples and standard at 280 nm.

Table 2: Comparisons of the EGCG contents in aqueous extracts at different time intervals (n = 3)

Extract	EGCG mean content (mg/g)				
	15 mins	20 mins	30 mins	45 mins	60 mins
GT 01	14.56 ± 1.11	15.92 ± 1.44	14.38 ± 1.11	14.78 ± 1.12	14.00 ± 1.05
GT 02	16.12 ± 0.68	16.67 ± 1.36	16.51 ± 1.16	16.47 ± 1.33	16.44 ± 1.22
GT 03	15.83 ± 0.45	17.09 ± 1.11	16.92 ± 1.28	16.86 ± 1.05	$16.72 \pm .0.88$
GT 04	18.64 ± 1.03	18.35 ± 1.03	17.94 ± 1.23	17.90 ± 1.12	16.96 ± 1.02

The values are presented as mean $\pm$ standard deviation, n = the number of replicates.

Discussion
Antibacterial activities

Green tea has been reported to be the only food product containing epigallocatechin gallate (EGCG) (Chu et al. 2017). Due to its high content of EGCG, green tea is used as a chemopreventive agent for various cancers such as liver, stomach, prostate, colon, oesophagus, bladder, pancreas, skin, and lung (Du et al. 2012, Reygaert 2018).

Previous pharmacological studies have reported that EGCG has antibacterial properties against various strains (Lee et al. 2017, Nikoo et al. 2018). The antibacterial activities shown in this study may be attributed to the presence of EGCG.

In this study, green tea aqueous extract was shown to exhibit significant antibacterial activities against various pathogenic bacteria. However, the degree of the inhibitory effects differs depending on the strain which is consistent with several previous reports (Si et al. 2006). *S. aureus*, gram-positive bacteria was highly susceptible to green tea extract. Among the gram-negative bacteria, *E. coli* was found to be more sensitive than *K. pneumoniae.* Regarding the inhibitory spectrum, the results are in line with several previous studies based on green tea (Chan et al. 2011). The differences in the antibacterial inhibition effect of tea can be attributed to variations in their chemical constituents arising from differences in their geographical locations/origins (Umashankar et al. 2018, Parvez et al. 2019).

Effects of brewing time on the content of EGCG

The amount of EGCG in tea products depends primarily on the variety of the plant, cultivation conditions, harvesting time, brewing temperature, and manufacturing process (Saklar et al. 2015, Wei et al. 2019). In this study, the hot water extraction method was applied because it is the most common way of brewing tea in Tanzania.

Varying the time was important to predict the maximum brewing time required for EGCG extraction. Brewing time beyond 20 minutes did not increase the EGCG extracted. However water prolonging extraction time may expose the EGCG to oxidative degradation (Sang et al. 2005, Wang et al. 2008). The EGCG concentration was observed to have decreased at 30–45 minutes.

Conclusion

Green tea showed strong inhibitory effects against Gram-positive and Gram-negative bacteria. The results on the antibacterial effects of Tanzanian green tea have shown that the potential for preventive and therapeutic purposes is present. The aqueous extraction of EGCG compound from GT 01, GT 02, GT 03, and GT 04 was influenced by time and it was found that the higher EGCG content was extracted at a shorter time of less than 20 minutes, hence brewing tea within 20 minutes is recommended.

Acknowledgment

Conflict of Interest
The authors declare no conflict of interest.

References

Al-Wahaibi LH, Amer AA, Marzouk AA, Gomaa HAM, Youssif BGM and Abdelhamid AA 2021 Design, synthesis, and antibacterial screening of some novel heteroaryl-based ciprofloxacin derivatives as DNA gyrase and topoisomerase iv inhibitors. *Pharmaceutials* 14(5): 399.

Anand J, Gautam P and Rai N 2015 Comparative study of antibacterial and anti-proliferative potential of green tea from different geographical locations in India. *Asian J. Pharm. Clin. Res*. 8(1): 253-258.

Boutayeb A 2006 The double burden of communicable and non-communicable diseases in developing countries. *Trans. R. Soc. Trop. Med. Hyg.*100(3): 191-199.

Boutayeb A and Boutayeb S 2005 The burden of non communicable diseases in developing countries. *Int. J. Equity Health* 4(1): 2.

Chalkley LJ and Koornhof HJ 1985 Antimicrobial activity of ciprofloxacin against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* determined by the killing curve method: Antibiotic comparisons and synergistic interactions. *Antimicrob. Agents Chemother*. 28(2): 331–342.

Chan EW, Soh EY, Tie PP and Law YP 2011 Antioxidant and antibacterial properties of green, black, and herbal teas of *Camellia sinensis*. *Pharmacognosy Res*. 3(4): 266-272.

Chanda S, Padalia H and Trivedi A 2016 Antimicrobial activity of some medicinal plant extracts and its synergistic interaction with some antibiotics. *J. Pharm. Res*. 1010(5): 211-220.

Chaturvedula VSP and Prakash I 2011 The aroma, taste, color and bioactive constituents of tea. *J. Med. Plants Res.* 5(11): 2110-2124.

Chu C, Deng J, Man Y and Qu Y 2017 Green tea extracts epigallocatechin-3-gallate for different treatments. *Biomed Res. Int* 2017: 5615647.

Colombo AL, Da Matta D, De Almeida LP and Rosas R 2002 Fluconazole susceptibility of Brazilian candida isolates

assessed by a disk diffusion method. *Braz. J. Infect. Dis.* 6(3): 118–123.

Cross SE, Jin YS, Lu QY, Rao J and Gimzewski JK 2011 Green tea extract selectively targets nanomechanics of live metastatic cancer cells. *Nanotechnol.* 22(21): 215101.

Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS and Wang CZ 2012 Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients* 4(11): 1679-1691.

Garipov MR, Pavelyev RS, Lisovskaya SA, Nikitina EV, Kayumov AR., Sabirova AE, Bondar OV, Malanyeva AG, Aimaletdinov AM, Iksanova AG, Balakin KV and Shtyrlin, YG 2017 Fluconazole-pyridoxine bis-triazolium compounds with potent activity against pathogenic bacteria and fungi including their biofilm-embedded forms. *J. Chem.* 2017: e4761650.

Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, Rodloff A, Soussy CJ, Steinbakk M, Soriano F and Stetsiouk O 2006 European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* 12(6): 501-503.

Kiehlbauch JA, Hannett GE, Salfinger M, Archinal W, Monserrat C, Carlyn C 2000 Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in New York State Laboratories. *J Clin Microbiol.* 38(9): 3341-3348.

Lee S, Razqan GS and Kwon DH 2017 Antibacterial activity of epigallocatechin-3-gallate (EGCG) and its synergism with β-lactam antibiotics sensitizing carbapenem-associated multidrug resistant clinical isolates of *Acinetobacter baumannii. Phytomedicine* 24: 49–55.

Liu SL, Jaw YM, Wang LF, Chuang GC, Zhuang ZY, Chen YS and Liou BK 2021 Evaluation of sensory quality for taiwanese specialty teas with cold infusion using cata and temporal cata by taiwanese consumers. *Foods* 10(10): 2344.

Michaud CM 2009 Global burden of infectious diseases. In: *Encycl. Microbiol* (pp. 444–454), Elsevier.

Ndhlala AR, Finnie JF and Van Staden J 2011 Plant composition, pharmacological properties and mutagenic evaluation of a commercial Zulu herbal mixture. *J. Ethnopharmacol.* 133(2): 663-674.

Nikoo M, Regenstein JM and Ahmadi Gavlighi H 2018 Antioxidant and antimicrobial activities of (-)-epigallocatechin-3-gallate (EGCG) and its potential to preserve the quality and safety of foods. *Compr. Rev. Food Sci. Food Saf.* 7(3): 732-753.

Pandey AK and Kumar S 2013 Perspective on plant products as antimicrobials agents: A Review. *Pharmacologia.* 4(7): 469-480.

Parvez MAK, Saha K, Rahman J, Munmun RA, Rahman MA, Dey SK, Rahman MS, Islam S and Shariare MH 2019 Antibacterial activities of green tea crude extracts and synergistic effects of epigallocatechingallate (EGCG) with gentamicin against MDR pathogens. *Heliyon* 5(7): e02126.

Reich E, Schibli A, Widmer V, Jorns R, Wolfram E and DeBatt A 2006 HPTLC methods for identification of green tea and green tea extract. *J. Liq. Chromatogr. Relat. Technol.* 29(14): 2141-2151.

Reygaert WC 2018 Green tea catechins: Their use in treating and preventing infectious diseases. *Biomed Res. Int.* 10: e 9105261.

Saklar S, Ertas E, Ozdemir IS and Karadeniz B 2015 Effects of different brewing conditions on catechin content and sensory acceptance in Turkish green tea infusions. *J. Food Sci. Technol.* 52(10): 6639-6646.

Sang S, Lee MJ, Hou Z, Ho CT and Yang CS 2005 Stability of tea polyphenol (-)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. *J. Agric. Food Chem.* 53(24): 9478-9484.

Si W, Gong J, Tsao R, Kalab M, Yang R and

Yin Y 2006 Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. *J. Chromatogr. A*. 1125(2): 204-210.

Song JM and Seong BL 2007 Tea catechins as a potential alternative anti-infectious agent. *Expert Rev. Anti. Infect. Ther*. 5(3): 497-506.

Subramani R, Narayanasamy M, Feussner KD 2017 Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. *Biotech*. 7(3): 172

Umashankar N, Pemmanda B, Gopkumar P, Hemalatha AJ, Sundar PK and Prashanth HV 2018 Effectiveness of topical green tea against multidrug-resistant Staphylococcus aureus in cases of primary pyoderma: An open controlled trial. *Indian J. Dermatol. Venereol. Leprol*. 84(2): 163-168.

Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y and Reinhart K 2009 International study of the prevalence and outcomes of infection in intensive care units. *J. Am. Med. Assoc*. 302(21): 2323-2329.

Wachira FN, Kamunya S, Karori S, Chalo R and Maritim T 2013 The tea plants: botanical aspects. In *Tea Heal. Dis. Prev* (pp 3–17), Elsevier.

Wang R, Zhou W and Jiang X 2008 Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. *J. Agric. Food Chem*. 56(8): 2694-2701.

Wei K, Ruan L, Li H, Wu L, Wang L, and Cheng H 2019 Estimation of the effects of major chemical components on the taste quality of green tea. *Int. Food Res. J*. 26(3): 869-876.

Zaveri NT 2006 Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sci*. 78: 2073-2080.