

Antimicrobial potential of aqueous extract of *Camellia sinensis* against representative microbes

Ishrat Khan^{1,2}, Tanveer Abbas^{1,2}, Komal Anjum^{1,2,3}, Syed Qamar Abbas⁴, Bibi Ibtesam Shagufta⁵, Sayed Asmat Ali Shah³, Najeeb Akhter³ and Syed Shams ul Hassan^{3,4*}

¹Food Safety Research Group, Lab # 115, Department of Microbiology, Faculty of Science, University of Karachi, Karachi, Pakistan

²Department of Microbiology, Faculty of Science, University of Karachi, Karachi, Pakistan

³Ocean College, Zhejiang University, Hangzhou, China

⁴Gomal University, DI Khan, KPK, Pakistan

⁵Department of Zoology, Kohat University of Science and Technology, KPK, Pakistan

Abstract: *Camellia sinensis* is being used for decades for its therapeutic efficacies against physiological problems and microbial infections. This study was undertaken to investigate the antibacterial and antifungal potential of aqueous extract of *Camellia sinensis*. Antibacterial activity was determined by disc and well diffusion assay. MIC and MBC were calculated by broth dilution method. Miles and Misra technique was used to find out colony forming unit per/ml. All the test organisms revealed a diverse range of vulnerability against aqueous extract. Among Gram positive, MRSA showed to be the most sensitive with least MIC and MBC while among Gram-negative *Pseudomonas aeruginosa* exhibited the highest sensitivity. In Miles and Misra, a progressive decline in log of CFU/ml was observed. In time-kill assay, a decline was noted in the viable count of *S.aureus* after exposure to 18% aqueous extract of *Camellia sinensis*. In the present study aqueous extract of *Camellia sinensis* found to be effective against Gram positive, Gram negative and fungi. The most important finding of this study is its aqueous extract inhibitory effect against drug-resistant microorganisms e.g. MRSA and *P. aeruginosa* and *Candida albicans*.

Keywords: *Camellia sinensis*, drug-resistant bacteria, crude extract, MIC and MBC, Miles and Misra.

INTRODUCTION

Human history has witnessed the use of natural products for curative purposes from ancient times (Majid *et al.*, 2015) According to a WHO report, natural products are being used in medicine all over the world, including the developed countries; as per Anjum *et al.*, (2016). *Camellia sinensis* has anti-inflammatory (Roccaro *et al.*, 2004), antibacterial (Abdel-Tawwab *et al.*, 2010; Weber *et al.*, 2003), antiviral (Weber *et al.*, 2003), antioxidant (Osada *et al.*, 2001), anticancer (Sartippour *et al.*, 2002), antiangiogenic (Weinreb *et al.*, 2004) and neuroprotective activities (Weinreb *et al.*, 2004).

Polyphenols constitute important components of *Camellia sinensis* such as flavanols, flavandiols, flavonoids, and phenolic acids. These phenols constitute about 30% of the dry weight of *Camellia sinensis*. Major phenols in *Camellia sinensis* are known as catechins (Sano *et al.*, 2001). The *Camellia sinensis* catechins contain epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate EGCG (Khokhar and Magnusdottir 2002; Raederstorff *et al.*, 2003). The antibacterial activity of *Camellia sinensis* has extensively studied and the aqueous extract has been found to be quite effective against various pathogens, including *Bacillus subtilis*, *Brevibacterium linens*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus epidermidis* (Padmini *et al.*, 2010). However,

Staphylococcus aureus and *Pseudomonas aeruginosa* are involved in many serious infections. According to recent studies, *Camellia sinensis* is reported active against methicillin-resistant *S. aureus* (MRSA) (Bauman 2010; Jones *et al.*, 2006; Hassan and Sheikh 2017). The *Camellia sinensis* polyphenol, EGCG has antibacterial activity against clinically important multi-resistant strains of *Acinetobacter baumannii* (Hara 2001).

Similarly, antifungal activity of *Camellia sinensis* and its components has also been studied and accordingly found to be effective against purified component catechin upto concentration of 2000mg/ml and pH dependent (Yoda 2004). *Candida albicans* is an opportunistic pathogen and it constitute normal flora of human G.I.T and vaginal tract and oral cavity (Hassan *et al.*, 2017). Constantly increasing resistance trend of microorganisms towards antibiotics and antifungal agents has leads us to focus more towards the search of new natural products having unique efficacy against infectious agents. The aim of this study is to investigate the antibacterial and antifungal potential of aqueous extract of *Camellia sinensis* (Green tea).

MATERIALS AND METHODS

Plant collection and identification

Camellia sinensis used in this study was purchased commercially and identified by College of Biological Sciences, Zhejiang University, Hangzhou, China.

*Corresponding author: e-mail: shams1327@yahoo.com

Preparation of aqueous extract of camellia sinensis

Aqueous extracts of *Camellia sinensis* were prepared according to a defined protocol with slight modification. According to required concentration, dried leaves of (100 gm) of *Camellia sinensis* were added in 100ml boiled distilled water, in order to prepare 100% aqueous extract. Using a pre-warm thermos flask (100°C), dried and the measured leaves were infused and shaken for 10 minutes. Then macerated to prepare its aqueous extract. After cooling at room temperature, the extract was first passed through cotton and then filtered through 0.22µm pore size membrane filter. The fresh extract was stored at 4°C until use (Spiro 1995).

Chemicals, glassware and media

Glassware was provided by Wertlab (Germany) and Borosil (Andheri East, Mumbai, India). The chemical used in this study such as methanol and salts (KH₂PO₄, Na₂HPO₄, NaCl) were provided by Scharlau (Spain). Different microbiological media (Nutrient Agar, MHA, Nutrient broth, BHI broth, MacConkeys agar) were used. All microbiological media were provided by Oxoid (UK).

Microbial isolates

In this study clinical isolates (both Gram positive and Gram negative) were used. Apart from that two ATCC strains and one CMCC strain were also used including *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922 and *Bacillus subtilis* [CMCC (B) 63501]. Clinical isolates, *K. pneumoniae*, *M. luteus*, *S. epidermidis*, *S. pyogenes*, *Salmonella typhi* and *P. aeruginosa* were provided by Department of Microbiology, University of Karachi, (Pakistan). *Candida albicans* was received from Agha Khan Hospital Karachi, Pakistan. ATCC and CMCC cultures of *S. aureus* ATCC 43300, *E. coli* ATCC 25922 and *Bacillus subtilis* [CMCC (B) 63501] were bought from ATCC and CMCC official web portal. Microorganisms were maintained on nutrient agar (NA), potato dextrose agar (PDA) slants and petri plates at 4°C. For assuring the purity of cultures, Gram staining was also done.

Extract susceptibility testing

Disc diffusion, well diffusion and spot methods were applied to monitor the sensitivity of the clinical isolates against aqueous extract of *Camellia sinensis*.

Disc diffusion method

This procedure was applied using pour plate technique using Muller Hinton Agar (MHA). The Inoculum of the test culture was measured for its turbidity by matching with 0.5 McFarland standards. Discs (for that purpose) were purchased commercially. Aqueous extract of *Camellia sinensis* of different percentages (20% to 40% for Gram positive and 35% to 60% for Gram negative) were used and the amount of the sample in each disc was

kept at 8µL. The plates were incubated for 24 hours at 37°C (Mbata *et al.*, 2008).

Well diffusion method

This method was followed using pour plate technique using Muller Hinton Agar (MHA). In this method, different percentages of aqueous extract (20% to 40% for Gram positive and 35% to 60% for Gram negative) of *Camellia sinensis* were used. The inocula of test cultures were maintained by matching the turbidity with 0.5 McFarland index. The diameter of the well was kept constant throughout the experiments (8mm) and the amount of the sample added in each well was 100µL. Plates were incubated at 37°C for 24 hours (Mbata *et al.*, 2008).

Spot method

Spot method was used to study antibacterial and antifungal activity of aqueous crude extract. Different percentages of aqueous extract were tested against two ATCC cultures including *E. coli* 25922, *S. aureus* 43300, one CMCC *Bacillus subtilis* 63501 and clinical isolate *Candida albicans*. The test inoculums were set by matching its turbidity with 0.5 McFarland. The volume of sample was kept constant i.e. 10µL. All the plates were incubated at 37°C for 24 hours.

MIC and MBC for aqueous extract of camellia sinensis

According to CLSI (Clinical and Laboratory Standards Institute), Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the aqueous extract of *Camellia sinensis* were ascertained by broth dilution method. In this method, the final volume of the tubes was kept constant throughout the experiments. The amount of culture added in each tube was 100µL. For MIC, tubes were incubated at 37°C for 24 hours. For MIC and MBC determination, streaking was done from all inoculated tubes on the nutrient agar plate, as MIC and MBC values are impossible to read with brown coloration and plates were incubated at 37°C for 24 hours. Then the presence and absence of growth was checked after an overnight (Sharma *et al.*, 2012).

Miles and misra technique

Miles and Misra technique was followed to determine the number of colonies forming units (CFU) of bacterial suspension in the presence of different concentrations of the aqueous extracts of *Camellia sinensis* (Anjum *et al.*, 2015). By using McFarland index 0.5, suspensions of the test organisms were prepared in PBS buffer (pH7) and the inoculum of cultures was set at 1.5×10⁸ cells/ml. The nutrient broth was used to form different concentrations of the aqueous extract of *Camellia sinensis*. The final volume of the tubes was kept constant throughout the experiment i.e. 1ml. Each tube was further diluted 10 fold in nutrient broth up to 10⁻⁶.

The amount of culture added in different concentration tubes was 10µL. In this method, nutrient agar was used. For each concentration, 10µL was inoculated on nutrient agar plate from every dilution tube. Nutrient agar plates were incubated at 37°C for 24 hours.

Time-kill assay

Time-kill assay was performed using *S.aureus* to determine (at which) time of growth, the test compound (aqueous extract of *Camellia sinensis*) exerts its killing effect. This experiment was performed by inoculating test organism in nutrient broth. After 24 hours of incubation at 37°C, broth culture was 10 fold diluted. The diluted test culture was inoculated in nutrient broth in the presence of 18% of an aqueous extract of *green tea* and further diluted up to 10⁻⁶ dilution. A 10µl volume was drawn from each dilution to inoculate on MHA plate at different time interval i.e.; at 0, 30, 60, 120, 240, and 1440 minutes of growth. The plates were incubated at 37°C for 24 hours. Control set was also run in which all the steps were same but the test compound was not added (referred as growth curve of *S. aureus*).

RESULTS

Camellia sinensis has effective antimicrobial activity against several Gram-positive and Gram-negative clinical isolates (Padmini et al., 2010). Antibacterial activity of *Camellia sinensis* against several pathogens had been checked (Betts et al., 2012), *Salmonella typhimurium* (Tiwari et al., 2005), *Acinetobacter baumannii* (Osterburg et al., 2009), *Escherichia coli* 0157 (Perumalla and Hettiarachchy 2011), methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee et al., 2013), *Shigella dysenteriae* and *Yersinia enterocolitica* (Tiwari et al., 2005). Similarly, several researchers have analyzed the antibacterial activity of *Camellia sinensis* by using purified polyphenols (Cui et al., 2012; Steinmann et al., 2013; Osterburg et al., 2009). In the present study *Camellia Sinensis* was used in the crude form and different classical methods were applied for analyzing the antimicrobial activity of *Camellia sinensis*.

Disc diffusion assay

Staphylococcus epidermidis showed the least sensitivity among Gram-positive organisms. *Salmonella typhi* revealed to be the most sensitive among the Gram-negative organism used in this study that it showed a largest zones of inhibition against different concentrations (table 1 & 2).

Well diffusion assay

S.aureus appeared to be very sensitive organism with a large diameter range of zone of inhibition (table 1 at supplementary data). In case of gram-negative organisms, *S.typhi* appeared to be the most sensitive organisms while

E. coli showed less sensitivity [(also in well diffusion assay) (table 2 at supplementary data)].

Spot method

Among all tested organisms, *Bacillus subtilis* appeared to be more sensitive against aqueous extract compared to *S. aureus* and *E. coli*. Aqueous extract has also manifested antifungal activity against *Candida albicans* (tables Available at supplementary data).

MIC and MBC of aqueous extracts of camellia sinensis

The test organisms suspensions were prepared in phosphate buffer solution (PBS) at pH7.0 and 1.5×10⁸ cells/ml diversity was adjusted by using McFarland index 0.5.

MIC is a recognized and well-used criterion for measurement of organism's susceptibility towards an antimicrobial compound. There are several factors which affect the MIC values including inoculum size, type of the test organisms, pH, and temperature.

According to the MIC and MBC values (table 3), the Gram-positive are more susceptible than the Gram-negative bacteria. This may be attributed to the difference in cell wall composition. In Gram-negative bacteria, the cell wall is surrounded by an outer envelope comprising lipopolysaccharides (LPS). This outer envelope restricts the penetration of the test compound. In Gram-positive organisms, the cell wall is not covered by an outer envelope LPS

Cumulatively aqueous extract of *Camellia sinensis* inhibited both Gram positive and Gram negative bacteria. Numerous studies have been conducted to find out the exact mode of action of *Camellia sinensis* polyphenols but it is still debatable and not well established. However, according to some studies, the structure and function of the bacterial cell membrane is the main target of *Camellia sinensis* catechin (Roccaro et al., 2004). Furthermore, crude form of *Camellia Sinensis* was examined and also tested for MIC and MBC so that's why it showed a little bit higher value of MIC and MBC as compared to reported literature (Abbas, 2011).

Miles and misra

In this technique, different concentrations of *Camellia Sinensis* aqueous extract were used against test microorganisms. Upon increasing the concentration of the aqueous extract, a gradual decrease in log of CFU/mL was seen in the case of all Gram positives. Among all Gram positives, *S. epidermidis* offered a high degree of sensitivity with low log of CFU/mL at 47% aqueous extract of *Camellia Sinensis*. In case of Gram negative, *P. aeruginosa* manifested high susceptibility with low log of CFU/mL at 55% aqueous extract of *Camellia Sinensis* (fig. 1).

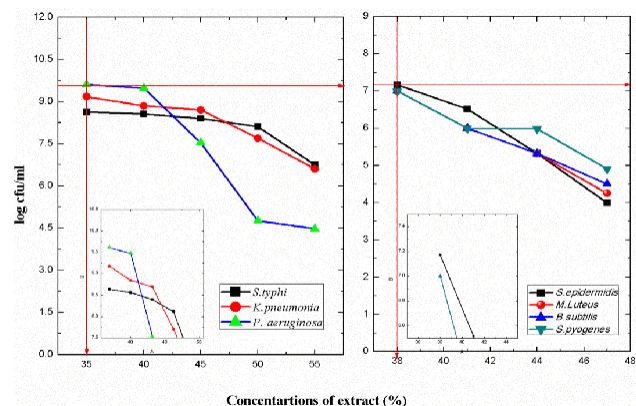


Fig. 1: Log of CFU/ml at different concentrations of aqueous extract of *Camellia sinensis* for Gram positive and Gram negative isolates. The small graphs show the zoom area in order to clearly observe the difference among the organism's results. The right side zoom picture shows two peaks, except *S. epidermidis* all the other three microorganisms have the same value and overlapped by other.

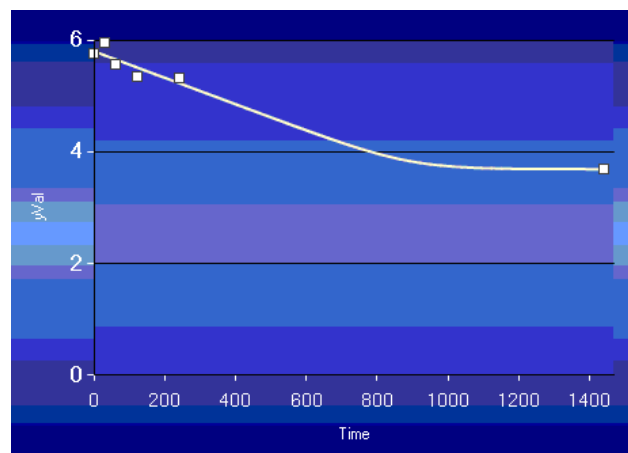


Fig. 2: Survival curve of *Staphylococcus aureus* with 18% aqueous extract of *Camellia sinensis* in DMFit model software, web edition (Dynamic modeling fit). $R^2 = 0.9470$ and SE of Fit = 0.1869

Time-kill assay

Time-kill assay was performed using 18% of an aqueous extract of *Camellia Sinensis* against *S. aureus* as test organism but in different studies EGCG was used to check the bactericidal effect of *Camellia sinensis* (Wongsrichanalai *et al.*, 2002; Abbas, 2011). Similarly, *Camellia sinensis* polyphenols were used in combination with oxacillin for conducting Time-kill assay against MRSA (Cho *et al.*, 2008). In contrast to our investigation, several studies focused the synergistic antibacterial effects of *Camellia sinensis* such as the antibacterial effect of *Camellia sinensis* polyphenols in combination with amoxicillin (Betts *et al.*, 2012) also in combination with oxacillin (Cho *et al.*, 2008). In this study, at 0 minute in the presence of the test compound the log of CFU/mL was

5.763. The organisms started growing and after 30 minutes log of CFU/mL increased to 5.968. Later on a drastic decrease was seen in the viable count of *Staphylococcus aureus* upon exposure to aqueous extract of *Camellia sinensis* as compared to the control i.e. after 24 hours it decreased to 3.698 Log CFU/m. This result suggested that the test compound exerted its killing effect after 30 minutes by acting on the growing cells (fig. 2). As drop in log of CFU/ml was started at 30 minutes hence no more samples collection were drawn after certain time interval i.e. no sample collection after 300 minutes.

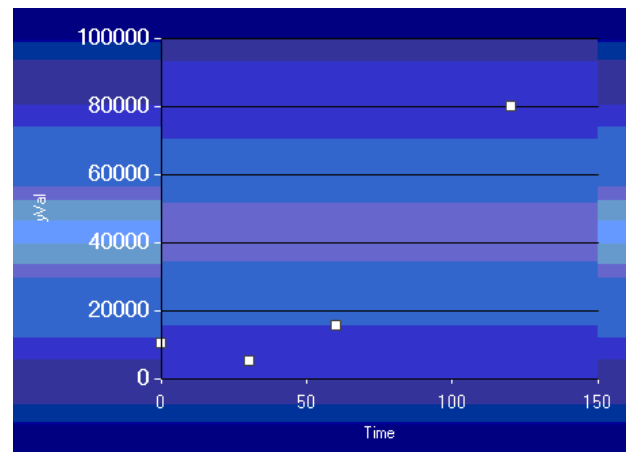


Fig. 3: Growth curve of *Staphylococcus aureus*. DMFit, web edition (Dynamic modeling fit)

Growth curve of staphylococcus aureus

Growth curve of *Staphylococcus aureus* was deduced (fig. 3). This experiment was performed by inoculating test organism in nutrient broth. After 24 hour incubation at 37°C, broth culture was 10 fold diluted to 10^{-3} dilution. Broth culture was further diluted up to 10^{-6} . A 10µl broth culture volume was inoculated on MHA plates at different time interval i.e. at 0 minute, after 30 minutes, 60 minutes, 120 minutes, 240 minutes, and 1440 minutes of growth. Plates were then incubated at 37°C for 24 hours.

According to this study aqueous extracts of *Camellia sinensis* found effective against different Gram-positive, Gram-negative and fungal clinical isolates. The inhibitory effect of aqueous extract of *Camellia sinensis* against the test organisms observed as decreasing log of CFU/ml upon increasing concentration. The *Camellia Sinensis* extracts also found to be very effective against multi drug-resistant *Pseudomonas aeruginosa* and *Candida albicans*.

DISCUSSION

Antimicrobial activity of aqueous extract of *Camellia Sinensis* was tested against clinical isolates. Extract concentration was ranged from 20 to 40% from gram

Table 1: Agar diffusion assay using different concentrations of aqueous extract of *Camellia sinensis* against Gram-positive isolates

Gram-Positive Organisms	Zone Of Inhibition (avg) using Well Diffusion Method (mm±SD)					
	20%	24%	28%	32%	36%	40%
<i>Staphylococcus aureus</i>	17.8±0.76	18.6±1.04	19.4±0.62	19.9±0.7	21±0.8	21.14±0.3
<i>Staphylococcus epidermidis</i>	12.3±0.9	12.6±1.2	13.3±0.5	14.5±0.7	15.6±1.5	16.5±1.4
<i>Bacillus subtilis</i>	15.2±0.35	16±0	17.1±1.23	17.3±1.2	18.2±0.3	20.7±2.1

Experiment run in duplicate and four times repeated (n=8). SD= Standard deviation
Standard deviation was calculated based on the number of repeats of experiment.

Table 2: Agar diffusion assay using different concentrations of aqueous extract of *Camellia sinensis* against Gram-negative isolates

Gram-Negative Organisms	Zone Of Inhibition (avg) using Well Diffusion Method (mm±SD)					
	35%	40%	45%	50%	55%	60%
<i>Pseudomonas aeruginosa</i>	16.5±0.88	16.72±0.74	17.2±0.3	17.8±0.2	18.1±0.5	18.8±0.1
<i>Klebsiella pneumoniae</i>	16.87±0.17	17.25±0	18±0.7	19±1.4	19.2±0.3	20.2±0.3
<i>Salmonella typhi</i>	14.62±0.17	14.87±0.88	16±0	16.3±1.2	19.5±0.7	21.1±0.1
<i>Escherichia coli</i>	12.8±0.88	14.12±1.23	15.2±1.41	15.5±0	16.12±0.88	16.7±1.06

Experiment run in duplicate and four times repeated (n=8). SD= Standard deviation
Standard deviation was calculated based on the number of repeats of experiment.

Table 3: Spot test using different concentrations of aqueous extract of *Camellia sinensis* against ATCC, CMCC and Clinical isolates

Organisms	Zone Of Inhibition (avg) using Spot Method (mm±SD)					
	50%	40%	30%	20%	10%	5%
<i>Bacillus subtilis</i> *	16.5±0.88	16.72±0.74	17.2±0.3	17.8±0.2	18.1±0.5	18.8±0.1
<i>E. coli</i> **	16.87±0.17	17.25±0	18±0.7	19±1.4	19.2±0.3	20.2±0.3
<i>Candida albicans</i> ***	14.62±0.17	14.87±0.88	16±0	16.3±1.2	19.5±0.7	21.1±0.1

Experiment run in duplicate and four times repeated (n=8). . CMCC, **ATCC, ***Clinical isolate
Standard deviation was calculated based on the number of repeats of experiment.

positive and 35 to 60% for gram negative. According to this study aqueous extracts of *Camellia Sinensis* are found to be effective against a wide range of gram-positive, gram-negative and fungal clinical isolates. The inhibitory effect of aqueous extract of *Camellia Sinensis* against tested organisms was recorded by lowering down log of CFU/ml upon increasing extract's concentration, showed by DM fit model. The *Camellia Sinensis* extracts are also found to be very effective against drug-resistant MRSA, *Pseudomonas aeruginosa* and *Candida albicans*.

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REFERENCES

Abbas T (2011). Activity of Tea Polyphenols against *Vibrio* Species. *Microbial. Sciences. Division*, University of Surrey, Guildford, Surrey, UK.

Abdel-Tawwab M, Ahmad M, Seden ME and Sakr SF (2010). Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of *Nile tilapia*, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection. *J. World Aquac. Soc.*, **41**(2): 203-213.

Anjum K, Abbas SQ, Shah SAA, Akhter N, Batool S, Hassan SSU (2016). Marine sponges as a Drug Treasure. *Biomol. Ther.*, **24**(4): 347-362.

Anjum K, Abbas T, Kazimi MR, Subhan SA, Mehmood N, Mujahid TA and Hassan N (2015). Antimicrobial activity of aqueous extract, methanolic extract and oil of *Nigella Sativa* (kalonji) against gram positives and gram negatives clinical isolates. *Int. J. Curr. Res.*, **7**(2): 12294-12299.

Bauman R (2007). Microbiology with Diseases by Taxonomy. *Pearson Benjamin Cummings*, San Francisco, CA., pp.300-600.

Betts I, Jonathan W, Murphy C, Kelly SM and Haswell SJ (2012). Synergistic antibacterial effects of tea flavin in combination with ampicillin against hospital isolates of *Stenotrophomonas maltophilia*. *J. Microbial.*, **2**(3): 1068-1078.

- Cho YS, Schiller NL and Oh KH (2008). Antibacterial effects of Green Tea Polyphenols on clinical isolates of methicillin resistant *Staphylococcus aureus*. *J. Curr. Microbial.*, **57**: 542-546.
- Cui Y, Oh YJ, Lim J, Youn M, Lee I, Pak HK and Park S (2012). AFM study of the differential inhibitory effects of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against Gram-positive and Gram-negative bacteria. *Food Microbial.*, **29**(1): 80-87.
- Hassan SSU, Anjum K, Abbas SQ, Akhter N, Shagufta BI, Shah SAA, Tasneem U (2017). Emerging biopharmaceuticals from marine actinobacteria. *Environ. Toxicol. Phar.*, **49**: 34-47.
- Hassan SSU and Shaikh AL (2017). Marine actinobacteria as a drug treasure house. *Biomed Pharmacother.*, **87**: 46-57.
- Hara Y (2001). *Green tea: health benefits and applications*. CRC press.
- Jones A, Morgan D, Walsh A, Turton J, Livermore D, Pitt T and Mortiboy D (2006). Importation of multidrug-resistant *Acinetobacter spp* infections with casualties from Iraq. *Lancet. Infect. Dis.*, **6**(6): 317-318.
- Khokhar S and Magnusdottir SGM (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *J. Agr. Food Chem.*, **50**(3): 565-570.
- Lee DS, Eom SH, Jeong SY, Shin HJ, Je JY, Lee EW and Lee MS (2013). Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) substance from the marine bacterium *Pseudomonas sp.* UJ-6. *Environ. Toxicol. Phar.*, **35**(2): 171-177.
- Majid, M, Khan MR, Shah NA, Haq IU, Farooq MA, Ullah S, Sharif A, Zahra Z, Younis T and Sajid M (2015). Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of *Euphorbia dracunculoides*. *J. Altern. Complement. Med.*, **15**: 349.
- Mbata TI, Debiao LU and Saikia A (2008). Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*. *Afr. J. Biotechnol.*, **7**(10): 1571-1573.
- Osada K, Takahashi M, Hoshina S, Nakamura M, Nakamura S and Sugano M (2001). Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein *in vitro*. *Comp. Biochem. Physiol. C.*, **128**(2): 153-164.
- Osterburg A, Gardner J, Hyon SH, Neely A and Babcock G (2009). Highly antibiotic-resistant *Acinetobacter baumannii* clinical isolates are killed by the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG). *Clin. Microbial. Infec.*, **15**(4): 341-346.
- Padmini E, Valarmathi A and Rani MU (2010). Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis*. *Asian J. Exp. Biol. Sci.*, **1**(4): 772-781.
- Perumalla AVS and Hettiarachchy NS (2011). Green tea and grape seed extracts Potential applications in food safety and quality. *Food. Res. Int.*, **44**(4): 827-839.
- Raederstorff DG, Schlachter MF, Elste V and Weber P (2003). Effect of EGCG on lipid absorption and plasma lipid levels in rats. *J. Nutr. Biochem.*, **14**(6): 326-332.
- Roccaro AS, Blanco AR, Giuliano F, Rusciano D and Enea V (2004). Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrob. Agents Chemother.*, **48**(6): 1968-1973.
- Sano M, Tabata M, Suzuki M, Degawa M, Miyase T and Maeda-Yamamoto M (2001). Simultaneous determination of twelve tea catechins by high-performance liquid chromatography with electrochemical detection. *Analyst*, **126**(6): 816-820.
- Sartippour, MR, Shao ZM, Heber D, Beatty P, Zhang L, Liu C and Brooks MN (2002). Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. *J. Nutr.*, **132**(8): 2307-2311.
- Sharma A, Gupta S, Sarethy IP, Dang S and Gabrani R (2012). Green tea extract: Possible mechanism and antibacterial activity on skin pathogens. *Food Chem.*, **135**(2): 672-675.
- Shepherd MG (1986). The pathogenesis and host defense mechanisms of oral candidiasis. *N. Zeal. Dent. J.*, **82**: 78-81.
- Spiro M (1995). What's in a nice cup of tea? *Education in Chemistry-London*. **32**(1): 11-13.
- Steinmann J, Buer J, Pietschmann T and Steinmann E (2013). Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. *Brit. J. Pharmacol.*, **168**(5): 1059-1073.
- Tiwari RP, Bharti SK, Kaur HD, Dikshit RP and Hoondal GS (2005). Synergistic antimicrobial activity of tea & antibiotics. *Indian J. Med. Res.*, **122**(1): 80.
- Weber JM, Ruzindana-Umunyana A, Imbeault L and Sircar S (2003). Inhibition of adenovirus infection and adenain by green tea catechins. *Antiviral. Research*, **58**(2): 167-173.
- Weinreb O, Mandel S, Amit T and Youdim MB (2004). Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J. Nutr. Biochem.*, **15**(9): 506-516.
- Wongsrichanalai C, Pickar AL, Wernsdorfer WH and Meshnick SR (2002). Epidemiology of drug-resistant malaria. *Lancet Infect. Dis.*, **2**(4): 209-218.
- Yoda Y, Hu ZQ, Zhao WH and Shimamura T (2004). Different susceptibilities of *Staphylococcus* and Gram-negative rods to epigallocatechin gallate. *J. Infect. Chemother.*, **10**(1): 55-58.