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Green Tea Versus Traditional Korean Teas: Antibacterial/Antifungal or Both?

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Abstract The feasibility of utilizing the antimicrobial activity of naturally available teas was studied. Eleven teas including 2 green teas and 9 other traditional Korean mixed teas were tested for their antimicrobial properties. Antibacterial and antifungal properties were assessed. The results showed that green teas possessed significant antifungal and antibacterial properties, while most of the mixed teas showed some amount of antifungal activity and almost insignificant antibacterial properties. Confocal microscopic imaging revealed mycelial damage as well as attack on sporophores rather than spores/spore germination to be the reason behind the antifungal activity. EGCG was identified as the crucial catechin for antimicrobial activity. The study confirmed that green tea had a clear edge over the traditional mixed teas when it comes to antimicrobial activity.

Keywords Green tea · Korean tea · Antibacterial · Antifungal · Inhibition · Catechins

Introduction

Tea is a very popular drink worldwide. It is produced from the plant *Camellia sinensis*, which is grown in at least 30 countries and grows best in certain tropical and subtropical regions [1]. Tea is mainly produced in four varieties: white, green, Oolong, and black. Green tea is available to consumers representing different processing methods, harvest times, varieties, and growing regions, all of which may contribute to different characteristics of tea [2].

Korean traditional tea is unique in that it includes a variety of herbal and plant infusions, including the leaves of the tea bush (*Camellia sinensis*). Like the usual brews made from tea leaves, Korean tea is prepared by infusing fruits, leaves, roots, or grains in hot water. Claims of holding high reputation for tea usually is on China or Japan or sometimes even India and Sri

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Lanka. These countries boast innumerable varieties and ancient tea-making traditions. However, South Korea does too, yet we hear less or even nothing about this. Korea produces a large number and varieties of herbal and fruit teas. Their blends and innovations and creativity makes sense when the richness of their landscape is considered: both hardwood deciduous and coniferous forests filled with fruits, roots, nuts, seeds, blossoms, leaves, herbs, and berries. All these make appearances in their teas. This is something unique and undiscovered by the international tea drinker's community.

As much as the flavor and the aroma of these traditional Korean teas are undiscovered to the international community, so much are their merits and promising attributes. Green tea which is the rather more prominent and an accomplished one is well established and reported for its health benefits. The health benefits that have been studied using green tea include the following: antioxidant [3, 4], anti-inflammatory [5], anticarcinogenic [3, 4, 6], for cardiovascular [3, 4] and oral health, and last but not the least, for its pronounced antimicrobial activity [7].

In the current paper, we compare the antibacterial and antifungal properties of green tea versus traditional Korean teas. Eleven teas were tested against 3 bacterial and fungal strains. The antimicrobial properties of green tea versus traditional mixed green teas have been systematically studied for the first time. The fact whether these teas are more antibacterial or more antifungal is probed into.

Materials and Methods

Eleven different traditional Korean teas were procured from a traditional supermarket in Seoul, Korea (Table. 1). They are coded as T1, T2, T3, T4, T5, T6, T7, T8, T9, T10 and T11. In this chronological order, we proceed to describe each of them. T1 is Jeju green tea (1.2 g/bag), a kind of organic green tea (100 % green tea) known for its good smell and taste. It is produced in Jeju Island, South Korea. T2 is James Teaspoon green tea (1.0 g), a 100 % green tea from Gyeong Sang Nam-Do, South Korea. T3 is traditionally known as Dong suh, a brown rice green tea (1.5 g) belonging to a traditional Korean tea type. It consists of 70 % brown rice and 30 % green tea. It is rich in catechins and has a good smell. Compared to 100 % green tea, brown rice green tea is believed to be less astringent. T4 is from Endorphin F&B Inc., a traditional Korean tea known as Corn silk tea (1.5 g), consisting of corn silk (15 % Korean, 82 % Australian) and *Polygonatum odoratum* (3 %). This tea has a positive effect on dieresis; it is also a good choice as diet tea. T5 is called Dong suh or Buckwheat tea (1.5 g); this tea contains 100 % buckwheat of Chinese origin. It is rich in rutin which can form vitamin P; it is good for body circulation. T6 is known as Sulloc brown rice green tea (1.5 g) and it has the composition of 70 % brown rice and 30 % green tea. T7 is once again Dong suh, same as T3 but from an earlier stock. T8 is Dong suh which is a *P. odoratum*-based tea (1.2 g), and this tea is 70 % *P. odoratum* and 30 % of brown rice; it is known to prevent cell aging. T9 is Chamomile (0.7 g), an organic herb tea, in which the chamomile used is imported from Germany. This tea possesses a sweet apple smell. T10 is Rooibos tea (0.8 g); this tea contains 100 % organic rooibos which is produced in South Africa. This herbal tea has no caffeine. T11 is Jasmine tea (1.0 g) with a composition of 98 % oolong tea and 2 % Jasmine flower, both imported from China.

Table 1 List of the different tea samples used in the study

Sample Code	Tea name	Tea type	Tea bag size (g/bag)	Composition
T1	Jeju tea	Green tea	1.2	100 % green tea
T2	James teaspoon green tea	Green tea	1.0	100 % green tea
T3	Brown rice green tea	Mixed green tea	1.5	70 % brown rice 30 % green tea
T4	Corn silk tea	Mixed tea	1.5	97 % corn silk 3 % *Polygonatum odoratum*
T5	Buckwheat tea	Mixed tea	1.5	
T6	Sulloc brown rice green tea	Mixed green tea	1.5	70 % brown rice 30 % green tea
T7	Brown rice green tea	Mixed green tea	1.5	70 % brown rice 30 % green tea
T8	Dong suh	Mixed tea	1.2	70 % *Polygonatum odoratum* 30 % brown rice
T9	Chamomile	Mixed tea	0.7	100 % Chamomile
T10	Rooibos	Mixed tea	0.8	100 % rooibos
T11	Jasmine	Mixed tea	1.0	98 % oolong tea 2 % jasmine

Concentrated tea extracts were obtained by allowing the tea bags to infuse in 30 mL of sterile water brought to rolling boil (boiled first then the tea bags dipped into). The bags were removed after 4 min of infusion. The extracts were used immediately once cooled down.

The antibacterial tests were performed using three bacterial species: *Salmonella enteritidis* 12021 (IFO 3313) and *Streptococcus mutans* 11823 (ATCC 25175) which were purchased from Korean Culture Center for Microorganisms, Seoul, South Korea. *Staphylococcus aureus sub. aureus* (KCTC 1928) was obtained from Korean Collection for Type Cultures, Seoul, South Korea. Brain heart infusion (BHI) broth was used in case of *S. mutans* and Luria-Bertani broth was used in case of the other two bacteria. Respective bacterial broth (2000 mL/3 mL) was used. T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 were incubated with *S. mutans*, *S. enteritidis*, and *S. aureus* for 12 h in a shaker cum incubator at 37 °C. Control (no addition of green tea extract) was also maintained for each of these bacteria. The total viable count, indicating the number of bacteria that survived after interaction with the different tea samples was enumerated by plate count method. The total viable count (TVC) was represented as colony forming unit/mL (cfu/mL) [8].

The antifungal tests were performed using three fungi: *Aspergillus niger*, *Botrytis cinerea* KB isolate, and *Penicillium spp*. The fungal spore suspension was prepared from mycelial mats grown on Petri plates. Triton X (0.001 %) (detergent to avoid spore aggregation) was spread on the mycelial mats and gently tapped to loosen and disperse the spores. The spores were collected and used as inoculum for the antifungal tests. Similar to the antibacterial tests, 2000 mL/3 mL concentrations of T1, T2, and T11 were allowed to interact with *A. niger*, *B. cinerea*, and *Penicillium spp.* for 12 h in a shaker cum incubator at 25 °C. The medium in this case was Potato Dextrose Broth (PDB). The TVC was enumerated by plate count method, where the colonies grown on PDA were counted after incubation at 25 °C after 48 h. The biomass of the fungi postincubation with the different teas was measured. The mycelia were filtered and dried in an oven at 55 °C and the dry weight measured and expressed in mg dry weight.

Postexposure to the tea samples, the acridine orange (AO) stained fungi and bacteria were imaged using a Olympus FluoView™ FV1000 confocal laser scanning microscope (CLSM), OLYMPUS AMERICA INC. Corporate Center Drive, Melville, NY, USA. To 500 μL of the interacted sample, 100 μL of acridine orange (0.1 % solution in distilled water) was added and incubated in dark for 10 min. After 10 min, the unbound stain was removed by centrifugation at 5000 rpm for 10 min. This washing was repeated thrice and the acridine orange-stained cells were finally suspended in 100 μL of sterile distilled water. Then, 10 μL of the respective cell suspensions were laid on glass slides and covered with a cover slip and viewed under CLSM. Acridine orange, a fluorescent dye, differentially stains single-stranded RNA and double-stranded DNA, fluorescing orange when intercalated with the former and green while complexing with the latter. In case of bacteria, the green fluorescing cells were said to depict the dead cells while the live cells fluoresce orange [9]. In case of fungi, extensive penetration of the dye is reported to depict cell wall damage [10].

The catechins in the teas were estimated using a Nanodrop ND-1000 v 3.3.1 spectrophotometer, (Nanodrop Technologies, Inc., Wilmington, USA). The absorbance was scanned from 220 to 700 nm.

Results and Discussion

Figure 1 displays the labels of the 11 teas tested, the first two were 100 % green teas, while the others were the traditional Korean mixed tea types. The antibacterial properties of the teas were tested against three bacterial species. Figure 2 a, b, c shows the results of these experiments. T1 and T2 green teas were observed to show distinct antibacterial effect on *S. aureus* (Fig. 2a) compared to T3, T4, T5, T6, T7, T8, T9, T10, and T11 which belong to the traditional Korean tea types. In case of *S. enteritidis*, it was observed (Fig. 2b) that the bactericidal effect was much lesser than that observed on *S. aureus*. However, the highest inhibition was observed from the T1 and T2 green tea extracts. With respect to antibacterial activity on *S. mutans*, it was observed (Fig. 2c) that of the three bacteria tested, the highest bactericidal activity was observed against this bacterium. Antibacterial activity once again was highest in T1 and T2 samples compared to the others. In case of *S. mutans*, it was observed that T3 and T11 also showed considerable antibacterial effect too. Thus, taking into consideration the activity of the teas against the three bacterial species, it can be consolidated that the green teas (T1 and T2) possessed significant antibacterial activity compared to the traditional mixed Korean teas.

With respect to the antifungal properties of tea extracts, Fig. 3a gives the results from the examination of three fungal species. As observed from Fig. 3a, highly significant inhibition (almost 95 % inhibition) was observed against *A. niger* in T1 and T2, while all the others showed no fungicidal activity at all. *B. cinerea* was susceptible to most of the tea types (T4, T5, T6, T7, T8, T9) including the green teas (T1 and T2). With T3 and T10 and T11, marginal activity was observed. *Penicillium spp*. was more resistant compared to the other two fungi toward T1

Fig. 1 Scheme showing labels of the 11 teas used in the study and the protocol followed

and T2 which showed only one order of inhibition. However, *Penicillium spp*. was significantly controlled by T3, T4, T5, T6, T7, and T10 too. T8, T9, and T11 showed no activity against this fungus.

With respect to the biomass (Fig. 3b), the mycelial weight after exposure to the different teas did not show any difference. T1 and T2 showed significant decrease (however, not proportionate to the inhibition observed via the TVC method), while the others did not show prominent reduction in biomass.

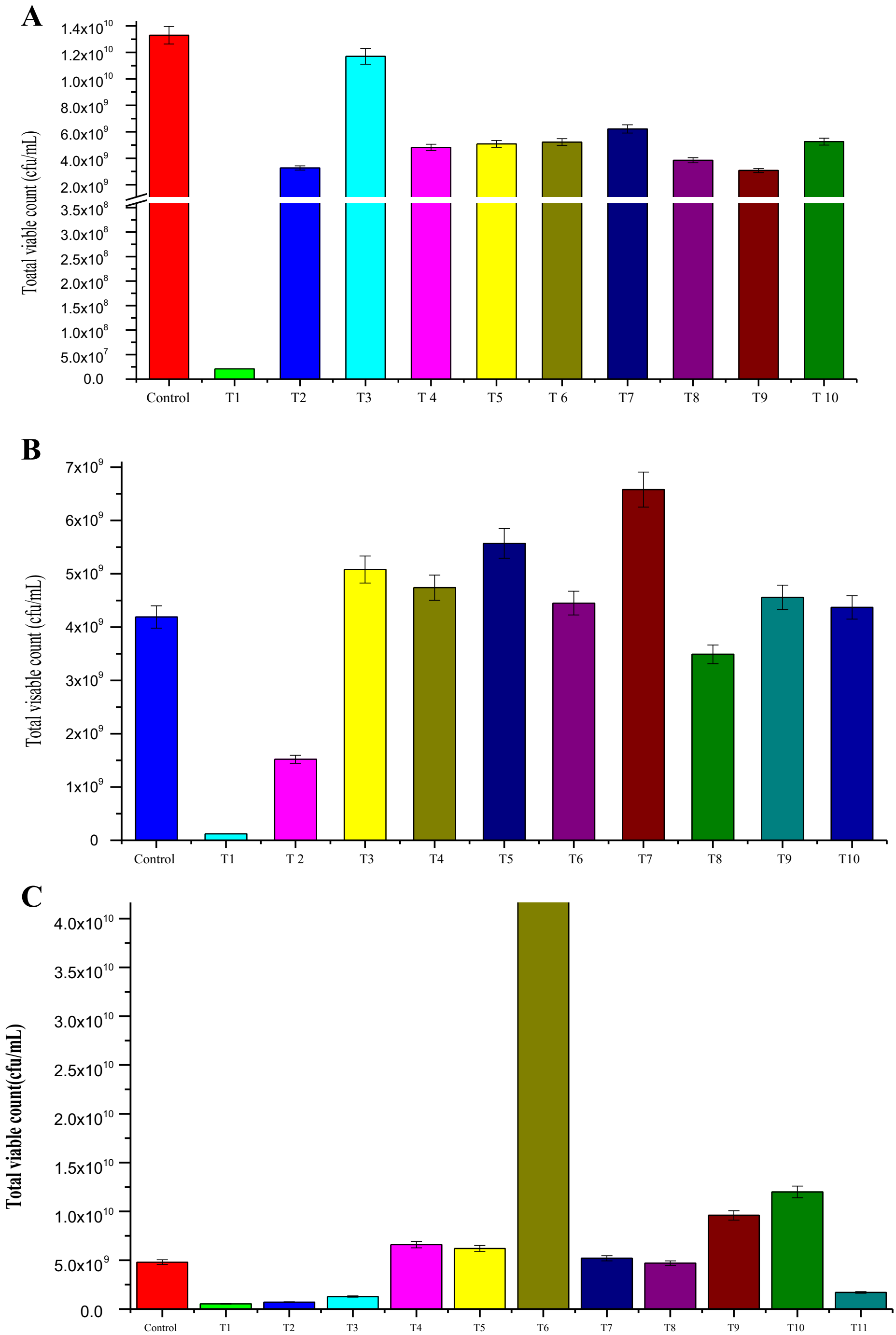

Fig. 2 Graph showing antibacterial activity of the teas against **a** *Staphylococcus aureus*, **b** *Salmonella enteritidis*, and **c** *Streptococcus mutans* based on the total viable counts of the bacteria surviving tea exposure

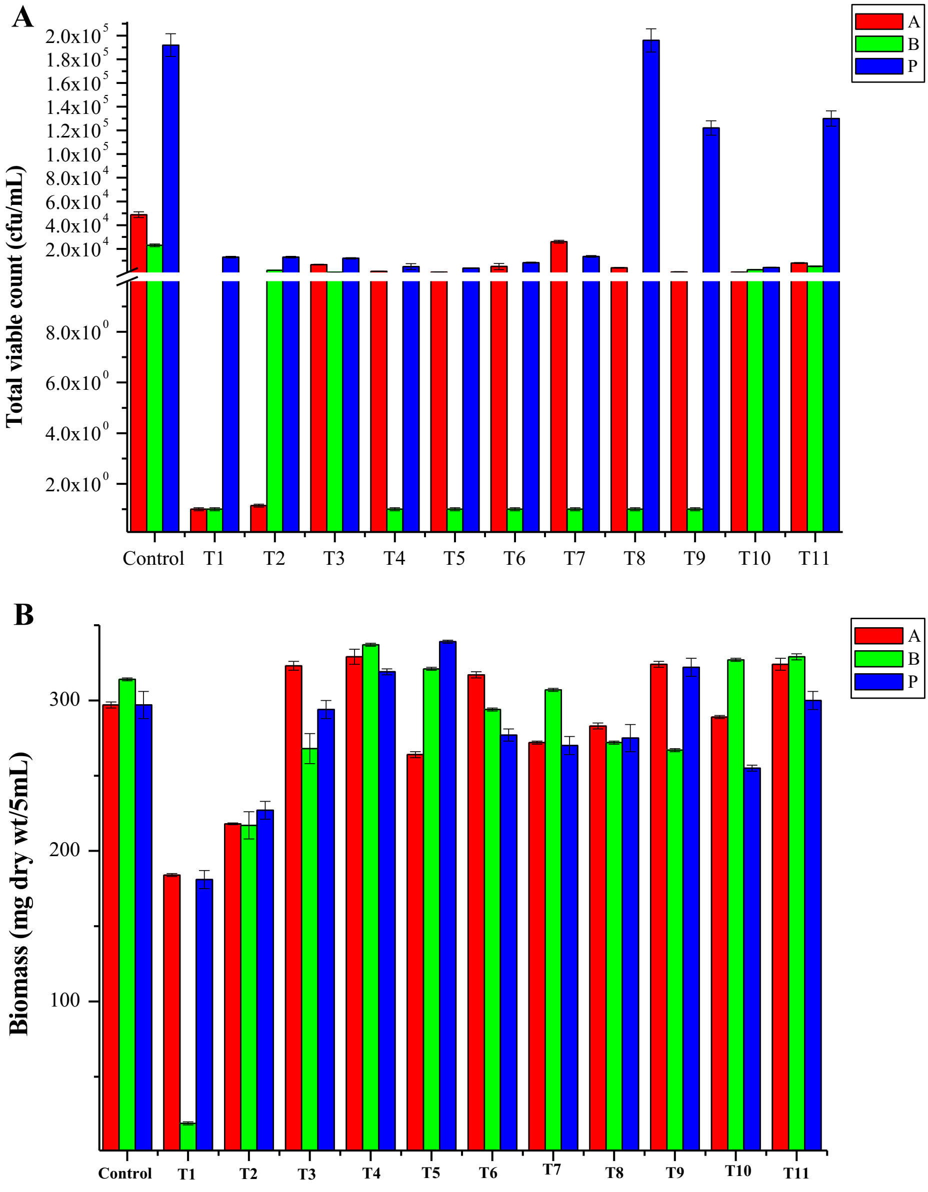

Fig. 3 Graph showing antifungal activity based on results from **a** plate count method and **b** biomass method

The fungal mycelia after exposure to tea extracts were imaged using CLSM following staining with AO. In case of bacteria, AO shows a distinct differentiation between live and dead cells. With dead cells fluorescing green and live cells orange. In case of fungi, such a demarcation is not reported; however, we could observe from Fig. 4 that after exposure to T1, sporangiophores or the spore-bearing ends of the mycelia were mostly empty in case of *A. niger* (a), *Botrytis* (b), and in case of *Penicillium* (c). Usually, the fungal mycelia stain

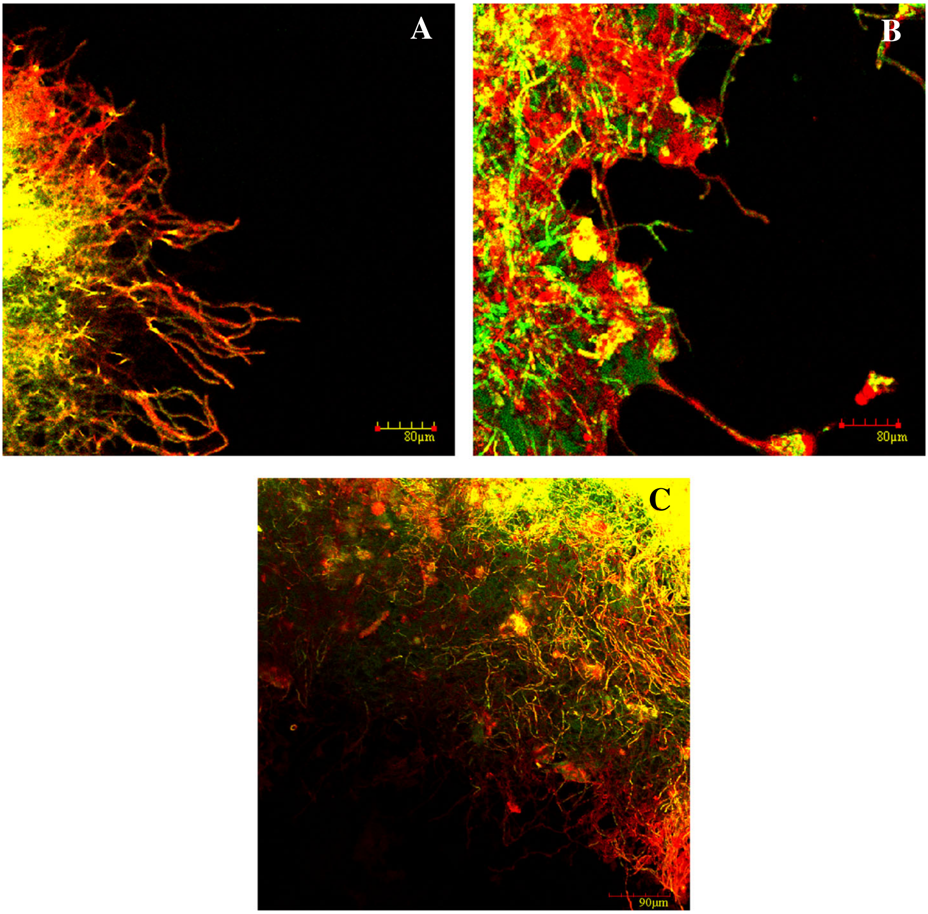

Fig. 4 CLSM image of **a** *Aspergillus niger*, **b** *Botrytis cinerea*, and **c** *Penicillium* spp. after exposure to T1

green; however, we could see orange red regions throughout the mycelial mat, indicating damage. It was interesting to observe that the spores following exposure to T1 stained red and also took in more stain, indicating cell wall breach (Fig. 5b) compared to the control or unexposed *Penicillium spp*. (Fig. 5a). It appears that the spore-bearing sporangiophores were the primary site of attack by the tea catechins.

Consolidating the antifungal results, it can be said that once again, the green teas have an edge over the traditional mixed teas; however, it should be noted that compared to the poor antibacterial properties of the mixed teas, the antifungal properties were significantly higher. Also, as observed from the biomass results not correlating with the TVC results, the mode of action appears not to be growth inhibition but through fungal mycelial damage and sporangiophore damage. This is understood, since if tea can damage the spore, then the spores once inoculated should not have germinated in the presence of the tea extracts in the first place. Although we do see that the spores germinated and developed mycelia, it appears that only the spore forming ability of the mycelia appears to have been curbed by the catechin effect. This is what resulted in decreased colonies in the plate count method.

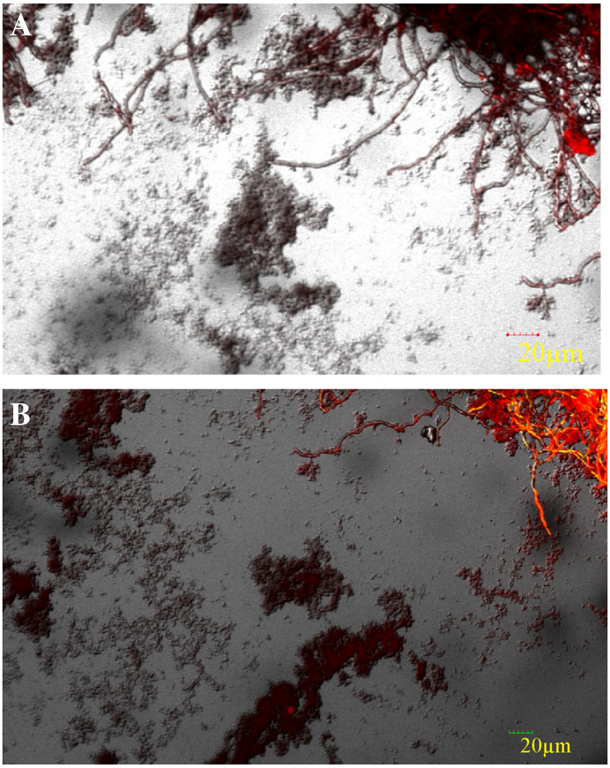

Fig. 5 **a** CLSM image showing unstained spores in control (untreated) *Penicillium* spp. and **b** more stained T1 treated *Penicillium* spp., which is an index that the cell integrity has been compromised allowing penetration of the stain

From these results, it can be concluded that green tea has a more pronounced antimicrobicidal activity compared to the mixed Korean teas. Green tea did show promisingly higher antifungal properties compared to antibacterial properties, in that it could result in complete inhibition of fungus, while in bacteria, few orders of magnitude reduction were only observed. Also, green tea was found to posses broad spectrum antifungal properties, while its antibacterial properties were much narrower and specific to specific strains. The mixed Korean teas (T3 to T11) showed higher antifungal than antibacterial properties. The antimicrobial activity against bacteria as well as fungi is not broad spectrum but highly species specific, in case of the mixed Korean teas. For instance, T8 and T9 show 100 % inhibition of *Botrytis spp*. while poor activity against *A. niger* and even poorer on *Penicillium spp*.

The mode of action or the mechanism of antibacterial or antifungal activity is obviously from the well-known catechins abounding in teas. There are four main catechins in tea. (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epi-gallocatechin-3- gallate (EGCG). In green tea, it is established that EGCG is the most abundant, representing approximately 59 % of the total catechins. EGC is next, making up approximately 19 %; followed by ECG, at 13.6 %; and lastly EC, at 6.4 % [4, 11]. It is also reported that, in addition to the type of tea, the amount of catechins can be affected by the following factors: geographic location of the tea plant, leaf processing, geographical location, growing conditions, and tea preparation [11–13].

The direct effect of tea catechins are reported to result from the binding of the catechins to the bacterial lipid bilayer cell membrane which then causes damage to

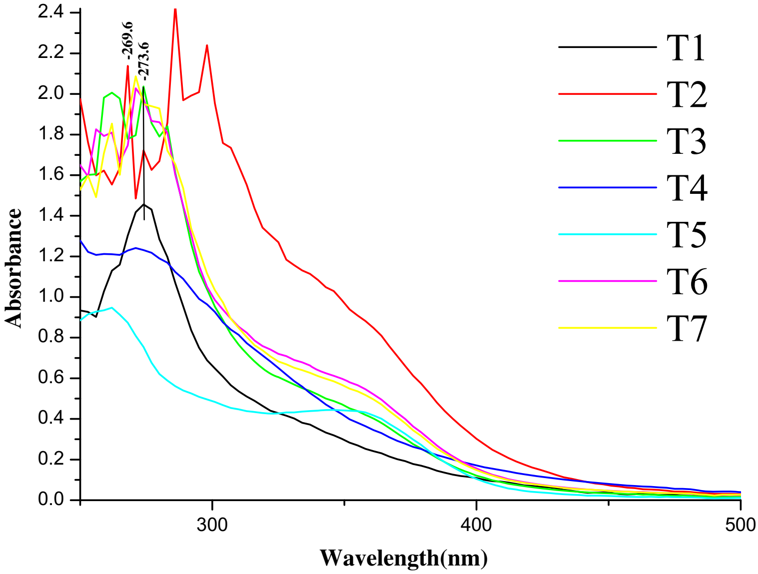

Fig. 6 UV–vis spectra showing catechin peaks in tea samples. EGCG peaks are seen at 273.6 nm and EGC peaks are observed at 269.6 nm

the membrane [14, 15]. This damage is said to trigger off a variety of miscellaneous antimicrobial effects.

In order to justify and directly correlate the catechin concentrations with the antimicrobial activity of the different teas, we have characterized the catechins in the different tea samples using UV–Vis spectrophotometric method. Atomssa and Gotlap [16] have reported the absorbance for the catechin family: EGCG shows an absorbance in the range of 248–361 nm in water with λ_{max} at 273.6 nm; ECG 246–363 nm λ_{max} at 276.8 nm; the spectral range of EGC in water is 254–378 nm and λ_{max} at 269.6 nm and that of EC is 252–328 nm with λ_{max} at 278.4 nm. Figure 6 shows the results of this study. As observed from the figure, T1 and T2 green teas that showed the highest activity showed a strong peak at 273 nm confirming the presence of EGCG. Of the catechin family EGCG is said to possess highly significant antimicrobial properties. The EGCG peak in T1 conformed to the exact pattern of the conventional EGCG peak and the EGC (269.6 nm) peak in T2 appeared more pronounced. With respect to T3, T6, and T7, which consisted of 30 % green tea, brief spikes of EGCG were evident. This is understandable since EGCG is reported highest in green teas, while the mixed teas mostly contain only lower compositions (30 %) of green tea. Figure 6 presents the spectra of T4 and T5, where no distinct peaks of EGCG were evident. This confirms the fact why the green teas showed better antimicrobial activity than did the mixed teas, Thus, it is confirmed that catechins especially EGCG are most vital when it comes to antimicrobial activity. Also, the phenolics and flavonoids known to abound in green teas are well established for their antimicrobial activity [17, 18]. The other properties claimed by the mixed traditional Korean teas cannot be questioned, but when it comes to antimicrobial activity, green tea tops the list.

Conclusion

For the first time, we compared the antimicrobial properties of green tea and Korean traditional teas. The study confirmed that green tea had a clear edge over traditional mixed Korean teas when it comes to antimicrobial activity. Green teas were both antibacterial and antifungal, while some of the mixed teas were anti-fungal than antibacterial. The presence of significant amounts of EGCG appears to be the key for the successful antimicrobial activity exhibited by green teas.

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