RESEARCH ARTICLE

Antimicrobial Effectiveness of Silver Nanoparticles enriched Tea Leaves

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ABSTRACT

Silver nanoparticles (AgNPs) are well recognized for their antimicrobial properties for many years. In the present study, AgNPs synthesized by a green method is investigated for its anti-microbial efficacy, when added in tea leaves. Further, the potential role of AgNPs in controlling the growth of foodborne pathogens was evaluated. Results indicate that AgNPs present in the tea liquor contributes about 50% higher anti-bacterial activity against the foodborne pathogens tested when compared with the untreated tea sample. A significant observation is that the microbial load in the tea reduced due to the presence of AgNPs . Collectively, this study indicates the importance of AgNPs as an anti-microbial agent in controlling the microbial growth associated with food spoilage. In addition, it is likely to enhance the quality and shelf life of tea.

Keywords: Antimicrobial, Food-borne, Preservatives, Silver nanoparticles.

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INTRODUCTION

In recent years, there has been growing interest in the application of nanotechnology in food products. The production of processed food employing anti-microbial agents as food preservatives has become extremely important because of the requirement of increased shelf life. Food preservatives play an important role in inhibitory action against a wide range of bacteria and fungi.[1,2] However, excess feeding of preservatives is unsafe to the consumers due to their serious side effects, such as, allergy, convulsions, hives, and hepatocellular damage (Nair, 2001).[3]

Tea is one of the widely consumed beverages worldwide. Tea is an aromatic beverage, commonly prepared by pouring boiling water over cured leaves of tea *Camellia sinensis*.[4,5] Regular consumption of tea has shown a promising effect in the prevention of many debilitating human diseases, mainly cardiovascular diseases, like atherosclerosis and coronary heart disease, and also has proven anti-cancer, anti-aging, and anti-diabetic properties.

Moisture content in the tea is a critical parameter with acceptable limits of < 3% w/w. If the moisture content is not controlled, microbial contamination in tea is very likely. Poor manufacturing practices, like improper tea leaves processing, drying, packaging, storage, and transport conditions, results in increased moisture content, which could further increase the bacterial and fungal growth. Microbes, like fungi, could release mycotoxins into the surrounding media and make the tea unfit for human consumption. China and India are the major tea producers in the list of the most aflatoxin affected countries.[6] The use of chemical preservatives, such as, sodium benzoate, benzoic acid, sodium sorbate, potassium sorbate, and sodium nitrite in higher amounts, may produce harmful effects in humans.[7]

There is an urgent need to develop a non-toxic, and potent anti-microbial agent in controlling microbial growth in food products. With the advent of nanotechnology, there has been tremendous improvement in the field of agriculture and the food industry. Green chemistry approaches of synthesis of Silver nanoparticles, using plants have been translated to applications, such as, food preservation, food packaging, dietary supplements, and cosmetics.[8] Having the advantage of green nanotechnology, the addition of Silver nanoparticles, prepared using plant extracts as an anti-microbial agent to the food products, such as, tea, could be highly beneficial. The present study aimed to investigate the anti-microbial effects of AgNPs in enriched tea leaves.

MATERIALS AND METHODS

Silver nanoparticles (AgNPs) used in the present study was synthesized as per our earlier report.[9]

Materials

Hot air oven, stainless steel (SS) spraying gun, spray coating pan, tray dryer, and AgNPs (Dhanvantari Nano Ayushadi Pvt. Ltd.)-B. No. TB1909001 of 340 ppm, were used in this study. Tea leaves of two different grades, *viz*., PF (tea dust), and silver tea (leaf tips) were used in the present study. Other materials

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include, trypcase soybean digest agar (TSA), sabouraud-dextrose agar (SDA), Petri plates, measuring cylinder, beaker, vacuum sealer, and bacterial cultures, like *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecium* ATCC 8459, and *Staphylococcus aureus* ATCC 25923.

Preparation of AgNPs enriched Tea Samples

Known amount of tea samples, PF tea and silver tea, were transferred to the SS coating pan. AgNPs was transferred to the holder in the SS sprayer gun. I) When the coating pan starts to rotate, 576 mL of AgNPs of 340 ppm was gently sprayed over 800 grams of PF tea to obtain 245 ppm silver concentration in PF tea. II) Similarly, 706 mL of AgNPs (B. No. TB1909001) of 340 ppm was gently sprayed over 415 grams of silver tea to achieve 340 ppm silver concentration in silver tea.

Allow the coating pan to slowly rotate, while spraying goes on continuously. Once the spraying is complete, spread the wet tea sample as a thin layer in the SS tray, and subject the tea sample to drying at 80ºC, till the moisture content (loss on drying) comes down to ≤ 3% w/w. AgNPs enriched tea samples are subjected to loss on drying analysis. The silver content in the AgNPs enriched tea samples and the tea liquor prepared from enriched tea is estimated using atomic absorption spectroscopy.

Evaluation of Antimicrobial Effect of AgNPs enriched Tea

Enumeration of total aerobic microbial count (TAMC) and total yeast mold count (TYMC) in the tea samples, before and after enrichment with AgNPs , was performed using the pour plate technique. A fixed amount of tea sample was dispersed in a constant volume of sterile saline solution and vortexed thoroughly. Serial dilution was performed, if necessary. From this, 1-mL of the test sample was added to 15 to 20 mL of TSA or SDA poured in Petri plates. TAMC plates were incubated at 34 ± 1ºC for 3 to 5 days, whereas TYMC plates were incubated at 22 ± 1ºC for 5 to 7 days. After the incubation period, the average colony count of two Petri plates was counted, and the number of colony forming unit (CFU) per milliliter of the sample was calculated.[10]

Preparation of Tea Liquor from AgNPs enriched Tea

About 2.5 grams of the tea was added to 150 mL of boiled purified water and kept for incubation. After 5 minutes, the mixture was passed through a nylon tea filter. Then, the tea residue was squeezed to remove excess liquid absorbed by the tea granules. The volume of the tea liquor collected was noted.

Studying Antimicrobial Effect of AgNPs in Tea Liquor of enriched Tea Leaves

In this study, the tea liquor prepared from the AgNPs enriched silver tea and raw silver tea (control) was tested against various food-borne pathogens, like *E. faecium* ATCC 8459, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. Anti-bacterial activity of tea liquor samples was evaluated by total plate count technique, using reference method (ISO 4833) at National accreditation board of testing and calibration laboratory (NABL).

About 10 mL of tea liquor sample is inoculated with 1-mL of 10^5 to 10^6 CFU/mL bacterial cells, and after 5 minutes of contact time, serial dilution has to be performed. From respective dilution tubes, 1 mL of inoculum to be poured in petriplate. After addition of media, plates have been incubated at 30ºC for 72 hours. Total plate count to be recorded after completion of 72 hours. The same procedure has been followed for each bacteria used in the study.[11] Simultaneously, the plating has to be performed for bacterial suspension alone to know the initial bacterial count present in the suspension. Difference in the initial and final microbial count will be interpreted to arrive at the anti-bacterial activity of tea liquor prepared from AgNPs enriched silver tea leaves.

RESULTS AND DISCUSSION

AgNPs used in this study was prepared using plant extract, following principles of green chemistry approach. AgNPs was detected in the enriched tea samples, using atomic absorption spectroscopy. Silver content was found to be 379.7 ppm in the enriched silver tea and 164.4 ppm in the enriched PF tea, respectively (Table 1). Loss on drying analysis showed that the enriched tea samples were completely dried, and the moisture content is found within the limits of < 3% w/w (Table 1). AgNPs enriched silver tea showed complete reduction in TAMC and TYMC, as compared to untreated control sample (Tables 2 and 3). Table 2 shows that AgNPs enriched PF tea showed about ~67% reduction in TAMC, as compared to untreated control sample. Reduction in microbial load in the tea is attributed to the presence of antimicrobial agent AgNPs , as a result of enrichment in PF and silver tea samples. Microbial analysis indicates that AgNPs enriched tea sample could strongly inhibit the **g**rowth of bacteria and fungi in the tea over the period of storage, and this could likely enhance the shelf life of the tea.

Within the contact time of 5 minutes, tea liquor of AgNPs enriched silver tea sample displayed maximum microbial reduction ~72 to 76% of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. faecium* ATCC 8459, and *S. aureus* ATCC 25923, which is shown in Table 4. Exception being *P. aeruginosa* ATCC 27853, where the tea liquor of AgNPs enriched silver tea sample reduced the bacterial count to 42%. Tea liquor sample prepared from untreated silver tea showed **min**imal anti-bacterial activity **(**23–26%) for all bacterial species tested (Table 4). Comparatively, tea liquor samples prepared from AgNPs enriched silver tea showed about additional 50% of microbial reduction against all bacterial species tested, which is attributed to the anti-

Table 1: Loss on drying and silver content of Silver nanoparticles enriched tea samples

Particulars	*Loss on drying (% w/w)*	*Silver content (ppm)*
Silver nanoparticles enriched silver tea	1.9	379.7
Silver nanoparticles enriched PF tea	0.56	164.35

Table 2: Total aerobic microbial count of control and AgNPs treated tea samples

Total aerobic microbial count			
S. No.	*Sample*	*CFU/mL*	*% microbe reduction*
1	Silver tea-control	285	100
2	AgNPs enriched silver tea	0	
3	PF tea-control	30	67
4	AgNPs enriched PF tea	10	

Table 3: Total yeast **mol**d count of control and AgNPs treated tea samples

Total yeast mold count			
S. No.	*Sample*	*CFU/mL*	*% microbe reduction*
1	Silver tea-control	65	100
2	AgNPs enriched silver tea	0	
3	PF tea-control	0	0
4	AgNPs enriched PF tea	0	

Table 4: Antibacterial activity of tea liquor of untreated silver tea and AgNPs enriched silver tea

Test microorganism	*Test sample*	*Initial culture concentration (CFU)*	*After 5 min (CFU)*	*% microbial reduction*
Escherichia coli ATCC 25922	AgNPs enriched silver tea	4,700,000	1,300,000	72.34
	AgNPs enriched silver tea-1:1 dilution	4,700,000	2,700,000	42.55
	Untreated silver tea	4,700,000	3,600,000	23.4
Pseudomonas aeruginosa ATCC 27853	AgNPs enriched silver tea	1,300,000	760,000	41.54
	AgNPs enriched silver tea-1:1 dilution	1,300,000	920,000	29.23
	Untreated silver tea	1,300,000	990,000	23.85
Enterococcus faecium ATCC 8459	AgNPs enriched silver tea	3,000,000	810,000	73
	AgNPs enriched silver tea-1:1 dilution	3,000,000	1,900,000	36.67
	Untreated silver tea	3,000,000	2,300,000	23.33
Staphylococcus aureus ATCC 25923	AgNPs enriched silver tea	2,300,000	530,000	76.96
	AgNPs enriched silver tea-1:1 dilution	2,300,000	1,400,000	39.13
	Untreated silver tea	2,300,000	1,700,000	26.09

bacterial effect of AgNPs **(**1-ppm) in it. When the concentration of AgNPs is 0.5 ppm in tea liquor, anti-bacterial activity is reduced to nearly 50%. By and large, there is significant anti-bacterial activity of upto 50%, infused by the addition of AgNPs to silver tea leaves, within 5 minutes of contact time, when compared with untreated samples.

AgNPs might form electrostatic bonds with the bacterial membrane. The interaction disrupts the bacterial membrane's integrity, resulting in cell death as a result of the bactericidal action. AgNPs binds with both the cell membrane and the mesosomes, leading to a disruption of mesosomal function, which leads to an increase in the production of reactive oxygen species (ROS), causing the cell to die.[12] AgNPs have been known to be most effective because of the acceptable antimicrobial activity against a broad spectrum of microorganisms.[13] Furthermore, AgNPs have many other advantages, such as, low toxicity to eukaryotic cells biocompatibility, high hydrophilicity, good complexity, and favourable solubility in water.[14] Due to these exciting properties, AgNPs serve as a potent antimicrobial agent and control the growth of food borne pathogens, and thereby, prevent the accumulation of undesirable substances, such as, mycotoxins released by molds.

CONCLUSION

In the present study, the importance of AgNPs in controlling the microbial load in tea is established. This study showed that AgNPs in the enriched tea is very effective against food borne pathogens, like *E. coli*, *E. faecium*, *P. aeruginosa*, and *S. aureus*. Thus, AgNPs play a significant role in preventing food spoilage and increase the quality of the food product. More importantly, these studies prove the potential of AgNPs as efficient antibacterial agents and make them suitable for applications as food preservatives. On the whole, enrichment of tea leaves with AgNPs control the growth of food borne pathogens and might improve the characteristics, such as, tea quality and the shelf life.

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