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Effect of light irradiation on the antibacterial activity of compounded papers containing wasted tea leaves

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Abstract The endurance of the antibacterial properties of compounded papers containing wasted green tea leaves needs to be examined before considering these papers for long-term use. Hence, compounded papers containing 60 wt% wasted green tea leaves were irradiated for 1–200 h using a xenon lamp to examine the effect of irradiation on antibacterial properties of the paper. Irradiation for 20 h (cumulative ultraviolet dose at 365 nm, 6.67×10^6 J/m^2) or longer was found to greatly increase the antibacterial activity of the paper to a level at which no bacterial cell was confirmed to be viable. The paper was also covered with various glass filters and irradiated for 1 h. Irradiation exclusively with visible rays did not significantly affect the antibacterial activity of the paper, whereas irradiation exclusively with ultraviolet rays, even for a short time, greatly increased the antibacterial activity.

Key words Ultraviolet radiation · Antibacterial activity · Compounded paper · Tea leaves

Introduction

Compounded papers[1,2] containing wasted green tea leaves have been reported to possess excellent antibacterial properties.[2–5] For their actual use, endurance of the functions is essential. Thus, it is necessary to examine whether the papers retain the antibacterial properties for a long time. In this context, the effect of sunlight on the chemical structure of the paper components is a matter of concern when considering their long-term use.[6,7] In this study, a xenon lamp, which reputedly resembles sunlight with respect to the spectral distribution of the visible rays and possesses good color-rendering properties, was used to accelerate the irradiation effect of sunlight on the compounded paper containing wasted green tea leaves to study the effects of irradiation on its antibacterial properties and color hue. In the irradiation experiment, various glass filters were used to cover the paper to examine the effects of rays of different wavelengths.

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Materials and methods

Samples

Preparation of wasted tea leaves

Green tea leaves are produced by promptly heating clipped leaves to inactivate enzymes for arresting oxidation in the leaves.[8] The most popular green tea is the one produced by steaming and then thoroughly rolling young leaves. In the present study, green tea leaves from Japan were used as the sample.[3] A prescribed amount of tea leaves was added to a volume of boiling distilled water in an enamel kettle and boiled for 30 min. A fine stainless steel mesh ball was used to filter the liquid. Wasted tea leaves were sampled under the extracting conditions described previously.[3]

Preparation of compounded papers containing wasted tea leaves

The pulp used was prepared by refining Canadian bleached conifer Kraft pulp to a standard freeness of 550 ml with a refiner. Aica Aibon RAX117 (Aica Kogyo), a latex binder (emulsion) made of styrene-butadiene rubber (SBR), was used to improve the adhesion between pulp and wasted tea leaves. Compounded papers containing wasted tea leaves were prepared as described previously.[3] First, the wasted tea leaf samples were ground under wet conditions using a Mass-Colloider (stone mill-type crusher) with a clearance of 40 μm. Then, the ground tea leaves were blended with a

prescribed amount of the bleached conifer Kraft pulp and the latex binder. The wasted tea leaf content was adjusted to 60 wt% in the formulation; the latex binder content was 0.3 wt% of the combined amount of the wasted tea leaves and the pulp in all the formulations. Distilled water was added to the mixtures of the foregoing ratios, which were then stirred with a mixer for 10 s to obtain slurries of even dispersion. An angular-sheeting machine (PU-401; Tester Sangyo) was used to adjust the slurries to a paper weight of 100 g/m^2. The sheets were pressed at 410 kPa at room temperature and dried at 120°C with a rotating drier to prepare compounded papers containing wasted tea leaves.[9]

Irradiation method

Figure 1 shows a schematic representation of the SUNTEST CPS, a xenon tester from Shimadzu Corporation. The xenon tester was used to irradiate the compounded paper containing wasted green tea leaves for 1–200 h.[10,11] Figure 2 depicts the wavelength spectrum of the light used for irradiation. A digital UV detector (UVX; Ultraviolet Products) was used to measure the irradiation intensity. The following measurements were obtained: 9.260 mW/cm^2 (365 nm), 8.420 mW/cm^2 (310 nm), and 1.291 mW/cm^2 (254 nm). These values translate into a cumulative UV dose per hour of 3.33×10^5 J/m^2 for 365 nm, 3.03×10^5 J/m^2 for 310 nm, and 4.65×10^4 J/m^2 for 254 nm, respectively. The atmospheric temperature in the irradiation chamber was 38.4°C during irradiation. Colored glass filters from Asahi Techno Glass Corporation were also used to cover the paper during irradiation.

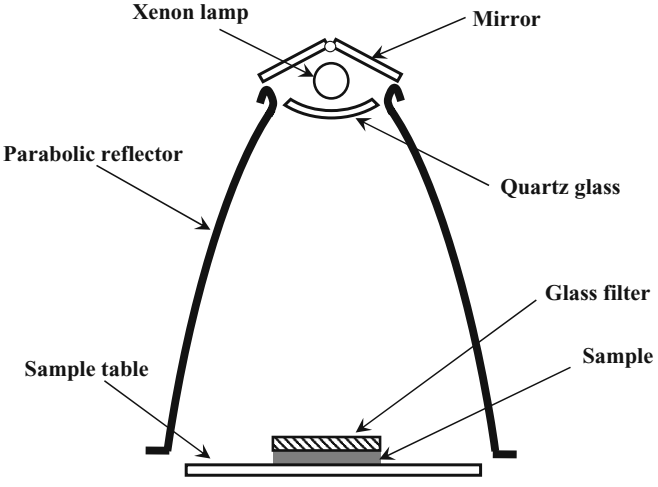

Fig. 1. Schematic representation of a light irradiation device

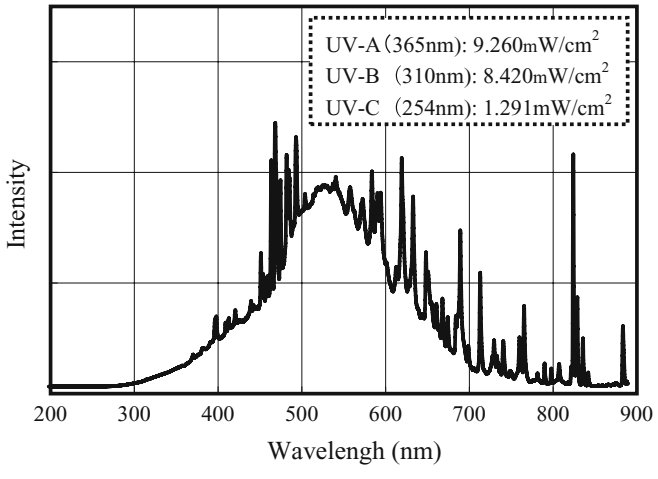

Fig. 2. Spectrophotometric distribution of a xenon arc lamp

Measurements

Tests of antibacterial properties

Staphylococcus aureus (NBRC 12732) used for the antibacterial evaluation was obtained from Incorporated Administrative Agency, National Institute of Technology and Evaluation. The bacterium is a gram-negative coccus that is known as a source of purulent diseases as well as a pathogen causing food poisoning. Japanese Industrial Standards was used as the reference for testing antibacterial properties.[12] A sample of 0.20 g from compounded papers containing the wasted tea leaves was transferred into a vial. The vials with the samples were autoclaved in a BS-245 autoclave (Tomy Kogyo) at 121°C for 15 min. Peptone (1.0 wt%) and yeast extract (0.5 wt%) (Becton Dickinson) and sodium chloride (0.5 wt%) were used to prepare peptone water of a prescribed concentration. The peptone water was used to prepare a *S. aureus* (NBRC 12732) suspension at a concentration of $1.0 \pm 0.3 \times 10^5$ colony-forming units/ml (hereafter, CFU/ml). Each autoclaved sample was inoculated with 0.10 ml suspension, tightly sealed, and incubated at 37° ± 1°C for 18 h.

To each of the incubated vials, 10 ml of rinsing physiological saline, which was adjusted to a prescribed concentration with sodium chloride (0.85 wt%) and Tween 80 (0.20 wt%) (Sigma Chemicals) was added, and the vials were shaken to disperse the bacterial cells. A physiological saline prepared with sodium chloride (0.85 wt%) was added to the stock dispersion of each sample to dilute it to the desired concentrations of up to 10^7 fold. A mannitol salt medium (Ganule; NISSUI Pharmaceutical), adjusted to 11.1 wt%, was inoculated with each of the diluted bacterial suspensions. The adopted inoculation method[13] entailed dropping 5 µl diluted suspension at five spots in each of the four sections of the medium, as described previously.[3] The Petri dishes were placed inverted in an incubator at 37° ± 1°C for 44 h. Grown colonies were counted and multiplied by the dilution ratios to calculate the numbers of viable cells.

To investigate the effect of irradiation alone, the paper was shielded from light with aluminum foil, left in an irradiation chamber for 20 h, and tested for antibacterial activity:

log C = the common logarithm value of viable cell number
Bacteriostatic activity = log N_2–log N_3
Bactericidal activity = log N_1–log N_3
N_1, initial cell number

N_2, viable cell number dropped on rayon fiber after 18 h incubation
N_3, viable cell number dropped on rayon fiber containing complex after 18 h incubation

Color hue evaluation

A spectrophotometer (UV-3100; Shimadzu) was used to measure the reflectance of compounded paper containing wasted green tea leaves at 250–750 nm with layers of standard white plates of barium sulfate placed behind the paper. The slit width was 2.0 nm, and the sampling pitch was 0.5 nm. A value of 100% was assigned to the reflectance of the standard white plate for calibration.

Infrared spectral analysis [Fourier transform infrared spectroscopy (FT-IR)]

An infrared spectrophotometer (FT-720; HORIBA) was used to measure the infrared absorption spectra. For measuring spectra of papers containing wasted tea leaves, a horizontal attenuated total reflectance device (ATR) was used. Measurement conditions were as follows for all the samples: measurement resolution, 4 cm^{-1}; measurement gain, AUTO; instrument function, H-G; scanning speed, 5.0 mm/s; number of scans, 1000; detector, TGS. Chlorophyll was measured by KBr pellet spectroscopy.

Results and discussion

Changes in the color hue of compounded paper containing wasted green tea leaves on light irradiation

A compounded paper containing 60 wt% wasted green tea leaves was irradiated to examine the effects of irradiation time on color hue. Figure 3 shows the changes in the color of the paper during irradiation. The paper became more reddish after 1 h of irradiation and continued to become intensely reddish as the irradiation time increased from 10 to 100 h. Eventually, the color lightened after 200 h, indicating that the color faded after longer hours of irradiation. In contrast, the paper that was left shielded with aluminum foil in a chamber to avoid incident light did not show a significant difference in color hue before and after irradiation. In other words, although the xenon lamp increased the surrounding air temperature in the irradiation chamber from room temperature to 38.4°C, the increased temperature did not affect the color hue of the paper. This finding indicated that light energy alone caused the paper to change in color hue in this experiment.

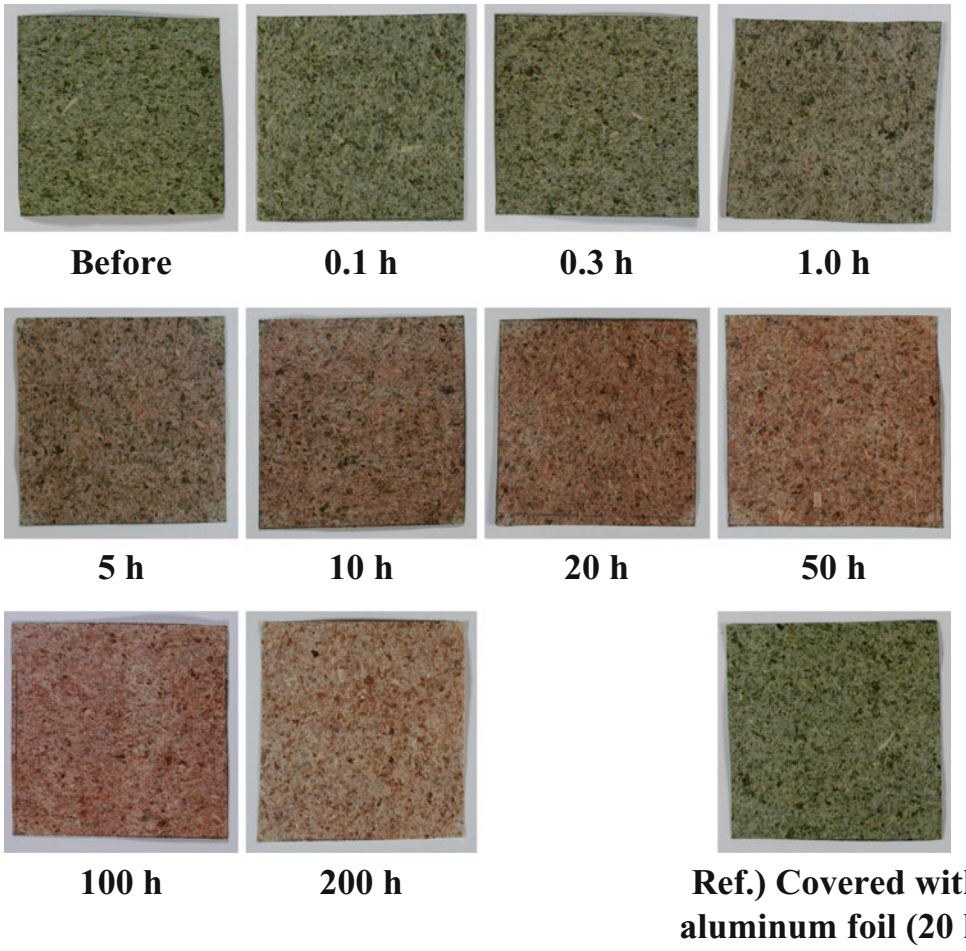

Fig. 3. Change in the color of papers containing 60 wt% of wasted green tea leaves on light irradiation for different durations

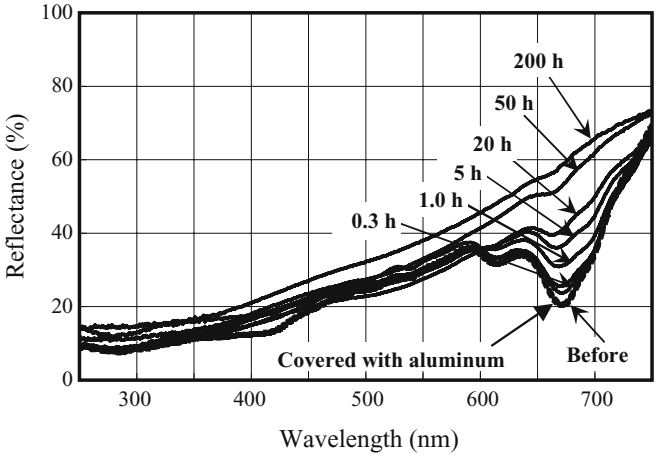

Fig. 4. Reflectance of papers containing 60 wt% of wasted green tea leaves versus wavelength on light irradiation for different durations

Then, to quantify the color hue changes, a UV-Vis spectrophotometer was used to measure the reflectance of the irradiated paper (Fig. 4). The obtained spectra showed that reflectance near the range of 600–750 nm increased with irradiation time. In general, the wavelength of visible rays ranges from 380 to 780 nm, and in particular, the rays of wavelengths 610–780 nm correspond to the red color.[14] The color hue of the paper quantitatively increased in reddishness with irradiation. This result closely agrees with the visual evaluation (see Fig. 3). The paper that was shielded from the incident light with aluminum foil (paper surface temperature, 35°C) did not show a significant increase in reflectance near the range of 600–750 nm. Thus, the effect of heat energy was proven to be negligible in this experiment.

Then, infrared absorption spectroscopy (FT-IR) was performed for the irradiated paper (Fig. 5). A remarkable change in the band appeared near 1720 cm^{-1} with an increase in UV irradiation time. Theaflavins differ from catechins in molecular structure in that all theaflavins have a ketone group (>C=O), which is not present in catechins. Therefore, the presence of an absorption peak corresponding to a ketone group suggests the presence of catechin products (theaflavin-like compounds). In fact, FT-IR measurement of the paper revealed that the peak corresponding to a ketone group near 1720 cm^{-1} in the absorption spectrum, which was not observed before irradiation, became higher with increase in the irradiation time (reference data of ketone group, 1755–1670 cm^{-1}).[15]

KBr crystal was used to prepare chlorophyll *a* tablets to measure absorption by FT-IR. A sharp peak was observed near 1735 cm^{-1}. From the results, the peak of the paper near 1735 cm^{-1} before irradiation was considered to represent absorption by chlorophyll *a*.

Effect of irradiation on the antibacterial properties of compounded paper containing wasted green tea leaves

To investigate the effect of irradiation on the antibacterial properties of compounded papers containing wasted green tea leaves, the irradiated samples were subjected to antibacterial activity tests. Figure 6 shows photographs of agar plates of irradiated papers for 10^2–10^5 fold dilutions. An agar plate of 100 wt% pulp paper that was not irradiated is shown for comparison.

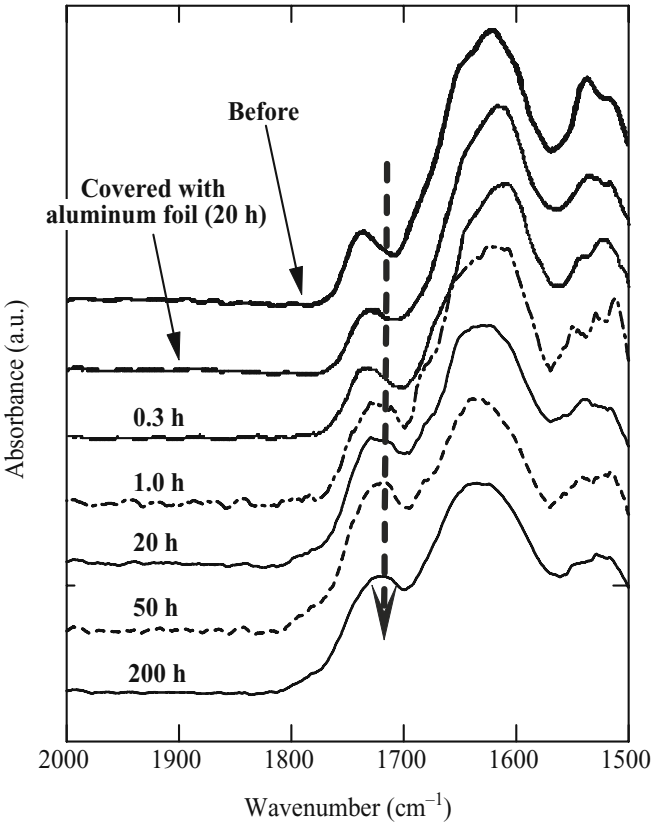

Fig. 5. Fourier transform-infrared (FT-IR) spectra of papers containing 60 wt% of wasted green tea leaves on light irradiation for different durations

The photographs in Figure 6 clearly show that bacterial growth was suppressed. Bacterial growth was further suppressed when the sample was irradiated for 5 h; only a few viable bacterial cells were observed on the specimen of the 10^2-fold-diluted sample. Irradiation for 20 h or longer increased the antibacterial activity of the paper to the extent that no viable bacterial cell was observed after culture. The high antibacterial activity was retained even after irradiation for 200 h, with no viable bacterial cell observed. In addition, when the paper was irradiated with UV for 0.3 h or was not irradiated at all, the medium turned yellow. The color change was the same as that of 100 wt% pulp paper. In contrast, when the paper was irradiated with UV for 1 h or longer, the medium maintained the original red color. A pH indicator was added to the mannitol salt medium used in this experiment. Organic acids produced by microbial growth turned the medium from red to yellow. In other words, the color change of the medium shows that UV irradiation for 1 h or longer suppresses microbial growth.

Table 1 gives evaluation results, showing that the number of viable bacterial cells was 4.68×10^7 CFU/ml for the nonir-

Table 1. Antibacterial properties against *Staphylococcus aureus* in papers containing 60 wt% of wasted green tea leaves after light irradiation for different durations

Sample	Wasted tea leaves content (wt%)	Irradiation time (h)	Incubation time (h)	Antibacterial properties			
				Viable bacteri (CFU/ml)	Log C	Bacteriostatic activity	Bactericidal activity
Initial	–	–	0	1.0×10^5	5	–	–
Papers containing wasted green tea leaves	60	0	18	4.68×10^7	7.62	0.92	–2.62
		0.3	18	9.64×10^7	7.98	0.56	–2.98
		1.0	18	1.20×10^4	4.08	4.46	0.92
		5	18	8.00×10^3	3.90	4.64	1.10
		10	18	8.00×10^3	3.90	4.64	1.10
		20	18	ND	–	–	–
		50	18	ND	–	–	–
		100	18	ND	–	–	–
		200	18	ND	–	–	–
		20 (covered with aluminum foil)	18	5.96×10^7	7.77	0.77	–2.77
Reference: Pulp paper	0	0	18	3.48×10^8	8.54	–	–

ND, not detected (<440 CFU/ml)

radiated compounded paper containing wasted green tea leaves. The value indicates only a little suppression of bacterial growth as compared to the suppression obtained with the 100 wt% pulp paper (3.48×10^8 CFU/ml). In contrast, the number of viable bacterial cells dropped as low as 1.20×10^4 CFU/ml for the irradiated paper, indicating an increase in antibacterial activity of the irradiated paper. The paper covered with aluminum foil yielded 5.96×10^7 CFU/ml of viable bacterial cells, which is approximately the same number as that before irradiation. In other words, temperatures up to 38.4°C were confirmed to make no contribution to the antibacterial activity.

As described here, irradiation clearly improved the antibacterial activity of the paper. It should be particularly noted that paper irradiated for 20 h or longer exhibited high antibacterial activity at which no viable bacterial cell was observed. The data indicate the desirable consequence that the antibacterial activity of compounded papers containing wasted green tea leaves increases on exposure to light during actual use.

Dependence of the hues and antibacterial activity on the UV wavelength used for irradiation

It was necessary to examine the effect of irradiation with rays of different wavelengths on the discoloration and antibacterial activity of the compounded paper containing wasted green tea leaves. Six kinds of glass filters that can filter out particular wavelengths were used to cover the paper during irradiation. In this way, the sample papers could be shielded from V rays of different wavelengths. Figure 7 shows the transmission spectra of the glass filters used for the spectrophotometric measurement. The R-64 filter completely shields the paper from visible rays of wavelengths less than 606 nm and UV rays. The L-42 filter completely shields the paper from UV rays of wavelengths less than 402 nm. The two filters transmit only visible rays. The UV-37, UV-33, and UV-29 filters transmit all visible rays and part of the UV rays, with transmitted rays decreasing in wavelength. The UV-D33S filter, a bandpass filter, transmits UV rays of wavelengths 230–430 nm.

Using these glass filters (R-64, L-42, UV-37, UV-33, UV-29, UV-D33S) for UV irradiation, the samples were selectively irradiated by the desired wavelengths. Figure 8 shows papers covered with various filters and irradiated for 1 h. Irradiation through the R-64 filter was found to change the color hue only slightly. When filters that only transmit all visible rays and some UV rays, such as the L-42, UV-37, UV-33, and UV-29 filters, were used, the color hue faded to grayish on irradiation. In contrast, when the UV-D33S filter or no filter was used, the greenish tone of the color hue was found to decrease and develop into a densely reddish color hue on irradiation.

To quantify the foregoing changes in the color hue, the reflectance wavelength of the paper was measured with a spectrophotometer. Figure 9 depicts the reflectance of paper covered with various filters and irradiated for 1 h. Irradiation did not yield a large difference between paper shielded with aluminum foil and that covered with an R-64 filter. In contrast, when an L-42 or an UV-37 filter was used, irradiation increased the overall reflectance of the paper. In other words, irradiation with visible rays was found to cause color fading of the paper, resulting in the most whitish color hue. When an UV-D33S or an UV-29 filter was used, irradiation changed the color of the paper to a reddish hue, decreasing the reflectance to a lower value than that obtained when an L-42 or an UV-37 filter was used. The level of reflectance was approximately the same as that obtained when no filter was used.

Furthermore, infrared spectroscopy (FT-IR) revealed some changes at 1720 cm^{-1} on irradiation of the papers for 1 h using the filter shielding (Fig. 10). When the UV-D33S filter was used, the peak of a ketone group near 1720 cm^{-1} increased in the absorption spectrum, as seen in the filter-free case. In contrast, when an R-64 or L-42 filter was used,

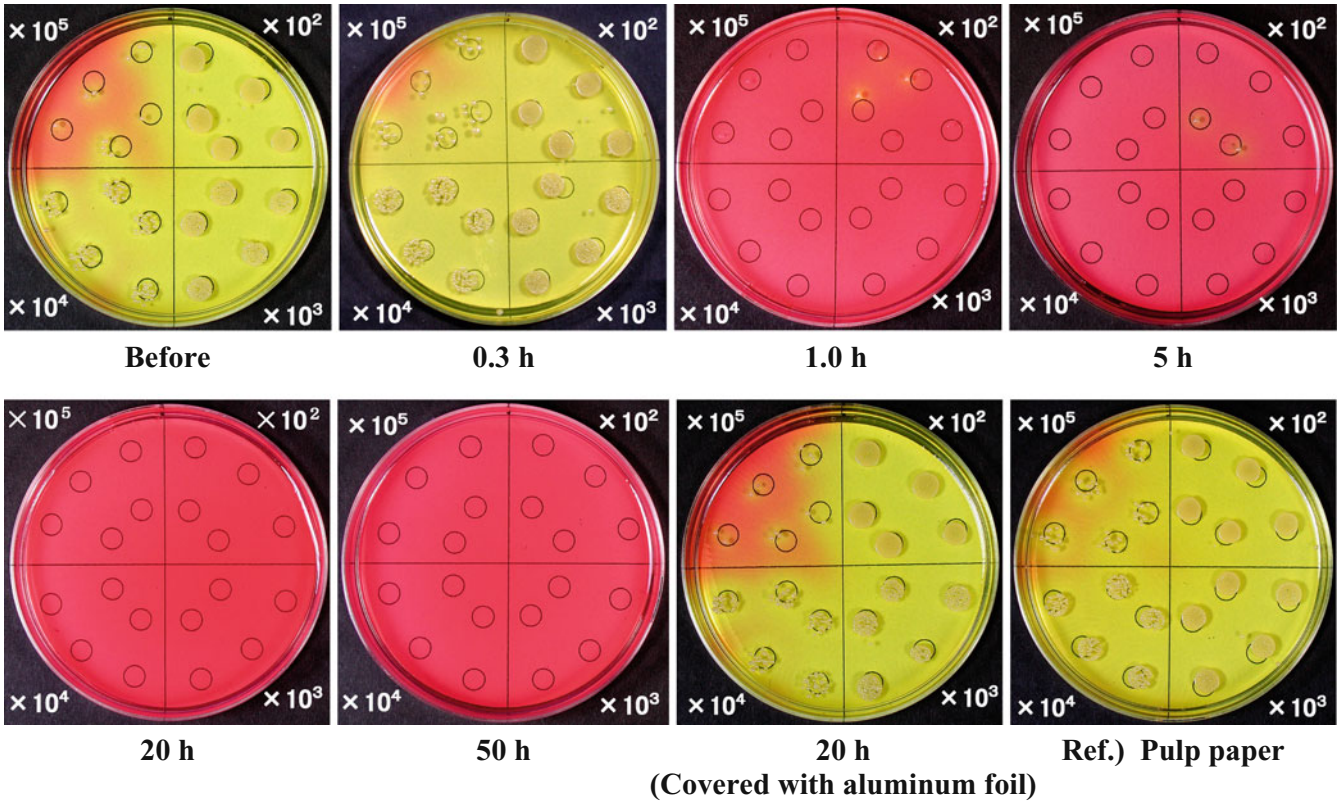

Fig. 6. Effect of light irradiations for different durations on the antibacterial properties against *Staphylococcus aureus* in papers containing 60 wt% of wasted green tea leaves. *Yellow color* of medium indicates microbial growth; *red color* of medium indicates no microbial growth

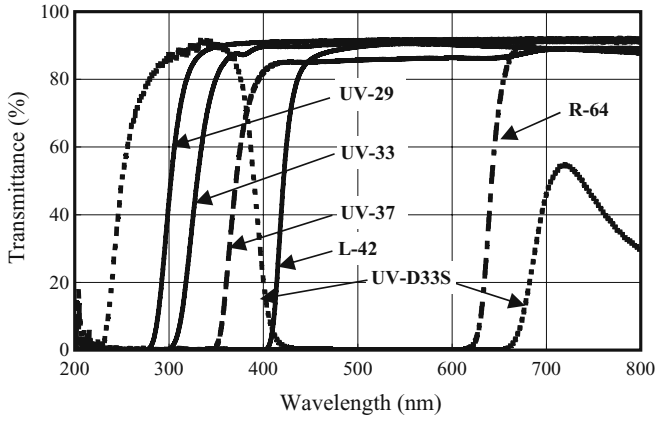

Fig. 7. Relationship between transmittance and wavelength for different filters

irradiation did not cause a significant change, indicating that irradiation with visible rays for a short time does not change catechins into their products.

When an R-64 or an L-42 filter was used, catechins did not change, thus limiting the development of the reddish color; as a result, the visible rays caused fading of the chlorophyll to develop a grayish color in the paper. However, compounded papers containing wasted green tea leaves turned reddish on irradiation for 20 h even when the L-42 filter was used.

The compounded paper containing wasted green tea leaves was covered with an R-64, L-42, UV-33, or UV-D33S

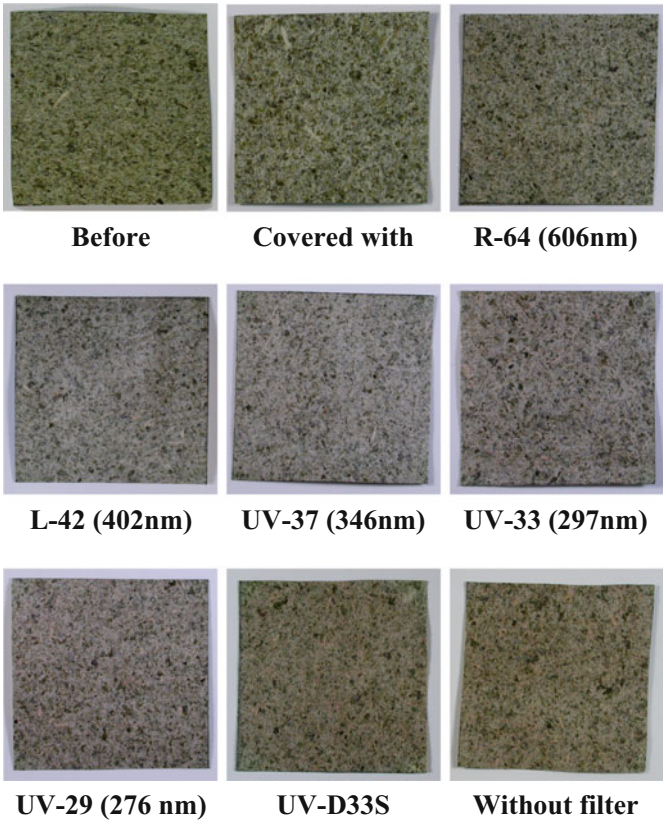

Fig. 8. Change in the color of papers containing 60 wt% of wasted green tea leaves on light irradiation for 1 h through different filters

filter and irradiated for 1 h to evaluate antibacterial activity using *S. aureus*. For comparison, a paper was shielded from light using aluminum foil and another paper was tested without irradiation. Figure 11 shows agar plates for 10^2–10^5 fold dilutions. UV irradiation turned the medium yellow when the medium was covered with an R-64 or L-42 filter. The color change is the same as that observed when the medium was not irradiated with UV at all. In contrast, when the medium was covered with a UV-D33S filter or not covered at all, UV irradiation did not change the color of the medium, which maintained the original red color. Thus, visual observation revealed that UV irradiation suppressed microbial growth in the medium that was covered with a UV-D33S filter or was not covered at all.

Table 2 shows antibacterial activity evaluation. The number of viable bacterial cells was 8.40×10^7 CFU/ml for the nonirradiated paper. In contrast, when the paper was covered with an R-64 or an L-42 filter, irradiation decreased the number of viable bacterial cells slightly but the number remained approximately 10^6 CFU/ml. When the UV-D33S filter or no filter was used, irradiation even for a short time (1 h) decreased the number of viable bacterial cells to as low as 10^4–10^5 CFU/ml, showing increased antibacterial activity. When aluminum foil was used to shield the paper from incident light, irradiation did not result in a significant difference in the number of viable bacterial cells, which remained approximately 5.60×10^7 CFU/ml.

The experimental results are favorable for the actual use of compounded papers containing wasted green tea leaves,

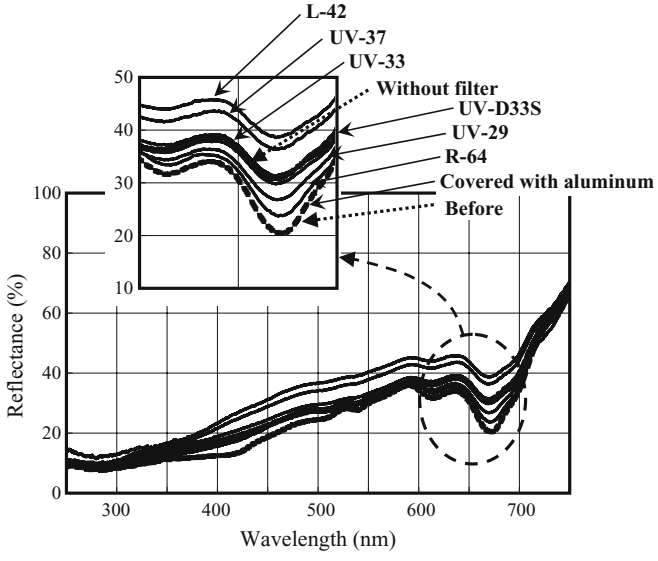

Fig. 9. Reflectance of papers containing 60 wt% of wasted green tea leaves versus wavelength after light irradiation for 1 h through different filters

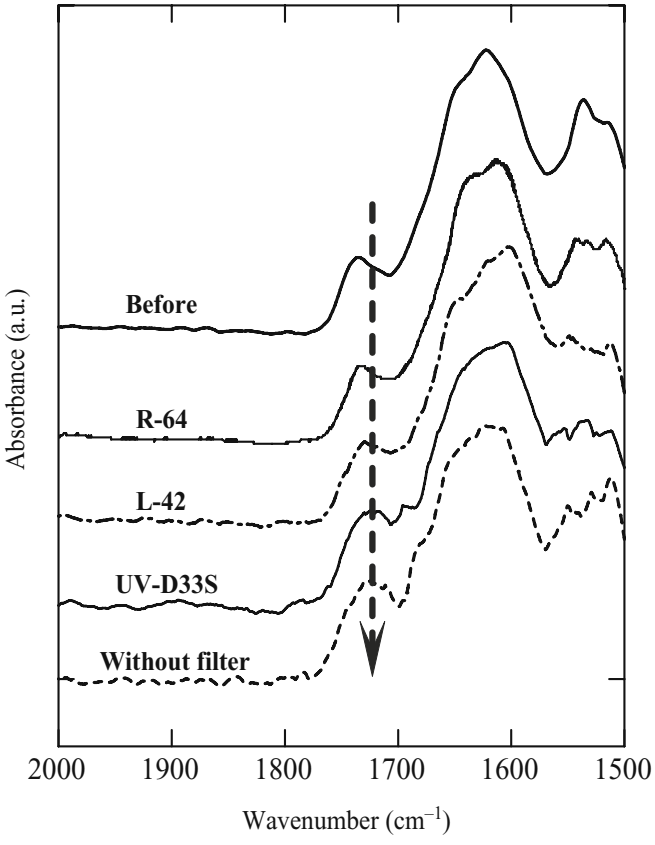

Fig. 10. FT-IR spectra of papers containing 60 wt% of wasted green tea leaves after light irradiation for 1 h through different filters

Table 2. Antibacterial properties against *S. aureus* in papers containing 60 wt% of wasted green tea leaves after light irradiation for 1 h through different filters

Sample	Wasted tea leaves content (wt%)	Filter	Incubation time (h)	Antibacterial properties			
				Viable bacteria (CFU/ml)	Log C	Bacteriostatic activity	Bactericidal activity
Initial	–	–	0	1.0×10^5	5	–	–
Papers containing wasted green tea leaves[a]	60	Before	18	8.40×10^7	7.92	1.59	–2.92
		R-64 (606 nm)	18	6.16×10^7	7.79	1.72	–2.79
		L-42 (402 nm)	18	2.32×10^7	7.37	2.14	–2.37
		UV-33 (297 nm)	18	3.08×10^6	6.49	3.02	–1.49
		UV-D33S (bandpass)	18	1.48×105	5.17	4.34	–0.17
		Without filter	18	1.20×104	4.08	4.46	0.92
		Covered with aluminum foil	18	5.60×10^7	7.75	1.76	–2.75
Bacteria only	0	0	18	3.24×10^9	9.51	–	–

[a] Latex binder content, 0.3wt%

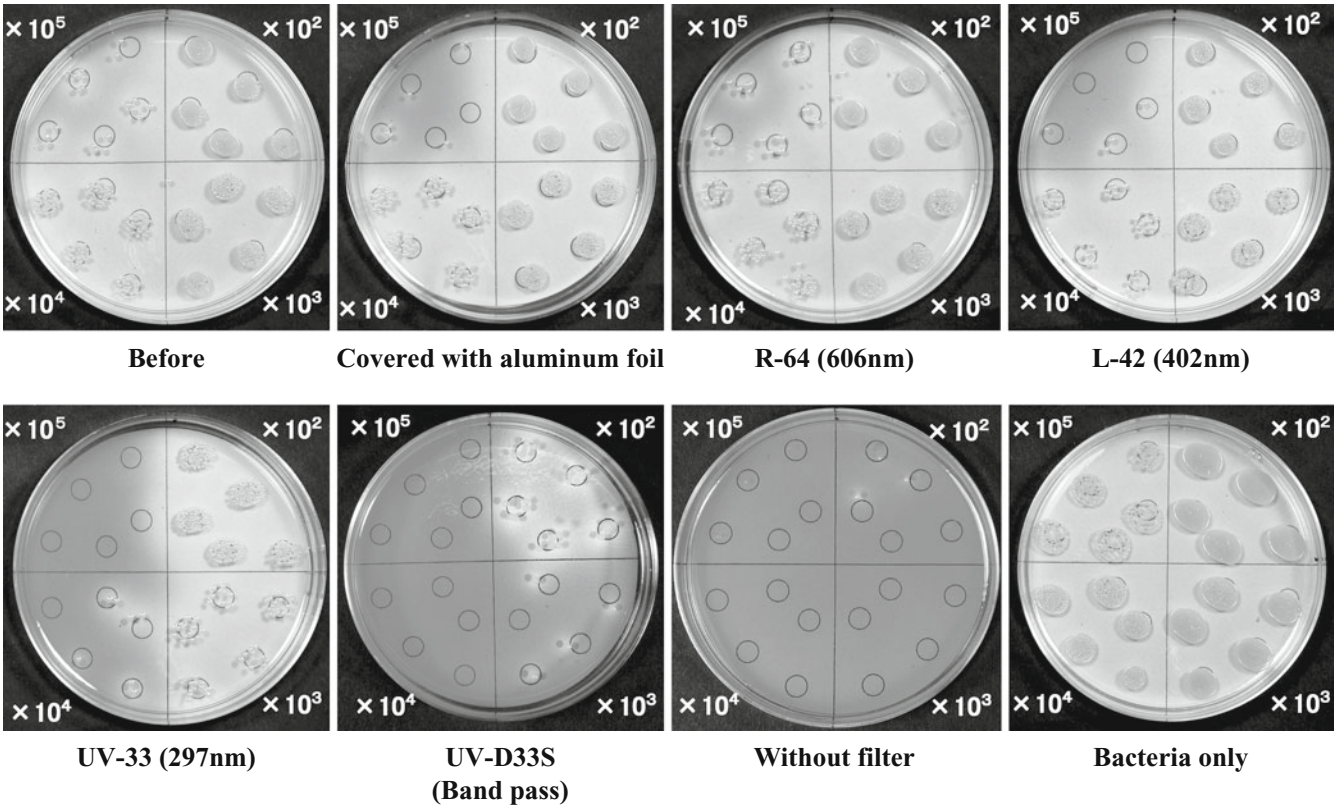

Fig. 11. Antibacterial properties against *S. aureus* in papers containing 60 wt% of wasted green tea leaves after light irradiation for 1 h through different glass filters

suggesting that exposure of the papers to the UV rays of sunlight does not decrease antibacterial activity but rather improves it.

Conclusions

Many functions of materials generally decrease with time. In contrast, the antimicrobial potency of the compounded papers prepared in this study increased on exposure to UV during actual use, which is very desirable from the aspect of product quality assurance. For example, wallpapers are exposed to sunlight for a long time. Functions such as antimicrobial potency and deodorant potency would be considered of great advantage, if the functions remained stable in the wallpaper for a long time. As described here, compounded papers containing wasted tea leaves are very promising, not only for the effective utilization of the wasted tea leaves, but also as durable functional paper.

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References

1. Takahashi T, Kasai W, Kondo T, Yokota H, Kunitake T (2008) Structure and mechanical properties of papers containing ground wasted tealeaves (in Japanese). Sen'i-Gakkaishi 64:252–258
2. Kumabe M (2008) Development of corrugated cardboard made from used tea leaves complying with food recycling law. JPI J 46:779–781
3. Takahashi T, Kondo T, Kasai W, Yokota H, Kunitake T (2007) Preparation of compounded papers using wasted tea leaves (in Japanese). Sen'i-Gakkaishi 63:256–263
4. Takahashi T, Kasai W, Kondo T, Yokota H, Kunitake T (2008) Antibacterial activity of compounded papers using wasted green tealeaves produced by paper-making method (in Japanese). Sen'i-Gakkaishi 64:358–365
5. Sato T, Morikawa Y. Published Japanese Patent, No. 2008-255515
6. Adelstein PZ, Zinn ED, Reilly J (2003) Effect of atmospheric pollution on paper stability. J Pulp Paper Sci 29:21–28
7. Seino T, Ona T, Yoshioka A, Takai M, Kojima Y, Ishikura Y, Ishida Y, Ohtani H (2004) Characterization of photo-yellowing trigger compounds repressing paper recyclability. Trans Mater Res Soc Jpn 29:2093–2096
8. Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea: a review. J Am Coll Nutr 25:79–99
9. Pulps: Preparation of laboratory sheets for physical testing. JIS-P8222
10. Building construction. Jointing products: Determination of adhesion/cohesion properties of sealants after exposure to heat, water and artificial light through glass. ISO-11431
11. Dentistry: Polymer-based restorative materials. ISO-4049
12. Testing for antibacterial activity textile products and efficacy. JIS-L1902
13. Gohya Y, Nakamura S (1994) Simplified and rapid enumeration of proteolytic bacteria in water using the plate-MPN technique. Suisanzoshoku 42:567–570
14. Kelling H, Hinz U (1990) Von der Farbempfindung zur Farbmessung. Getreide Mehl Brot 44:314–316
15. Dyer JR (1991) Infrared spectroscopy. In: Applications of absorption spectroscopy of organic compounds. Prentice Hall, Englewood Cliffs, NJ, pp 22–57