A *Paulownia coreana* Box for Storage of Annals of Joseon Dynasty: Its Efficacy and Functionality Evaluations of Temperature and Relative Humidity Control, and Microbe and Insect Repellent Activity

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ABSTRACT *Paulownia coreana* has various advantages as a convenient workability, aesthetic outlook, beautiful patterns, low weight and high strength, and its permeability. *P. coreana* has been widely used for storage but there are no empirical researches proving its functionality in a field of conservation science until now. In this study, the seasonal and daily temperature and relative humidity control, and microbe and insect repellent activity were evaluated under the controlled and uncontrolled circumstances from 2015 to 2016. The results showed to be mainly excellent in relative humidity control and the buffering effect was good to adjust the average daily relative humidity range from the outside. With respect to the antimicrobial properties of *P. coreana*, we observed that its water-soluble extract produced visible zones of inhibition against five bacteria. However, it was difficult to predict the antimicrobial and/or insecticidal properties.

Key Words *Paulownia coreana* box, Storage box, Functionality, Temperature and relative humidity control, Microbe and insect repellent activity

1. INTRODUCTION

The storage boxes made with wood materials have been traditionally used to preserve the paper and textile objects for a long-term storage in not only Korea but also near countries as China and Japan. The wood has been used as a variety of household goods, furnitures, and architecture materials since ancient period due to its various advantages as convenient workability, aesthetic outlook and outstanding patterns, low weight and high strength, and its permeability. Following the analysis results of eighty-two wooden species from the furnitures dated from late Joseon Dynasty and possessed by

the Seoul Museum of History, 7% of wooden species was identified as *Paulownia* spp. It is mainly used for inner drawers' material because of its lightweight, low-specific gravity and low strain rate (Song *et al.*, 2010). Among them, *P. coreana* belongs to the family of *Scrophulariaceae* with a very clear ring and the colour is either light purple or pale rose pink with unclear distinction between the sapwood and heartwood. *P. coreana* is widely used in various appliances and furnitures because of its fast growth and low absorbency. Following the published researches, it is known well that the air-dried gravity of *P. coreana* is low to 0.24 and also easy to control humidity (Lee *et al.*, 2004). So it has been applied

for making the boxes and furniture in storage and/or display spaces to preserve the small-sized artifacts such as costumes, papers, archives and paintings (Park, 2005; Chung *et al.*, 2008; Cho, 2011).

Even though it is known well that it has peculiar characteristics such as low weight, little cracking and/or splitting, relative humidity control and insect repellent, an empirical researches has not been reported to probe its functionality of the material itself. In this study, we tried to verify empirically its functionality by evaluating the temperature and relative humidity control, and microbe and insect repellent activity of *P. coreana* box.

2. MATERIAL AND METHOD

2.1. Temperature and Relative Humidity Control

Two comparative spaces were set up to identify the temperature and relative humidity control of *P. coreana*: (1) a national storage room controlled with thermo-hygrostat equipment at the Kyujanggak Institute for Korean Studies (Seoul National University, Seoul) and (2) the Korean traditional architecture without any thermo-hygrostat systems at the Anguk-dong detached palace (Korea National University of Cultural Heritage, Buyeo). Each 5 data loggers (174H, Testo, DEU) were installed and recorded with 30 minutes

intervals from October 2015 to September 2016 at the Kyujanggak Institute for Korean Studies and Anguk-dong detached palace.

The locations were selected to measure the controlled temperature and relative humidity at the Kyujanggak Institute for Korean Studies: inner newly-fabricated *P. coreana* box (W930 × L380 × H312 mm, T15 mm), inner old *P. coreana* box (W930 × L380 × H312 mm, T15 mm) that had been used to store the Annal of Joseon Dynasty in the past (Accession number: storage box of Annal of the Joseon Dynasty, Sil-03), inner newly fabricated *Pseudotsuga menziesii* storage box (W930 × L380 × H312 mm, T15 mm) as a control group, *P. coreana* corridor as an open space, and inner sealed *P. coreana* furniture as a close space (Figure 1). Also, 5 locations were chosen to identify its self-regulating control under uncontrolled circumstances without any thermo-hygrostat equipment at the Anguk-dong detached palace: inner newly-fabricated *P. coreana* and *P. menziesii* wooden box located in room, inner room, inner newly-fabricated *P. coreana* box located outdoor, and the outside (Figure 2).

2.2. Active ingredient extraction from wood materials

Prior to evaluating the microbe and insect repellent activity of *P. coreana*, active ingredient was extracted from wood materials and *P. menziesii* was used as a control group.

Figure 1. Indoor controlled storage space with thermo-hygrostat at the Kyujanggak Institute for Korean Studies (A: *P. coreana* and *P. menziesii* box, B: Sealed *P. coreana* furniture, C: *P. coreana* corridor, D: Old *P. coreana* box, and E: Newly fabricated *P. coreana* box).

Figure 2. Indoor and outdoor uncontrolled traditional architecture without thermo-hygrostat at Anguk-dong detached palace, The Korea National University of Cultural Heritage (A: Overall building, B: Inner room, C: Newly fabricated *P. coreana* and *P. menziesii* box in a room, D: Newly fabricated *P. coreana* box in outdoors, and E: Inner *P. coreana* box with data logger).

Before extraction process, the wood materials (sap wood) were sufficiently dried, cut into 1 to 3 cm, and then powdered up into 700 meshes with a universal cutting mill (PULVERISETTE 19, FRITSCH, DEU) for an experiment.

The extracting process was divided into water-soluble extraction and volatile extraction to extract the active ingredient (Jeong *et al.*, 2018). The water-soluble extraction was made by mixing 100 g of dried wood powder and 1000 mL of distilled water at 30°C for 72 hours. Then, the mixture of dried powers and extract was separated from the filter and volatile condensation was conducted with evaporator under the 50 to 70°C. For volatile extraction, 100 g of wood powder was distilled into 125 mL of dichloromethane (CH₂Cl₂) that had been re-extruded from the SDE device. And then sodium anhydrous sulfate (Na₂SO₄) was added into the extract to remove the moisture and then condensed with N₂ gas.

2.3. Microbe repellent activity and antimicrobial activity test

On 23th June of 2016, the microbe was collected with air-collector (Buck Bio-Culture™ Pump, A.P. Buck, Inc, USA) sampling 100 L of air twice through PDA (Potato Dextrose Agar) at a national storage room of the Kyujanggak Institute for Korean Studies where the Annals of the Joseon Dynasty has been preserved until now. Total 5 locations were selected to collect the microbe: 3 locations in *P. corridor* of storage room as open areas and 2 locations in sealed *P. coreana* furniture as a close area. The collected microbe was cultured in a 28°C incubator for 3 to 5 days; and after extracted DNA was analyzed with PCR and Automatic DNA sequencer, BlastN search was conducted at the genebank (NCBI, National Center for Biotechnology Information).

To evaluate the microbe repellent activity test, 150 µl of a microbial suspension was inoculated on PDA and NA medium of which *P. coreana* samples (20 × 20 × 15 mm) were placed in middle and cultured to confirm the inhibition zone of the microbe formed around it. To assess the antimicrobial activity test with extract, 150 µl of a microbial suspension was applied soaking on a paper disc and cultured in 28°C to confirm the inhibition growth formed around the paper disc.

2.4. Insect repellent activity test

Two typical species of insects were provided and used at the Institute of Preventive Conservation for Cultural Property, Korea National University of Cultural Heritage for insect repellent activity test: *Reticulitermes speratus kyushuensis* and *Sitophilus oryzae*. *R. speratus kyushuensis* was known as one of the main pests that have damaged the wooden cultural heritage and *S. oryzae* was often used for judging the fumigation effect in a field of conservation science. *R. speratus kyushuensis* was collected from Hapjeong-ri, Buyeo on April of 2016 and only repletes were used after breeding at room temperature and RH 90±5%. *S. oryzae* was selected without distinction of their gender at 25±3°C and RH 75±5% at a laboratory.

In this study, the insect repellent activity tests were divided into contact toxicity, fumigation toxicity and insect repellent tests. 0.5 µl of extract was directly contacted to the thorax of 40 insects to identify whether they are confronted by death for the contact toxicity test. A close container method was applied for fumigation toxicity test; a filter paper soaking 100 µl of extract was put in the bottom of lower plastic container (65 × 65 × 100 mm) and then 40 insects and their small amount of their food (*R. speratus kyushuensis*: 2 × 2 cm filter paper, *S. oryzae*: grains of rice) were put together to the upper container; test was conducted at RH 90±5% for 72 hours with 24 hours intervals with 3 times repeats. In a T-shaped container, both treated area with water-soluble and volatile extracts and untreated area with distilled water and dichloromethane were placed on both sides. And then 40 insects were placed in the middle of container for the repellent tests. The absorption process with extracts upon the treated and untreated area was carried out in same way with a fumigation toxicity test. The repellent test was conducted on water-soluble and volatile extract of *P. coreana* in the dark room for 24 hours with 3 repeats.

3. RESULT

3.1. Temperature and relative humidity control

A national storage room of the Kyujanggak Institute for Korean Studies showed little monthly average temperature difference because the temperature and relative humidity have

kept constant with thermo-hygrostat equipment from October of 2015 to September of 2016. The monthly average temperature ranged minimum 19.0°C to maximum 21.3°C; and monthly average relative humidity was in range of 52.6% to 60.7% inside the controlled storage room. There was little temperature difference depending on its locations from uncontrolled traditional space without any thermo-hygrostat systems at Anguk-dong detached palace and it indicates the outside circumstances influenced the monthly average temperature of the indoor and outdoor spaces (Figure 3).

Measuring the monthly average relative humidity for 4 seasons under uncontrolled spaces, inner *P. coreana* boxes exposed to the outside followed the relative humidity of the outside. They are measured higher in fall season (77.7%) than

hot and humid summer season (72.7%) and the lowest was in spring season (61.0%). And under the controlled circumstances, there was no significant difference between the old *P. coreana* box and the newly fabricated *P. coreana* and *P. menziesii* box (Figure 4).

The monthly average daily temperature range of the outside was the highest in spring (March) and lowest in fall season (September). On spring of 2016, the average daily temperature range was 13.5°C in outside and 11.4°C in the inner *P. coreana* boxes exposed to the outside; that of inner *P. coreana* boxes installed in the uncontrolled room was 9.8°C; and those of inner *P. coreana* furniture and boxes installed in a controlled storage room ranged from 0.5°C to 0.9°C. It indicates that this was based on the outside weather (13.5°C)

Figure 3. Result of seasonal average temperature of *P. coreana* and *P. menziesii* under the controlled and uncontrolled surroundings in 2015 and 2016.

Figure 4. Result of seasonal average relative humidity of *P. coreana* and *P. menziesii* under the controlled and uncontrolled surroundings in 2015 and 2016.

and *P. coreana* boxes show a good buffering effect to adjust the monthly average daily temperature range from the outside; the different buffering degrees of a daily temperature range was confirmed depending on the locations: inner *P. coreana* wooden boxes installed in the outside (2.1°C), inner *P. coreana* and *P. menziesii* storage wooden box in uncontrolled room (3.7°C and 3.8°C) under the uncontrolled

circumstances. And, the average daily temperature range of inner old *P. coreana* heritage and newly fabricated *P. coreana* box was same under the controlled space (Figure 5).

Overall, the average daily relative humidity ranges were as high in a row of spring (March), summer (June), winter (December) and fall (September) season of 2015 and 2016. In December, the humidity self-control capacity of inner boxes

Figure 5. Result of average daily temperature range of inner *P. coreana* and *P. menziesii* in controlled and uncontrolled surrounding (°C).

Figure 6. Result of average daily relative humidity range of inner *P. coreana* and *P. menziesii* in controlled and uncontrolled surrounding (%).

(3.7% of average daily temperature range) was confirmed to 0.5% lower than that of the *P. menziesii* storage box (3.2%). In March, the humidity control capacity was increased under the controlled storage in the order of the newly fabricated *P. coreana* box (1.0%), new *P. menziesii* box (0.9%) and old *P. coreana* box (0.7%). In the spring season when the daily relative humidity range was the highest, buffering capacity for a daily humidity range showed 41.0% of the inner newly-fabricated *P. coreana* box installed in the outside as an uncontrolled space while daily relative humidity variability of the outdoor was 48.6%; and those of inner newly-fabricated *P. coreana* and *P. menziesii* box in an uncontrolled room were 43.6% and 43.9%. And the humidity control capacity of the *P. coreana* corridor where the indoor air moved inside the controlled storage was 0.6% lower than inner *P. coreana* furniture or box in a controlled storage room during the summer season of 2016 (Figure 6).

3.2. Microbe repellent activity and antimicrobial activity tests

Fungi was inoculated in a PDA medium and bacteria was conducted in a NA (Nutrient Agar) for 3 to 5 days in 28°C incubator until the microbial growth was visible to the naked eye. Through morphological separation, airborne microorganisms were isolated into 4 fungi and 5 bacteria, and the genetic analysis showed that the analyzed species were *Phaeosphaeriaceae* sp., *Staphylococcus aureus* and so on (Table 1).

The microbe repellent activity test was conducted to evaluate that the extracted materials can control the growth of microorganisms that target the surface wood, leading to contamination and/or discoloration. The result of the microbe repellent activity confirms that *P. coreana* and *P. menziesii* do not have any microbe repellent substances so the growth of inhibition zones was not formed for 4 fungi and 5 bacteria (Figure 7).

Table 1. Result of genetic analysis with microbe and bacteria

No.	Description	Accession	No.	Description	Accession
JF1	<i>Phaeosphaeriaceae</i> sp.	KU255073.1	JB1	<i>Staphylococcus aureus</i>	LC107806.1
JF2	<i>Phoma</i> sp.	JQ838011.1	JB2	<i>Bacillus cereus</i>	LN827673.1
JF3	<i>Ascomycota</i> sp.	JN850978.1	JB3	<i>Chryseobacterium hominis</i>	AM423087.1
JF4	<i>Pseudotaeniolina globosa</i>	KC311489.1	JB4	<i>Staphylococcus hominis</i>	EU337115.1
-	-	-	JB5	<i>Dermaococcus</i> sp.	JF905611.1

Figure 7. Result of microbe repellent activity of *P. coreana* (A: CF1, B: JF1, C: JB3, and D: JB5).

Figure 8. Result of antimicrobial activity of JF3 (left) and JF5 (right) with water-soluble extraction (A: distilled water, B: *P. coreana*, and C: *P. menziesii*).

Figure 9. Result of antimicrobial activity of JF2 (left) and JB2 (right) with volatile extraction (A: dichloromethane, B: *P. coreana*, and C: *P. menziesii*).

Table 2. Result of microbe repellent activity and antimicrobial activity of the extract (Growth and deterioration activity grade: none(-), weak(+), average(++), strong(+++))

No.	Description	Microbe repellent activity		Antimicrobial activity					
		<i>P. coreana</i>	<i>P. menziesii</i>	Water-soluble extraction			Volatile extraction		
				Distilled water	<i>P. coreana</i>	<i>P. menziesii</i>	Dichloro-methane (CH ₂ Cl ₂)	<i>P. coreana</i>	<i>P. menziesii</i>
CF1	<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-
JF1	<i>Phaeosphaeriaceae</i> sp.	-	-	-	-	-	-	-	-
JF2	<i>Phoma</i> sp.	-	-	-	-	-	-	-	-
JF3	<i>Ascomycota</i> sp.	-	-	-	-	-	-	-	-
JF4	<i>Pseudotaeniolina globosa</i>	-	-	-	-	-	-	-	-
JB1	<i>Staphylococcus aureus</i>	-	-	-	+	-	-	-	-
JB2	<i>Bacillus cereus</i>	-	-	-	+	-	-	-	-
JB3	<i>Chryseobacterium hominis</i>	-	-	-	++	-	-	-	-
JB4	<i>Staphylococcus hominis</i>	-	-	-	+	-	-	-	-
JB5	<i>Dermacoccus</i> sp.	-	-	-	++	-	-	-	-

Antimicrobial activity tests were performed to confirm whether the wood extract inhibited the growth of micro-organisms. In case of the fungi tested, the water-soluble extracts of *P. coreana* and *P. menziesii* did not produce clear zones of growth inhibition, indicating that these extracts do not have any antifungal properties. Meanwhile, the antibacterial activity tests revealed growth inhibition of five bacteria by the water-soluble extract of *P. coreana*, with clear zones ranging from 0.05 to 0.5 cm around the paper disc containing the extract (Figure 8). However, no antibacterial activity was observed for the water-soluble extract of *P. menziesii*. Notably, the volatile extracts from *P. coreana* and *P. menziesii*, including dichloromethane, did not produce growth inhibition zones in the antimicrobial activity tests. Thus, based on the antifungal and antibacterial activity tests, it was concluded that the volatile extracts from both *P. coreana* and *P. menziesii* do not affect the growth of fungi and bacteria (Figure 9, Table 2). Moreover, we determined that antifungal

substances were not present in these extracts.

3.3. Insect repellent activity

In the contact toxicity test, it was clear that *R. speratus kyushuensis* and *S. oryzae* were not identified toward the water-soluble extract whereas a large number of insects were identified with volatile extracts. This seems to be influenced by the solvent rather than the extract components since there is no significant difference from a control group, dichloromethane itself. The contact toxicity with both *R. speratus kyushuensis* and *S. oryzae* was equally ineffective in water-soluble extracts, and the effect from the volatile extracts was confirmed to be due to extracting solvent (Figure 10).

Monitoring for 3 days with fumigation toxicity tests of water-soluble extracts, *R. speratus kyushuensis* was active after 3 days and *S. oryzae* was reduced to average 3.2 insects after 3 days. This was confirmed that the numbers of both

Figure 10. Result of contact toxicity of *R. speratus kyushuensis* and *S. oryzae* with extract.

Figure 11. Result of fumigation toxicity of *R. speratus kyushuensis* and *S. oryzae* with extract.

R. speratus kyushuensis and *S. oryzae* were reduced by natural death, not by extracts; volatile extract is also considered to have no fumigation toxicity effect due to the small numbers of dead insects (Figure 11). Also, both *R. speratus kyushuensis* and *S. oryzae* freely moved toward both areas where the water-soluble and volatile extracts were absorbed and control areas with distilled water or dichloromethane in the repellent test. Therefore, these extracts from *P. coreana* and *P. menziesii* do not have any insect repellent so *R. speratus kyushuensis* and *S. oryzae* do not avoid the extracts.

4. CONCLUSION

The temperature and relative humidity control of *P. coreana*, which is widely used for the storage of the cultural heritage, was evaluated under the controlled and uncontrolled circumstances from 2015 to 2016. In this study, seasonal and daily change of temperature and relative humidity was mea-

sured to identify the self-control ability of *P. coreana* and it shows to be mainly excellent in humidity control. In spring season, when the daily relative humidity range was the highest, buffering capacity for a daily humidity range showed 41.0% of the inner newly-fabricated *P. coreana* box installed in the outside as an uncontrolled space while daily relative humidity range of the outdoor was 48.6%; and those of inner newly-fabricated *P. coreana* and *P. menziesii* box in an uncontrolled room were 43.6% and 43.9%. In winter and summer seasons, inner newly-fabricated *P. coreana* box installed outside shortened 32.5% gap of monthly average daily relative humidity range from the outside.

These results suggest that the circumstance control ability of wood box can shorten a sudden change period in case of an emergency situation where it is difficult to operate or control the constant temperature and relative humidity by unexpected breakdown or shutdown of the thermo-hygrostat in museums, libraries or art galleries. Based on the environmental data established in this, it is considered that further researches on the humidity control ability are needed with more detailed environment conditions whether there are archival and traditional binding books in a storage box or how many books are preserved in it because the papers can serve as a positive buffer agents with good humidity control ability together along the storage boxes. And then it is expected that these results will be applied in various ways to produce the manuals for integrated management and long-term preservation of cultural heritage in near future.

Also, microbe repellent activity, antimicrobial activity test and insect repellent test were experimented with water-soluble and volatile extracts from *P. coreana*. Its results concluded that *P. coreana* has no antifungal and antibacterial substances toward 9 different types of microbe collected at a storage space and inner furnitures made with its material. However, growth inhibition sized to 0.05 to 0.5 cm was observed around the paper disc applying with the water-soluble extract of *P. coreana* toward 5 bacteria; in particular, JB3 and JB5 showed growth inhibition zones so that this indicates water-soluble extract of *P. coreana* had a weak antibacterial activity.

The water-soluble extract of *P. coreana* wasn't effective to 2 types of insects as *R. speratus kyushuensis* and *S. oryzae* in the contact toxicity test; and water-soluble and volatile extracts also could not affect the fumigation effect to insects

in the fumigation toxicity test. And insects can approach freely toward the material itself extracted from *P. coreana* without any repellent in a repellent test. Following the published research, it coincides the conventional heritage storage box material, *P. coreana*, was not good enough for antimicrobial property (Chung *et al.*, 2008). Additionally, despite its customary use, it reported there are many disadvantages on electromagnetic wave blocking, gas absorption, alkali resistance, low strength for storing heavy materials, weather resistance and gas adsorption (National Research Institute of Cultural Heritage, 2009).

Summarizing these results, it can be judged that *P. coreana* does not prevent the biological deterioration when the pest comes into contact and/or attempted to attack it. Based on the integrated results, we cannot expect *P. coreana* boxes, which are widely used for the preservation of heritage materials, to have insecticidal or antibacterial properties. So we are planning to study further on the correlation between humidity and microbial growth in the future.

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