ISSN 1738-8716(Print)
ISSN 2287-8130(Online)
Particle and Aerosol Research
Part. Aerosol Res. Vol. 18, No. 2: June 2022 pp. 37-50
http://dx.doi.org/10.11629/jpaar.2022.18.2.037

지하철 객실 적용을 위한 황칠 추출물 소독제의 항균특성 및 안전성 평가

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(2022년 2월 4일 투고, 2022년 3월 18일 수정, 2022년 4월 9일 게재확정)

Anti-bacterial properties and safety evaluation of disinfectant using *Dendropanax morbifera* (Hwangchil) extract for passenger cabin in the subway

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(Received 4 Feb 2022; Revised 18 Mar 2022; Accepted 9 Apr 2022)

Abstract

Due to the syndrome coronavirus 2 (SARS-CoV-2) pandemic, the subway passenger cabin should be continuously sterilized. However, a disinfectant such as chlorine is toxic and can lead to different issues to human health. In this paper, we introduced a novel disinfectant based on natural product (Dendropanax morbifera extract). Via ultra-high performance liquid chromatography - mass spectrometer (UHPLC-MS), different compounds from Dendropanax morbifera extract showed antivirus potentials. Antimicrobial experiments confirmed that the air-disinfectant containing Dendropanax morbifera can eliminate harmful microorganisms including Gram (-), Gram (+), and yeast within 5 mins. The as-prepared air-disinfectant also showed high antivirus activity against H1N1, HRV, and EV71.
Deodorization test also indicates that the as-prepared air-disinfectant can lower the harmful gas such as ammonia and trimethylamine in the atmosphere. To evaluate the potential of air-disinfectant containing Dendropanax morbifera in practical applications, different safety tests including acute oral toxicity, acute skin irritation, and eye irritation were conducted. Results showed that the as-prepared disinfectant did not negatively affect tested animals during these safety investigations.

Keywords: *Dendropanax morbifera*, antivirus, antimicrobial, disinfectants, natural product, passenger cabin

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1. Introduction

The 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a global health emergency (Abdelrahman et al., 2020). Until July of 2021, the pandemic has infected more than 190 million people worldwide, and resulted in the death of more than 4 million people (mortality of 2.1%). In Korea, SARS-CoV-2 infected 179,203 people and lead to the death of 2,058 cases (source: Johns Hopkins University, access day: 2021/07/20). In Seoul, 40% of commuters use the subway system every day - the largest share among all transportation modes. Therefore, the subway system can accelerate the spread of the coronavirus. It is noticed that the passenger cabin is a closed environment, and people easily contact each other, especially during rush hour. From May 2020, subway passengers will be required to wear face masks, and from the end of November 2020, late-night subway services were reduced by 20%, starting from 10 p.m. as part of efforts to control coronavirus spreading. Therefore, a method to reduce the coronavirus inside the passenger cabin should be found. There is a need to directly spread the antivirus agents inside the passenger cabin with no harmful and annoying passengers. However, a popular disinfectants agent, such as chlorine, is toxic and can lead to human health problems. The sanitizing process should be conducted only when the passenger cabin is empty. Thus, finding alternative antivirus agents is necessary.

Dendropanax morbifera (Hwangchil) is an endemic species in Korea. Extracts from its leaf and stems could be a source of anti-oxidant and anti-cancer compounds (Hyun et al., 2013). Similar to *Houttuynia cordata*, we believed that quercetin and rutin from *Dendropanax morbifera* (*D. morbifera*) could be used to act against coronavirus (Chiow et al., 2016). Also, organic compounds with a caffeoyl moiety (e.g., caffeic acid, rosmarinic, chicoric acid, etc.) have antivirus properties towards herpes simplex (HSV), influenza, and immunodeficiency viruses (HIV) (Langland et al., 2018). Other compounds, such as myricetin, have antivirus properties towards SARS coronavirus helicase by affecting the ATPase activity (Yu et al., 2012). Meanwhile, kaempferol has antivirus activity against the 3a channel protein (Schwarz et al., 2014), and hesperetin could affect the 3C-like protease of SARS-CoV (Lin et al., 2005). Also, *D. morbifera* extract contains saponins to support improving immunity, and its effectiveness could be applied in various fields such as medicine, colorant, and tea ingredients. In this study, we used a mixture of D. morbifera extract, ethanol, and glycerin to inhibit different types of harmful bacteria and viruses. It is believed that *D. morbifera* extract's purifier is a good candidate as a direct disinfectant for subway passenger cabin.

2. Materials and Methods

2.1 Preparation and characterization of *D. morbifera* extract via microwave–assisted method

Dry leaves and the body of *D. morbifera* were washed with water, chopped into small pieces, and ground into a homogeneous mixture in a blender. The *D. morbifera* was irradiated in the microwave at 800 W for 10 min. After irradiation, the sample was cool down at room temperature. Then 15 mL of DI water was added to dissolve active agents, and insoluble impurities were removed by centrifugation. The pure extract solution was obtained via filtration by a 0.2 μm centrifuge tube filter. The active compounds of *D. morbifera* were characterized by an ultra-high performance liquid chromatography system coupled to a mass spectrometer (UHPLC-MS) equipped with an electrospray ionization source (ESI). The HPLC separation was conducted on a ACQUITY UPLC HSS T3 column (1.8 μm) with the following parameters: runtime: 25 min; solvent A: formic Acid; solvent B: acetonitrile; flow rate: 0.5 mL/min; gradient program: 0 min: 97% A, 0-1 min, 97% A,

1-15 min: 100% B, 15-16 min: 100% B, 16-19 min: 97% A, 19-25 min: 97% A; wavelength: 265 nm; column temperature: 35 oC; sample temperature: 12 oC; injection volume: 5 μL. The mass spectrometer was operated with the following parameters: capillary voltage: 3.0 kV (positive) and 2.8 kV (negative); resolution: 20,000; mass range scanned: 50-1200 m/z; nebulizer pressure: 6.4 Bar; drying gas temperature: 500 oC; drying gas flow rate: 800 L/h.

Freeze-drying process was used to produce pure extract powder. The extract yield (Y %) was calculated around 33.5% by the following equation:

$$Y(\%) = \frac{W_1}{W_2} \times 100$$

where W_1 is the weight (g) of pure extract powder, W_2 is the weight (g) of the initial *D. morbifera* powder.

2.2 Preparation of air-disinfectant containing *D. morbifera* extract

The air-disinfectant was produced by mixing ethanol, purified water, glycerin, (d)-limonene, dipropylene glycol, and *D. morbifera* extract compound. The concentration of each ingredient in the air-disinfectant was shown in Table 1. Although the concentration of ethanol at 60-95% generally inactivated most microorganisms, some microorganisms are still resistant to ethanol even at very high concentrations (~95%) (Kampf, 2018). In addition, *D. morbifera* extract is claimed to have antibacterial activity at 0.04 - 0.1 mg/mL (Kim et al., 2016). Based on this information, we select the concentration of ethanol and *D. morbifera* extract at 65% and 0.32 % (~3.2 mg/mL) to develop the air-disinfectant mixture to utilize the antimicrobial effects of both components.

Table 1. Ingredients of air-disinfectant containing *D. morbifera*.

Ingredients	CAS No.	Concentration (vol. %)
Ethyl alcohol	64-17-5	65
Purified water	77320-18-5	32.68
Glycerin	56-81-5	1.5
D. morbifera extract	977071-52-5	0.32
(d)-Limonene	5989-27-5	0.1
Dipropylene glycol	25265-71-8	0.1
Other	-	0.3

2.3 Antimicrobial and antiviral of air-disinfectant containing *D. morbifera* extract

The antimicrobial of air-disinfectant containing *D. morbifera* extract was conducted by Korea Institute of Construction and Living Environment Testing with KCL-FTR-1002:2018 standard method. Harmful bacteria and yeast including *Escherichia coli* (*E. coli*; Gram-negative (-)), *Klebsiella pneumoniae* (*K. peneumoniae*; Gram (-)), Methicillin-resistant *Staphylococcus aureus* (MRSA; Gram-positive (+)), *Staphylococcus aureus* (*S. aureus*; Gram (+)), *Candida albicans* (*C. albicans*, yeast), *Streptococcus mutans* (*S. mutans*, Gram (+)), *Pseudomonas aeruginosa* (*P. aeruginosa*; Gram (-)), and *Bacillus cereus* (*B. cereus*, Gram (+)).

Besides bacteria, different harmful viruses including Influenza A (H1N1), Human Rhinovirus A (HRV), and enterovirus 71 (EV71) were used to test the antiviral efficiencies of air-disinfectant containing *D. morbifera* extract. For this experiment, HeLa cells (2×10^4) were placed on each well of 96-well plate and cultured for 24 hours. After 24 hours, the culture supernatant from each well was removed, and virus solution was added to each well, and then air-disinfectant containing *D. morbifera* at concentration of 1 μg/mL were introduced. After the infection was completed, 100 μL of trichloroacetic acid (TCA,

10%) was added to each well of 96-well plate and left at 4oC for 1 hour, following by washing with DI water several times. After drying at room temperature, 100 μL of a 0.4 wt.% sulforhodamine B (SRB) solution in 1% (v/v) acid acetic was added and stained for 30 min. SRB staining solution that was not bound to cells was washed several times with 1% (v/v) acetic acid and dried again. Next, 100 μL of 10 mM Tris solution (pH 10.5) was added to each well to sufficiently dissolve the cell-bound dye, and then absorbance was measured at 560 nm. Each treatment group was denoted as an untreated group (*A*), a group treated with air-disinfectant containing *D. morbifera* (*B*), a group treated with only the virus (*C*), and a group treated with both virus and air-disinfectant containing *D. morbifera* (*D*). The cell viability (%) and the antiproliferative ability (%) of air-disinfectant containing *D. morbifera* against viruses were calculated according to the following equations:

$$Cell\ viability\ (\%) = \frac{A}{D} \times 100$$

$$Antiproliferative\ ability\ (\%) = \frac{D - C}{B - C} \times 100$$

Additionally, for evaluation of the antimicroorganism in reality, we applied the air-disinfectant containing *D. morbifera* into the dirty positions (testing area: 10 × 10 cm) including working desk and floor in the Bionano Research Institute 5th floor, Gachon University, Seongnam City, Korea. The adenosine triphosphate (ATP) levels of tested spots before and after applying air-disinfectant were measured by Clean-Q Real time Hygiene Monitoring System (Teltron Inc., Daejon, Korea). ATP is an energy source of all living organisms and can be used as an indicator for the existence of microorganisms.

2.4. Deodorization test

The deodorization test was characterized by KCL-EK608:2018 standard by Korea Institute of Construction and Living Environment Testing. Briefly, 10 mL of ammonia, 5 mL of trimethylamine, 20 mL of hydrogen sulfide, and 20 mL of methyl mercaptan were exposed to the tested room and purified with air-disinfectant containing *D. morbifera* extract, and the reduction of their concentration was measured.

2.5. Safety characterizations of air–disinfectant containing *D. morbifera* extract

2.5.1. Acute oral toxicity

The acute oral toxicity test was performed according to the National Institute of Environmental Sciences Notice No. 2020-08 with Animal Ethics Committee Approval No. IAC2020-2526. 12 female rats were divided into 3 groups, 2 groups (G1-G2) were fed with air-disinfectant containing *D. morbifera* extract at a dose of 300 mg/kg body weight (B.W.) and 2 groups (G3-G4) were fed at a dose of 2,000 mg/kg B.W. After administration, mortality, general symptoms, and weight changes were observed for 14 days. Surviving animals were additionally necropsied to check for organ abnormalities visually.

2.5.2. Acute skin irritation and corrosion test

Acute skin irritation and corrosion of the components of air-disinfectant containing *D. morbifera* extract were performed according to the National Institute of Environmental Sciences Notice No. 2019-23 with Animal Ethics Committee Approval No. IAC2020-2524. 0.5 mL of the test substance was administered to the back of a New Zealand white (NZW) rabbit for 4 hours. Mortality, general symptoms, weight change and skin irritation and corrosion were evaluated up to 72 hours after patch removal. The stimuli were scored according to Table S1.

2.5.3. Eye irritation and corrosion test

Eye irritation and corrosion of air-disinfectant containing *D. morbifera* extract were performed

Table S1. Generalsymptoms and skin irritiation evaluation table.

Erythema and Eschar Formation		Oedema Formation	
Symptoms	**Score**	**Symptoms**	**Score**
No erythema	0	No oedema	0
Very mild erythema (nearly non-discernible by naked eyes)	1	Very mild oedema (nearly non-discernible by naked eyes)	1
Clear erythema	2	Mild oedema (the swelling area is clear enough to be distinguished	2
Moderate erythema	3	Moderate oedema (the swelling area is ~1 mm)	3
Severe erythema	4	Severe oedema (the swelling area is 〉 1 mm	4
Highest score: 4		Highest score: 4	

according to the National Institute of Environmental Sciences Notice No. 2019-23 with Animal Ethics Committee Approval No. IAC2020-2525 and IAC2020-2545. After administering 0.1 mL of the test substance to the right eye (conjunctival sac) of a NZW rabbit, general symptoms, weight change, mortality, eye irritation, and corrosion were evaluated for 6 days. Eye mucosal reactions were observed up to 1, 24, 48, 72 hours, and 5 days in the initial test and 1, 24, 48, 72 hours, and 6 days in the confirmatory test. At 1, 24, 48, and 72 hours, the stimuli were scored according to Table S2.

3. Results and Discussion

3.1 Characterization of *D. morbifera* extract

UHPLC-MS chromatograms identified 33 main compounds of *D. morbifera* extract and their suggested beneficials (Fig. 1, Table S3). From Table S3, Quinic acid, 1-O-Caffeoylquinic acid, 4-O-Caffeoylquinic acid, and Apigenin-6-C-glucosylglucoside (Isovitexin) were identified as main antiviral compounds in the *D. morbifera* extract while 7-O-β-D-gluocopyranosyl-kaempferol, Kaemferol-3,7-di-O-β-D-glucopyranoside, Genistein-7,4'-di-O-β-D-glucoside, Aloe-emodin 8-glucoside, and 3-Hydroxyl baicalein were identified as possible antiviral compounds. For example, caffeic acid is proved to have antiviral activity against herpes simplex virus (HSV), influenza A, and human immunodeficiency virus (HIV) (Langland et al., 2018). Caffeic acid is believed to inactivate HSV via inhibiting viral-host cell interaction (Astani et al., 2012; Astani et al., 2014). Ge et al. (2018) found that most caffeoylquinic acid can inhibit the secretion of HBsAg and HBeAg and the replication of hepatitis B virus (HBV) (Ge et al., 2018). For example, at 100 μg/mL, monocaffeoylquinic acid 9 can inhibit HBsAg and HBeAg secretion and HBV DNA replication by 83.82, 70.76, and 33.96% compared to the control. Via the relationship between structure and activity, they found that caffeoylquinic acids containing one caffeoyl group have better inhibitory activities (Ge et al., 2018). Like caffeic acid, quinic acid could also inhibit the replication of HBV-DNA and the production of HBsAg (Wang et al., 2009). Vitexin has antiviral effects against rotavirus and parainfluenza type 3 virus (Li et al., 2002; Knipping et al., 2012). However, the antiviral mechanisms are unclear (Knipping et al., 2012). As mentioned above, Kaempferol can inhibit the 3a channel protein of coronavirus (Schwarz et al., 2014), baicalein can prevent the production of influenza A nucleoprotein

Table S2. Scorecriteria according to the classification of eye damage.

Cornea	Score
No ulcer or turbidity	0
Dispersed or dense turbidity. The end of iris is clearly observed	1
The translucent areas is easily observed. The end of iris is slightly indistinct	2
Perl luster is showed. The end of iris is not observed, the size of pupil is barely observed	3
The iris cannot be observed because the cornea is opaque	4
Iris	**Score**
Normal	0
Significant wrinkle formation, congestion, swelling, moderate hyperemia around the cornea, the iris responds to light	1
No responds to light, bleeding, mostly destroyed	2
Conjunctivae (Redness)	**Score**
Normal	0
Some blood vessels are clearly congested	1
Wide range of crimsom color, individual blood vessels are not easily observed	2
Wide range of red color (diffuse beefy red color)	3
Chemosis (Swelling)	**Score**
Normal	0
Slightly swelling (including labial membrane)	1
Significant swelling with partial abduction of the eyelid	2
Swelling of the eyelid to the extent that half of the eyelids is closed	3
Swelling of the eyelid to the extent that more than half of the eyelids is closed	4

in human lung epithelial (A549) cells infected with influenza H5N1 virus (Sithisarn et al., 2013), aloe-emodin can inactivate SARS-CoV via the 3CL protease inhibition (Lin et al., 2005), and genistein can inhibit the ion channel activity of vir HIV-1 protein Vpu of HIV (Sauter et al., 2014). Therefore, their derivatives (7-O-β-D-gluocopyranosyl-kaempferol, Kaempferol-3,7-di-O-β-D-glucopyranoside,

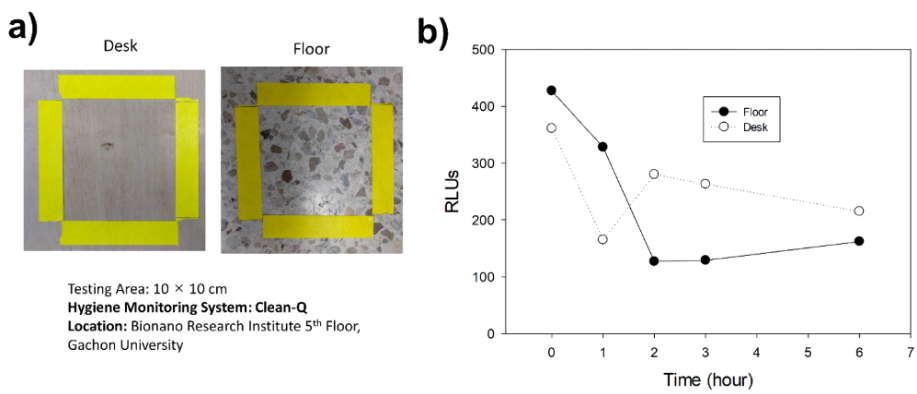

Figure S1. Anti-ATP efficiency of the air-disinfectant containing *Dendropanax morbifera* extract: a) Tested points; b) Result.

Table S3. Identified compounds in *Dendropanax morbifera* extract.

No	Compound	Formula	Ion mode	Retention time	Extract Mass (m/z)	Suggested role
1	**Quinic acid**	**$C_7H_{12}O_6$**	**-**	**0.59**	**191.0558**	**Astringent, anti-viral**
2	**1-O-Caffeoylquinic acid**	**$C_{16}H_{18}O_9$**	**-**	**2.95**	**353.0874**	**Phenolic acid, antioxidant, antibacterial, anticancer, antihistamic, anti-viral**
3	**4-O-Caffeoylquinic acid**	**$C_{16}H_{18}O_9$**	**-**	**3.52**	**353.0871**	
4	3-[(4-O-Acetyl-6-deoxy-alpha-L-mannopyranosyl)-oxyl]-2-(3,4-dihydroxyphenyl)-5-hydroxy-6-methoxy-4-oxo-4H-chromen-7-yl 2-O-acetyl-6-deoxy-alpha L-mannopyranoside	$C_{32}H_{36}O_{18}$	-	3.43	707.1825	Flavonoids
	Benzyl alcohol xylopyranosyl(1->6)glucopyranoside	$C_{18}H_{26}O_{10}$	-	3.71	401.1437	Tea aroma glycosidic precursor bioactivation
6	**Apigenin-6-C-glucosylglucoside (Isovitexin)**	**$C_{27}H_{30}O_{15}$**	**-**	**3.79**	**593.1497**	**Anti-inflammatory, antioxidant, antibacterial, anti-Alzheimer's disease, anti-diabetic, anti-viral**
			+	3.79	595.2663	
7	Apocynoside I	$C_{19}H_{30}O_8$	-	3.86	431.1901	Inhibition of CYP2C9 - causing aging and lowered disease resistance
8	Corchoionoside C	$C_{19}H_{30}O_8$	-	3.86	431.1909	Flavonoids
9	1,3-Dihydroxy-2-hydromethylanthraquinone-3-B-β-D-xylopyranose(1->6)-β-D-glucopyranoside	$C_{16}H_{28}O_{14}$	-	4	563.1395	N/A
			+	3.99	565.2557	
10	Apiin	$C_{16}H_{28}O_{14}$	-	4.09	563.1402	Anxiolytic, anti-inflammatory, anti-cancer, anti-fungal
			+	4.09	565.1562	
11	Isochaftoside	$C_{16}H_{28}O_{14}$	-	4.23	563.1399	Flavones
12	***7-O-β-D-gluocopyranosylkaempferol***	***$C_{21}H_{20}O_{11}$***	**-**	***4.24***	***447.0924***	***Anti-inflammatory, anti-cancer, anti-diabetes Inhibit vascular endothelial inflammation Protect the cranial nerve and heart function Treat fibropoliferative disorders, anti-viral***
			+	**4.13**	**449.1083**	
13	***Kaempferol-3,7-di-O-β-D-glucopyranoside***	***$C_{27}H_{30}O_{16}$***	**-**	***4.46***	***609.1453***	
			+	***4.46***	***611.1618***	
14	Nelumboroside A	$C_{27}H_{30}O_{16}$	-	4.57	609.1450	Antioxidant
15	3,8-Di-C-glucosylapigenin	$C_{27}H_{30}O_{15}$	-	4.79	593.1499	N/A
16	***Genistein-7,4'-di-O-β-D-glucoside***	***$C_{27}H_{30}O_{15}$***	**-**	***4.79***	***593.1499***	***Prevent hypertension, anti-cancer, maintaining bone mineral density, anti-Alzheimer's disease, anti-viral***
17	Terestigmine	$C_{21}H_{33}N_3O_3$	-	9.08	374.2436	Cholinesterase inhibitor (treatment of cognition disorders)

18	N–(3–Methoxy–5–nitrophenyl)–2–(5–methyl–3,4–dinitro–1H–pyrazol–1–yl)acetamide	$C_{13}H_{12}N_6O_8$	+	0.56	381.0795	N/A
19	Guanine	$C_5H_5N_5O$	+	1.45	152.0566	Nucleobases
20	Daidzein–4',7–diglucoside	$C_{27}H_{30}O_{14}$	+	4.42	579.1728	Phytoestrogen
21	Viscidulin I	C15H10O7	+	4.46	303.0502	Inhibitor of hepatocellular carinoma cell (protein Glypican–3)
22	Viscumneoside III	$C_{25}H_{26}O_{13}$	+	4.47	535.1454	Tyrosinase inhibition (skin whitening)
23	***Aloe emodin 8-glucoside***	***$C_{21}H_{20}O_{10}$***	-	***4.51***	***431.0971***	***Anti-diabetic, DNA targeting molecule, anti-viral***
			+	***4.51***	***433.1132***	
24	3,7,8,3',4'–Pentahydroxyflavone (Melanoxetin)	$C_{15}H_{10}O_7$	+	4.63	303.0499	The strong antioxidant flavonoids, xanthine oxidase inhibition, antihyperruricemic, anti–inflammatory, enhance immune–regulation
25	Rubianic acid (dithiooxamide)	$C_{25}H_{26}O_{13}$	+	4.65	535.1451	Chelating agent (detection of copper), building block in the synthesis of cyclen
26	***3-Hydroxy baicalein***	***$C_{15}H_{10}O_6$***	+	***4.79***	***287.0548***	***Anxiolytic, antiestrogen, anti-inflammatory, anti-cancer, antibacterial, anti-viral***
27	7–Hydroxy–1–methoxy–2–methoxyxanthone	$C_{15}H_{10}O_6$	+	4.79	287.0548	N/A
28	6,6'–Iminobis(2,2–dimethyl–1–hexanol)	$C_{16}H_{35}NO_2$	+	7.7	274.2738	N/A
29	N~2~–[(2S,4S,5S)–5–Amino–6–cyclohexyl–4–hydroxy–2–isopropylhexanoyl]–N–[2–pyridinylmethyl)–L–isoleucinamide	$C_{27}H_{46}N_4O_3$	+	9.08	475.3646	N/A
30	4–{5–[(4–Methyl–1–piperidinyl)methyl]–1H–tetrazol–1–yl}–N–([1,2,4]triazolo[4,3–a]pyridin–3–ylmethyl)butanamide	$C_{19}H_{27}N_9O$	+	9.08	398.2415	N/A
31	1–Methyl–2–[(Z)–8–tetradecenyl]–4(1H)–quinolone	$C_{24}H_{35}NO$	+	9.08	276.2596	N/A
32	O–Benzyl–N–[9–(1H–imidazol–1–yl)nonanoyl]–L–seryl–N~6~–[(benzyloxy)carbonyl]–N–(2–cyclohexylethyl)–L–lysinamide)	$C_{44}H_{64}N_6O_6$	+	9.08	773.4956	N/A

Bold: Main anti-viral compounds
Italic and Bold: Potential anti-viralcompounds

Genistein-7,4'-di-O-β-D-glucoside, Aloe-emodin 8-glucoside, and 3-Hydroxyl baicalein) may also have antivirus activity. However, the antivirus potentials of these agents should be more confirmed.

3.2 Antimicrobial and antiviral of air–disinfectant containing *D. morbifera* extract

The antimicrobial of air-disinfectant containing *D. morbifera* extract was shown in Table 2 and Fig. 2.

It is clearly recognized that the air-disinfectant containing *D. morbifera* extract can eliminate harmful microorganisms after contacting for 5 min and have antimicrobial effects in both Gram (-) and Gram (+) bacteria as well as yeast. Table 3 indicated that the air-disinfectant containing *D. morbifera* extract almost inhibits the growth of harmful viruses with the antiproliferative ability (%) of 95.29 %, 96.12%, and 95.83% for H1N1, HRV, and EV71, respectively. In reality, the air-disinfectant can immediately reduce the ATP level of the dirty desk and floor surface. Furthermore, the anti-ATP efficiency can remain up to 4 hours (Fig. S1). This result indicated the potential of the air-disinfectant containing *D. morbifera* extract in practical applications. In the upcoming work, we will investigate the antimicrobial activities of the air-disinfectant by reaction time.

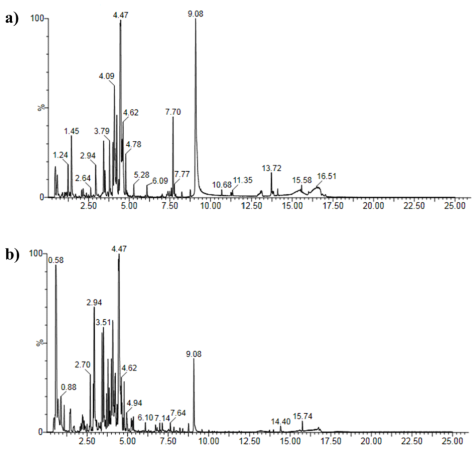

Figure 1. High resolution extractedion chromatograms for *D. morbifera* extract: a) positive ion mode; b) negative ion mode.

3.3. Deodorization test

The deodorization of air-disinfectant containing *D. morbifera* extract was summarized in Table S4. In general, the as-prepared air-disinfectant has high performance in the removal of toxic gas such as ammonia (97.5%) and trimethylamine (75.0%,) and can slightly reduce the concentration of hydrogen sulfide (6.0%) and methyl mercaptan (25.0%).

Table 2. Antimicrobial activities of air-disinfectant containing *D. morbifera* extract.

Species	Samples	Initial concentration (CFU/mL)	Concentration after 5 min reacted with a tested solution	Antibacterial efficiency (%)
E. coli	Blank	8.3×10^4	8.3×10^4	-
	Air-disinfectant	8.3×10^4	< 10	99.9
K. pneumoniae	Blank	9.6×10^4	9.6×10^4	-
	Air-disinfectant	9.6×10^4	< 10	99.9
MRSA	Blank	9.0×10^4	9.0×10^4	-
	Air-disinfectant	9.0×10^4	< 10	99.9
S. aureus	Blank	1.8×10^4	1.8×10^4	-
	Air-disinfectant	1.8×10^4	< 10	99.9
C. albicans	Blank	1.5×10^4	1.5×10^4	-
	Air-disinfectant	1.5×10^4	< 10	99.9
S. mutans	Blank	4.0×10^4	4.0×10^4	-
	Air-disinfectant	4.0×10^4	< 10	99.9
P. aeruginosa	Blank	5.5×10^4	5.5×10^4	-
	Air-disinfectant	5.5×10^4	< 10	99.9
B. cereus	Blank	5.6×10^4	5.6×10^4	-
	Air-disinfectant	5.6×10^4	< 10	99.9

Table 3. Antiviralability of air-disinfectant containing *D. morbifera* extract.

	H1N1	HRV	EV71
Cell viability (%)	175.8	179.1	182.0
Antiproliferative ability (%)	95.29	96.12	95.83

Table S4. Deodorization performances of air-disinfectant containing *Dendropanax morbifera* extract.

Odor	Deodorization performance (%)	Condition
Ammonia	75.0	Temperature: 20.8 ±0.3 ℃ Humidity: 43.8 ±0.8%
Trimethylamine	97.5	
Hydrogen sulfide	6.0	
Methyl mercaptan	25.0	

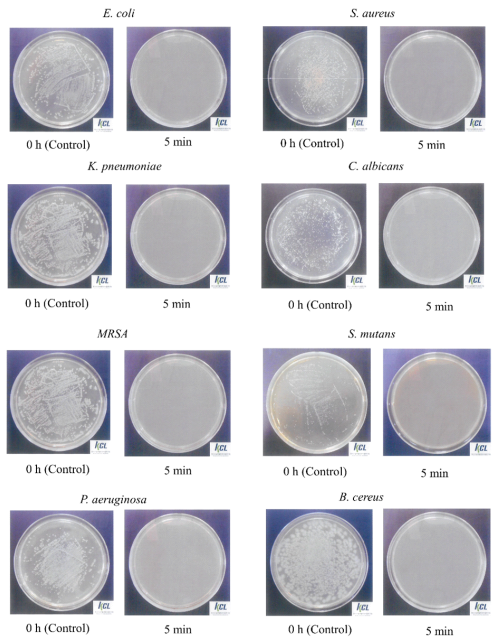

Figure 2. Antimicrobial efficiency of air-disinfectant containing *D. morbifera* against *E. coli*, *K. pneumoniae*, MRSA, *P. aeruginosa*, *S. aureus*, *C. albicans*, *S. mutans*, and *B. cereus*.

3.4. Safety characterizations of air-disinfectant containing *D. morbifera* extract

3.4.1. Acute oral toxicity

Table S5 indicated that no abnormal findings were observed 14 days after administering air-disinfectant containing *D. morbifera* extract. In addition, there is no significant change in B.W. of tested animals. The additional necropsy experiments confirmed no abnormal findings were observed in any groups, even at a high concentration dose (2,000 mg/kg B.W.)

3.4.2. Acute skin irritation and corrosive test

During the experimental period, no animals were died due to the administration of the test substance. Also, no specific abnormal symptoms and changes of B.W. were observed in all experimental rabbits. Skin irritation and corrosion were not observed in the initial test, and corrosion was observed at 72 hours after patch removal in the confirmatory test (Fig. 3). The mean score of "Erthyma and Eschar Formation" was "0.0" in the initial test and "0.7" in one confirmatory test (1201). Corrosion was not observed in one confirmatory test at 72 hours. Therefore, it is impossible to calculate the average skin response score (TableS6).

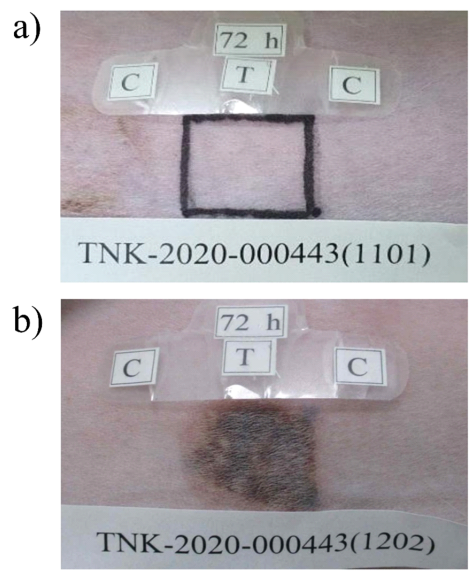

Figure 3. Skin photograph at 72 hours after application of test substance: a) initial test; b) confirmatory test.

Table S5. Results of acute oral toxicity test.

Group	Dose (mg/kg B.W.)	Mortality (%)	Animal Number	Days after administration								Necrospy findings
				0		1		7		14		
				Clinical signs	B.W.	Clinical signs	B.W.	Clinical signs	B.W.	Clinical signs	B.W.	
G1	300	0	2101	N*	218.8	N	239.2	N	260.2	N	276.2	N
			2102	N	217.8	N	240.9	N	244.9	N	244.6	N
			2103	N	213.0	N	233.2	N	256.5	N	270.4	N
G2	300	0	2201	N	226.6	N	248.5	N	269.5	N	269.3	N
			2202	N	228.1	N	256.5	N	273.4	N	272.0	N
			2203	N	239.0	N	276.6	N	288.0	N	292.7	N
G3	2,000	0	2301	N	218.8	N	240.2	N	244.2	N	256.9	N
			2302	N	199.7	N	218.3	N	225.2	N	232.9	N
			2303	N	217.8	N	244.3	N	253.3	N	275.6	N
G4	2,000	0	2401	N	222.3	N	239.1	N	262.3	N	264.7	N
			2402	N	239.8	N	255.0	N	287.0	N	286.9	N
			2403	N	233.0	N	255.7	N	272.1	N	277.2	N

Table S6. Results of skin irritation and corrosive test.

Symptoms	Erythema and Eschar Formation			Oedema Formation		
Group	G1	G2		G1	G2	
Observation time/Animal number	1101	1201	1202	1101	1201	1202
1 h	0	0	0	0	0	0
24 h	0	0	0	0	0	0
48 h	0	1	1	0	0	0
72 h	0	1	–[(a)]	0	0	–[(a)]

3.4.3. Eye irritation and corrosion test

During the experimental period, no animals were died due to the administration of the test substance. Lacrimation was observed in the initial test, while no weight loss was observed due to the administration of the test substance. Conjunctivae redness and conjunctivae edema (chemosis) were observed. However, the eye mucous membrane irritation was recovered within 5 days in the initial test and within 6 days in the confirmatory test, and reversibility was confirmed. The mean ocular mucosal response score for conjunctival flare was calculated as “1.7” in the initial test and “0.3 and “1.0” in the confirmatory test. The means score of the ocular mucosal reaction for conjunctival edema was calculated as “0.0” in the initial test and “0.7” and “0.7” in the confirmatory

Table S7. Results of eye irritation and corrosive test.

Group	Animal Number	Observation Time	Cornea (Opacity)	Iris	Conjunctivae (Redness)	Chemosis (Swelling)
G1	1101	1 h	0	0	2	3
		24 h	0	0	2	0
		48 h	0	0	2	0
		72 h	0	0	1	0
		All eye irritation symptoms were recovered within 5 days after administration (Conjunctivae: 1 h – 4 days, Chemosis: 1h)				
G2	1201	1 h	0	0	1	2
		24 h	0	0	1	2
		48 h	0	0	0	0
		72 h	0	0	0	0
		All eye irritation symptoms were recovered within 3 days after administration (Conjunctivae: 1 h – 24 h, Chemosis: 1 h – 24 h)				
	1202	1 h	0	0	1	2
		24 h	0	0	1	2
		48 h	0	0	1	0
		72 h	0	0	1	0
		All eye irritation symptoms were recovered within 6 days after administration (Conjunctivae: 1 h – 5 days, Chemosis: 1 h – 5 days)				

test. The average ocular mucosal response score is shown in Table S7.

3.5. Potentials of air–disinfectant containing *D. morbifera* extract for usage in subway passenger cabin

According to the safety test results of air-disinfectant containing natural products such as *D. morbifera* extract, it has potential to be used in the subway passenger cabin even with passengers inside. However, in order to ensure the safety of this product before installation, the evaluation of inhalation toxicity should be further conducted. Air-disinfectant containing *D. morbifera* can be inserted in the humidifier (Fig. 4), designed to own a nozzle that allows the disinfectant agent to spread to the subway passenger cabin with the suspended size of ~0.48 μm. The suspended size is tested by Korea Testing Certification. With the cabin size of ~40 m^2, the spray interval time can be automatically set every 45 minutes for 10 seconds and only cost 200 mL of air-disinfectant each month. However, depends on the number of passengers, the spray interval can be manually adjusted. Also, the humidifier with the slim design and the maximum acoustical volume used at power-up is < 35 dB, it can be installed and operated in the subway cabin without the notice of passengers.

4. Conclusion

Antivirus disinfectant containing *D. morbifera* extract was developed. Different antibacterial and antivirus compounds from *D. morbifera* extract have been identified. From antimicrobial experiment,

antivirus disinfectant containing *D. morbifera* extract can eliminate various harmful microorganisms after 5 min of contact. Similar to bacteria, dangerous viruses including H1N1, HRV, and EV71 were also inhibited when interacting with the as-prepared antivirus disinfectant. The as-prepared can also strongly reduce ammonia and trimethylamine in the atmosphere. Via different safety tests including acute oral toxicity, skin irritation, and eye irritation, antivirus disinfectant containing *D. morbifera* is believed to be safe for use in practical applications. In the upcoming research, after evaluating the inhalation toxicity, antivirus disinfectant containing *D. morbifera* extract will be embedded into a humidifier and confirm its efficiency in the subway passenger cabin.

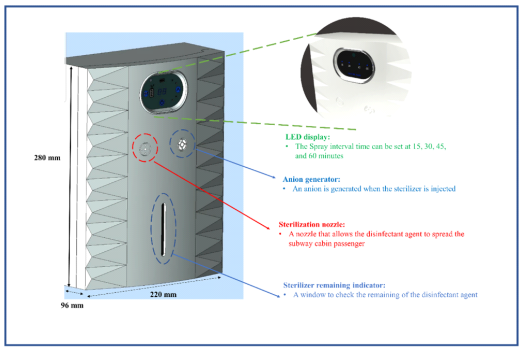

Figure 4. Design of humidifier containing air-disinfectant containing *D. morbifera* (Hwangchil) extract for built-in passenger cabin.

Acknowledgement

서울특별시 서울산업진흥원 <서울글로벌챌린지 2021> 사업을 통해 우수 논문으로 선정되어 작성된 논문이다.

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