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Houttuynia cordata (HC) has been commonly used as many traditional remedies in local areas of Japan. Although many pharmacological activities of HC have been reported, the mechanism underlying the effect of HC remains unknown. We conducted the interview survey in Japan to verify how HC was actually used. The interview survey revealed that HC poultice (HCP) prepared from smothering fresh leaves of HC was most frequently used for the treatment of purulent skin diseases including furuncle and carbuncle with high effectiveness. Ethanol extract of HCP (eHCP) showed anti-bacterial effects against methicillin-resistant *Staphylococcus aureus* (MRSA), and showed an anti-biofilm activity against MRSA. eHCP showed dose-dependent inhibition of *S. aureus* lipoteichoic acid (LTA)-induced interleukin-8 and CCL20 production in human keratinocyte without any cytotoxicity. These results suggest that HCP is effective for skin abscess and its underlying mechanism might be the complicated multiple activities for both bacteria and host cells.

Key words: *Houttuynia cordata* poultice; methicillin-resistant *Staphylococcus aureus*; anti-biofilm activity; interleukin-8; CCL20

Houttuynia cordata (HC) Thunberg is a flowering perennial herb with peculiar smell. It is native to Japan, Korea, China, Southeast Asia, and Himalayas. HC belongs to Saururaceae family, and *Houttuynia* is a monotypic genus. HC has been used medicinally in Asia, and its shoots are edible in China and Malaysia.[1–3] HC has been also commonly used as a traditional remedy for treatment of various diseases or symptoms in local areas of Japan.

Houttuynia Herb was first listed since 1961 in the 7th Revised Japanese Pharmacopoeia. In the 16th Revised Japanese Pharmacopoeia guidebook (2011), it was listed the dried areal parts of HC for the treatment of constipation and chronic skin diseases, and was showed diuretic and anti-inflammatory effects by drinking a decoction. It was reported that HC contains many constituents such as essential oil, flavonoids and other polyphenols, alkaloids, organic acid and fatty acid, sterols and microelements, and has various pharmacological activities including diuretic, anti-bacterial, anti-viral, anti-cancer/-tumor, anti-inflammatory, anti-oxidative, anti-diabetic, anti-allergic, and anti-mutagenic effects.[4,5] However, although many pharmacological activities of HC have been reported,[4–13] the mechanism underlying the effect of HC remains unknown.

In the present study, we conducted the interview survey to verify how HC was actually used for the treatment of various diseases and investigated its underlying mechanisms. Our data showed that HC poultice (HCP) prepared from smothering fresh leaves of HC in hot ashes or over charcoal fire wrapped with big leaves or Japanese paper was most frequently used for the treatment of purulent skin diseases including skin abscess caused by *S. aureus*[14,15] with high effectiveness. We also demonstrated that the ethanol extract from HCP (eHCP) had an anti-bacterial effect against *S. aureus* including MRSA and an anti-biofilm effect against MRSA, and further suppressed *S. aureus* LTA-induced IL-8 and CCL20 production in human keratinocytes. These results suggest that HCP is effective for skin abscess and its underlying mechanism might

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be the complicated multiple activities for both bacteria and host cells.

Materials and methods

The interview survey. An interview survey using a questionnaire (Fig. 1) was performed for local residents of Tsuno-cho, Kochi Prefecture. In Kochi Prefecture, past records using HC for many treatments of diseases and symptoms are stored and HC was growing wild. Our interview survey was approved by the ethics committee of Tokushima University Hospital (approved number: 2344). Participants in this study provided written informed consent in the presence of the facilitator. We asked all assentients about their age and whether or not to experience using HC as traditional remedies. We made digitization in seven phase evaluations of beneficial effects: 3; highly effective, 2; effective, 1; some effective, 0; ineffective, −1; some aggravation, −2; aggravation, −3; highly aggravation.

Plant materials and sample preparations. HC was identified by Dr. K. Fujikawa of the Kochi Prefectural Makino Botanical Garden, and voucher specimens (FOS-005024, FOS-005025 and FOS-007536) were deposited there. Native-grown HC was obtained in Kochi, and was cryopreserved at −80 °C until just before use. eHCP was prepared as follows: approximately 20 g of fresh leaves of HC wrapped in aluminum foil was heated at 450 W for 6 min using an electromagnetic cooker to obtain HCP. It was shaken with 5 mL of ethanol for 10 min and then centrifuged for 20 min at 1500 g. After centrifugation, the clear top layer was recovered and stored at −20 °C until assayed. A water extract of HCP was prepared as follows: 10 g of HCP was shaken with 10 mL of sterile purified water for 15 min, and then centrifuged for 20 min at 1,500 g. After centrifugation, the clear top layer was recovered. A decoction of HCP was prepared as follows: 50 g of HCP was decocted with sterile purified water for 30 min at 90–95°C (final 40 mL, EK-SA 10, ZOJIRUSHI, Osaka, Japan), and then centrifuged for 20 min at 1,500 g. After centrifugation, the clear top layer was recovered.

Bacterial strains and growth conditions. The bacterial strains used in this study are shown in Table 1. *Enterococcus faecalis*, *Escherichia coli*, *Serratia marcescens,* and *Pseudomonas aeruginosa* were grown in Muller-Hinton broth (MHB, Becton Dickinson, Sparks, MD, USA) supplemented with 50 µg/mL of $CaCl_2$, 25 µg/mL of $MgCl_2$, and *S. aureus* (methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* MRSA) and *S. epidermidis* were grown in MHB supplemented with 25 µg/mL of $CaCl_2$, 12.5 µg/mL of $MgCl_2$ and 2% NaCl.[16] *Streptococcus pyogenes*, *S. mitis*, *S. agalactiae,* and *S. constellatas* were grown in brain heart infusion (Becton Dickinson). For time kill assay of MRSA, trypticase soy broth (TSB, Becton Dickinson) and TSB agar (Wako, Osaka, Japan) were used, and for biofilm formation assay of MRSA, TSB supplemented with 0.3% glucose was used.

Anti-bacterial assay. The bacterial cell suspension of MRSA COL was adjusted to approximately 1×10^8 colony forming unit (CFU)/mL with saline. Fresh

Fig. 1. Questionnaire used in the survey.

Table 1. Bacterial strains.

Strain	Source
Methicillin-sensitive *Staphylococcus aureus* T-MSSA 1–11 strains	Clinical isolates
Methicillin-resistant *Staphylococcus aureus* T-MRSA 1–61 strains	Clinical isolates
MRSA COL	Wild type
Staphylococcus epidermidis TSE1	Clinical isolate
Streptococcus pyogenes TSP2	Clinical isolate
Streptococcus mitis JCM12971	Type strain
Streptococcus agalactiae TSA1	Clinical isolate
Streptococcus constellatas 4528	Clinical isolate
Enterococcus faecalis TEF1	Clinical isolate
Pseudomonas aeruginosa PAO1	Wild type
Escherichia coli K1	Wild type
Serratia marcescens TSM1	Clinical isolate

leaves (2.56 ± 0.03 g) of HC and lump (2.56 ± 0.03 g) of HCP were put on the MHB agar plates where bacteria was spread and then these plates were incubated for 24 h at 37 °C.

Susceptibility assay. The minimum inhibitory concentration (MIC) of eHCP was determined by a microbial broth dilution method. Approximately 10^6 CFU/mL of bacterial culture was inoculated into 100 μL of medium containing a twofold serial dilution of eHCP in 96-well culture plate (TPP, Trasadingen, Switzerland) and incubated for 20 h at 37 °C. The MIC of eHCP was defined as the lowest concentration showing no bacterial growth.

Time kill assay of MRSA. Approximately 10^6 CFU/mL of MRSA COL was cultured in fresh TSB supplemented with 10% eHCP for various periods of time at 37 °C with aeration and then spread onto TSB agar. After 24-h incubation at 37 °C, CFU was enumerated. 10% ethanol was used as negative control.

Biofilm formation assay. A crystal violet biofilm assay was performed to quantify the biofilm mass as previously described.[17] T-MRSA 31 strain (2×10^6 CFU) was added into 150 μL of TSB supplemented with 0.3% glucose and 1% eHCP in a U-bottomed 96-well plate (Cellstar, Greiner-bio-one, Germany) and incubated for 24 h at 37 °C. 1% Ethanol was used as a negative control. After incubation, formed biofilms were washed with purified water twice without disturbing the adherent biofilm, stained with 150 μL of 0.1% crystal violet for 10 min at room temperature, and excess stain was removed by gentle washes with purified water twice. After being dried, the stained biofilm was extracted from the well by adding 150 μL of ethanol and the absorbance of the extract from stained biofilm was measured at 595 nm using a microplate reader (model 680; Bio-Rad Laboratories, Hercules, CA, USA).

Cell culture. RT-7 cells, an immortalized human keratinocyte cell line, kindly provided by Dr. N. Kamata (Hiroshima University, Japan) as described previously[18] were cultured in Keratinocyte-SFM (Gibco

BRL, Gaithersburg, MD, USA) supplemented with 100 U/mL of penicillin and 100 μg/mL of streptomycin (Gibco BRL) at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2. Confluent monolayers were cultured with 1 μg/mL of purified *S. aureus* LTA (InvivoGen, San Diego, CA, USA) and/or 0.1, 0.5, 1% eHCP.

Lactate dehydrogenase cytotoxicity assay. The effect of eHCP on cell cytotoxicity was determined using Lactate dehydrogenase (LDH) assay. Confluent RT-7 cell monolayers in a 24-well plate were cultured in Kerationocyte-SFM medium supplemented with 0.1, 0.5, 1% eHCP for 24 h at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2. As a positive control, RT-7 cells were treated with 0.1% Triton X-100 and gently shaken for 10 min at room temperature. For the cytotoxicity assay, the levels of LDH in the recovered cell culture supernatants were determined using LDH cytotoxicity assay kit (Cayman Chemical, Ann Arbor, MI, USA) in accordance with the manufacturer's instructions. Absorbance was measured at 490 nm using a microplate reader (Bio-Rad Laboratories).

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay kits were used to quantify interleukin (IL)-8 and CCL20/macrophage inflammatory protein-3α (R&D Systems, Minneapolis, MN, USA) in cell culture supernatants.

Statistical analysis. All statistical analyses were performed using the unpaired Student's *t* test. Differences were considered significant when the probability value was less than 5% ($p < 0.05$).

Results

The interview survey. We performed the interview survey, and obtained 96 assentients (49 females and 47 males; response rate 86.5% of 111 people), and 85.4% of 96 assentients were over 60 years old. In 96 assentients, we acquired 58 people who experienced using HC (PEHC, 28 females and 30 males; 60.4% of 96 assentients).
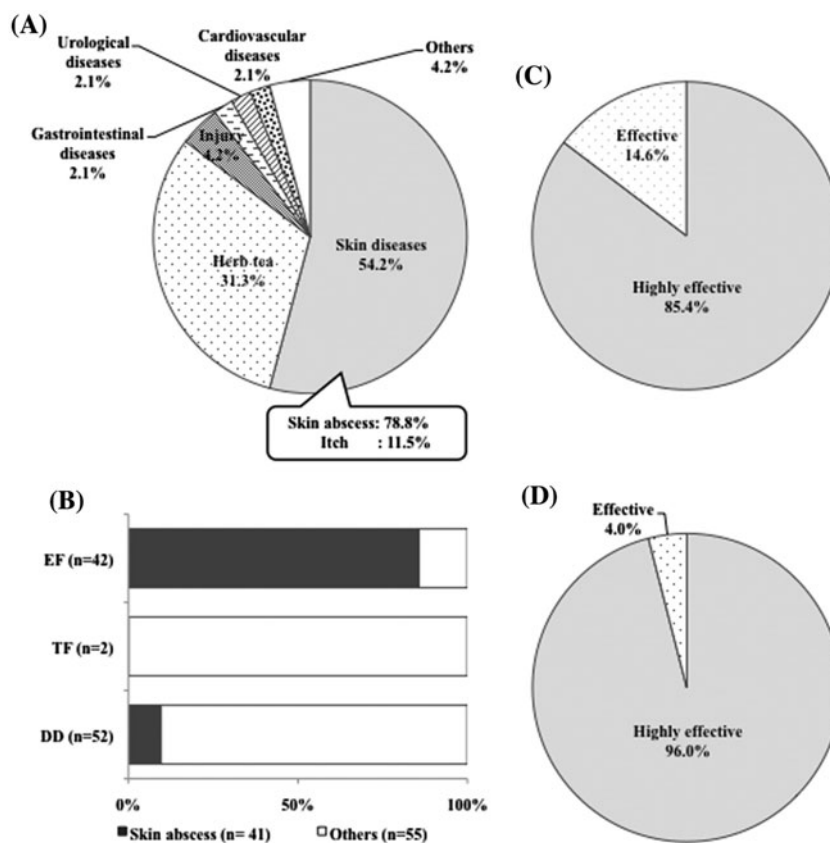
Fig. 2.　Interview survey results.

Notes: (A) Pie chart represents the purposes of HC application. (B) Bar chart represents the mode of application of HC (*n* = 96). EF: the external use of fresh leaves, TF: taking fresh leaves, DD: drinking the decoction of dried aerial parts or whole parts. (C) Pie chart represents effectiveness of HC for skin abscess (*n* = 41). (D) Pie chart represents effectiveness of HCP for skin abscess (*n* = 25).

We obtained 96 answers from 58 PEHC by multiple answers allowed in the questionnaire. Fig. 2(A) shows the classification of the purpose of applications from PEHC. Especially, the purpose of applications of skin abscess accounted for 42.7% of 96 answers and it occupied 78.8% in skin diseases.

Fig. 2(B) shows the modes of applications of skin abscess and others. The modes of applications from PEHC were the external use of fresh leaves (EF), taking fresh leaves (TF), and drinking the decoction of dried aerial parts or whole parts (DD). 85.7% of EF was applied for skin abscess. EF for skin abscess was further categorized into three: (1) the external use of smothered (in hot ashes or over charcoal fire) fresh leaves of HC wrapped with big leaves such as butterburs, mulberries, persimmons, Japanese paper, or aluminum foil (HCP, 69.4%); (2) the external use of fresh leaves of HC warmed over fire (11.1%) and (3) the external use of crumpled fresh leaves of HC (19.4%).

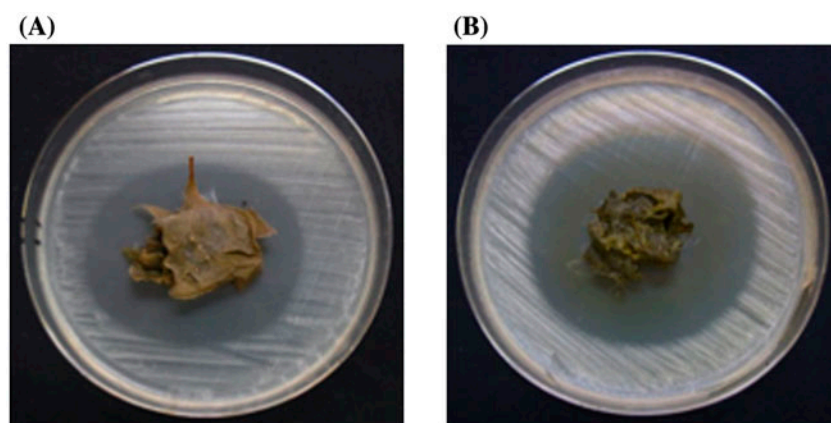Fig. 3.　Growth inhibitory effect of HCP against MRSA.

Notes: Fresh leaves (2.56 ± 0.03 g) of HC (A) or Lump (2.56 ± 0.03 g) of HCP (B) were put on the MHB agar plates where the bacterial cell suspension (approximately 1×10^8 CFU/mL) of MRSA COL was spread. After 24-h incubation at 37 °C, the size of growth-inhibitory zone was measured. Representative data from three separate experiments are shown.

Table 2. MIC of eHCP, water extract and decoction of HCP against MRSA COL.

	MIC	
	(%)	(µg/mL)
Ethanol extract of HCP (eHCP)	0.6	110
Water extract of HCP (shaking in water for 15 min)	>10	ND[a]
Decoction of HCP (30 min at 90–95 °C)	>10	ND[a]

[a]ND: not determined.

Regarding the effectiveness of HC for the treatment of skin diseases, HC was also shown to be effective for skin abscess (Fig. 2(C)). Furthermore, HCP was shown to be high effective for the treatment of skin abscess (Fig. 2(D)). It should be noted that there were no responses of adverse reactions by using HC. However, it was found that HCP is currently seldom used and only elderly people had the experience of using it. According these findings, HCP was found to have been used for the treatment of skin abscess with high effectiveness.

Effects of H. cordata *poultice on susceptibilities of MSSA and MRSA.* We first determined whether HCP and fresh leaves of HC have the growth inhibitory effect against MRSA COL. We could observe the growth-inhibitory zone of HCP and fresh leaves of HC
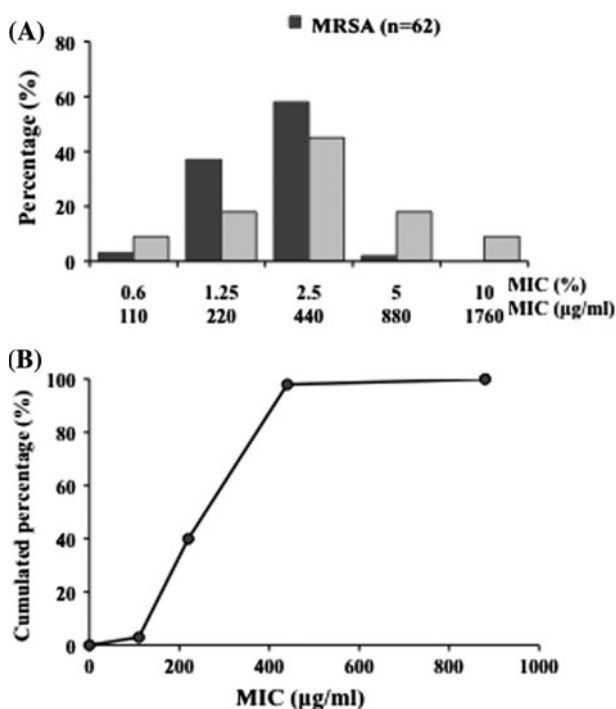

Fig. 4. Effects of eHCP on susceptibilities against MRSA and MSSA.

Notes: Bacterial culture (10^6 CFU/mL) was inoculated into 100 µL of medium containing a twofold serial dilution of eHCP in a 96-well culture plate and incubated for 20 h at 37 °C. The MIC of eHCP was defined as the lowest concentration showing no bacterial growth. The percentages of total strains (MRSA COL, 61 MRSA, and 11 MSSA clinical isolates) (A) and the cumulated percentages of 62 MRSA (MRSA COL and 61 MRSA clinical isolates) (B) at the indicated MIC were presented.

(Fig. 3). Then, we determined anti-bacterial effects of eHCP, water extract of HCP, and decoction of HCP against MRSA COL by a microbial broth dilution method. This result demonstrated that eHCP, not water extract and decoction of HCP, have the anti-bacterial activity against MRSA COL (Table 2). Next, we determined anti-bacterial effects of eHCP against MSSA and other MRSA clinical isolates. As shown in Fig. 4, MIC range of eHCP for both MSSA and MRSA was from 0.6% (110 µg/mL) to 10% (1,760 µg/mL). More than 70% of MICs for MRSA and MSSA clinical isolates were below 2.5% (440 µg/mL). We also determined the anti-bacterial effect of eHCP against other bacteria, which causes cutaneous infections such as furuncle, impetigo, and bedsore,[19–23] by the same susceptibility assay. eHCP also had antibiotic effects for *S. epidermidis*, *S. pyogenes*, *S. mitis,* and *E. faecalis*, not for *S. agalactiae*, *S. constellatas*, *S. marcescens*, *E. coli,* and *P. aeruginosa* (Table 3).

Bactericidal effect of ethanol extract of H. cordata *poultice for MRSA.* We performed the time kill assay to determine whether the anti-bacterial effects of eHCP for MRSA COL are bactericidal or bacteriostatic. Fig. 5 shows that the number of viable cells after 10 h-incubations with 10% eHCP (16 times MIC) was about 10 times lower than that of control (10% ethanol). This result demonstrated that eHCP had a bactericidal effect for MRSA but this activity was not so strong.

Anti-biofilm effects of ethanol extract of H. cordata *poultice.* Next, we tried to examine the anti-biofilm effects of eHCP for MRSA. For this experiment, we used the culture of T-MRSA 31 clinical isolate because MRSA COL had less biofilm formation activity than T-MRSA 31 (data not shown) and confirmed that MIC of eHCP against T-MRSA 31 was 2.5% (440 µg/mL). The results of biofilm formation assay showed that 1% eHCP significantly inhibited biofilm formation (Fig. 6).

No cytotoxic effect of ethanol extract of H. cordata *poultice on keratinocytes.* To confirm the absence of any cytotoxic effect of eHCP on keratinocytes, we measured the level of LDH released from RT-7 cells. As a positive control, keratinocytes were treated with 0.1% Triton X-100 for 10 min. No cytotoxic effect of

Table 3. MIC of eHCP against cutaneous infections-related bacteria.

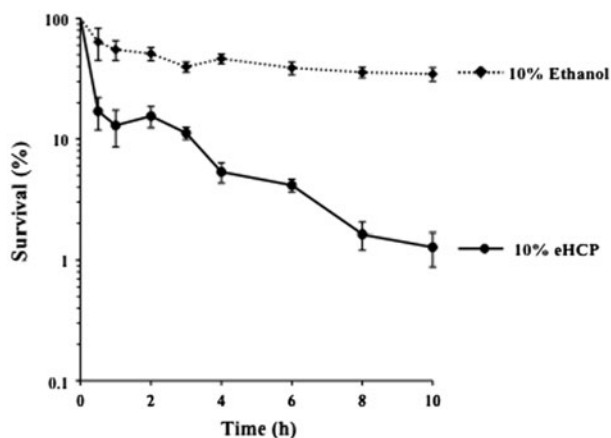| Bacterial strain	MIC	
	(%)	(µg/mL)
Staphylococcus epidermidis TSE1	2.5	440
Streptococcus pyogenes TSP2	10	1760
Streptococcus mitis JCM12971	1.25	220
Streptococcus agalactiae TSA1	>10	>1760
Streptococcus constellatas 4528	>10	>1760
Enterococcus faecalis TEF1	5	880
Pseudomonas aeruginosa PAO1	>10	>1760
Escherichia coli K1	>10	>1760
Serratia marcescens TSM1	>10	>1760

Fig. 5.   Bactericidal effect of eHCP for MRSA COL.

Notes: Approximately 10^6 CFU/mL of MRSA COL strain was cultured in TSB supplemented with 10% eHCP (solid lines) for various periods of time at 37 °C with aeration and then spread onto TSB agar. After 24-h incubation at 37 °C, CFU was enumerated. Ethanol (10%) was used as negative control (dotted line). The survival percentages were calculated by the CFU number compared to 100% when the bacterial cell culture was started. Data are presented as mean ± standard deviation (*n* = 3).

eHCP (up to 1.0%) was observed because the level of LDH was almost same as negative controls (up to 1.0% ethanol; data not shown).

Inhibitory effect of ethanol extract of H. cordata *poultice on chemokines production in keratinocytes.* Toll-like receptor (TLR)-2, a pattern recognition receptor specifically for LTA from Gram-positive bacteria such as *S. aureus*, plays a major role in Gram-positive bacterial infection.[24] Keratinocytes secrete pro-inflammatory mediators in response to various stimuli including microbial infections, chemical, and thermal irritations. LTA from *S. aureus* activates host cells thorough TLR-2 pathway and stimulates the excessive production of pro-inflammatory cytokines and chemokines including IL-8 and CCL20.[25–29] Therefore, we examined the inhibitory effects of eHCP (0.1, 0.5, and 1%) on IL-8 and CCL20 productions in *S. aureus* LTA-stimulated RT-7 cells. These results showed that eHCP significantly inhibited IL-8 and CCL20 productions in a concentration-dependent manner (Fig. 7).

Discussion

Although many pharmacological activities of HC have been reported,[4,5] the underlying mechanism of its activities remains unknown. In the present study, we conducted the interview survey to verify how HC was actually used for the treatment of various diseases or symptoms and investigated the mechanism involved in its effects. The interview survey revealed that HCP was most frequently used for the treatment of skin abscess with high effectiveness.

We first confirmed that HCP has growth-inhibitory effect against MRSA COL using anti-bacterial assay (Fig. 3), suggesting that the lump of HCP has the anti-bacterial activity against MRSA. Previous reports have
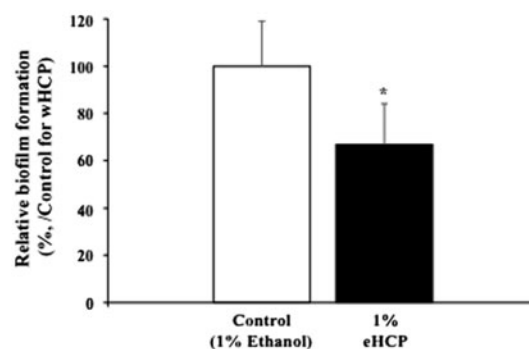

Fig. 6.   Anti-biofilm effects of eHCP on T-MRSA 31 clinical isolate.

Notes: T-MRSA 31 strain (2 μL, 10^9 CFU/mL) was added into 150 μL of TSB supplemented with 0.3% glucose and 1% eHCP in a U-bottomed 96-well plate and incubated for 24 h at 37 °C. Ethanol (1%) was used as a control. After incubation, formed biofilms were washed, stained with 50 μL of 0.1% crystal violet, incubated for 10 min at room temperature, and excess stain was removed by gentle washes. After being dried, the stained biofilm was extracted from the well by adding 150 μL of ethanol and the absorbance of the extract from stained biofilm was measured at 595 nm. The relative biofilm formation was calculated by the absorbance compared with the absorbance of control. Data are presented as mean ± standard deviation (*n* = 8). Asterisk shows significant difference versus control group (**p* < 0.01 vs. control group).
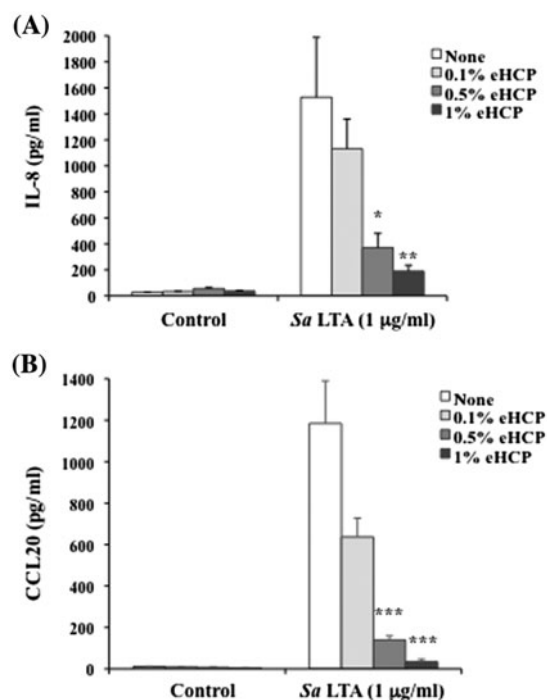

Fig. 7.   Inhibitory effect of eHCP on chemokines production in *S. aureus* LTA-stimulated RT-7 cells.

Notes: Confluent RT-7 cell monolayers cultured in a 24-well plate were stimulated with 1 μg/mL of *S. aureus* LTA and eHCP (0.1, 0.5 or 1.0%) for 24 h at 37 °C. The production levels of IL-8 and CCL20 in the recovered cell culture supernatants were determined. Data are presented as mean ± standard deviation (*n* = 4). Asterisks show significant differences vs. no eHCP group (None) (**p* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. no eHCP group).

shown that essential oil from HC, which contains aldehydes such as capric aldehyde (decanal), lauryl aldehyde (dodecanal), and decanoyl acetaldehyde (3-oxo-dodecanal, houttuynin), has an anti-bacterial

activity against Gram-positive bacteria including *S. aureus*.[6–9] Decanoyl acetaldehyde had better anti-bacterial activity than other aldehyde, but is less stable than others.[8] We considered that a process of smothering fresh leaves of HC wrapped with aluminum foil is similar to collecting its essential oil by steam distillation for enhancing the anti-bacterial activity of HC. This study also showed that eHCP has anti-bacterial effects against 61 MRSA clinical isolates as well as MRSA COL (Table 2, Fig. 4). Interestingly, eHCP also had a bactericidal effect against MRSA COL (Fig. 5).

S. aureus expresses several virulence factors, including cell wall-associated teichoic acid and cell surface proteins that promote adherence to host tissues. *S. aureus* can also form a slimy layer as a biofilm after adhering to tissue surface and cause chronic and recurrent infections.[14,15,19] Most of adhesion factors are staphylococcal surface-attachment proteins such as fibronectin binding proteins.[30,31] This study demonstrated that eHCP could inhibit the biofilm formation (Fig. 6). Therefore, we infer that eHCP may inhibit an expression of surface-attachment proteins or extracellular matrix productions.

Previous study reported that quercitrin (3-rhamnosyl-quercetin), which is one of flavonoid glycosides of HC, had an inhibitory effect on acute inflammatory edema induced by various phlogistic agents, such as histamine and serotonin.[11] Other studies demonstrated that essential oil purified from dried aerial parts of HC mediated an inhibition of cyclooxygenase-2 by a similar mechanism to that of nonsteroidal anti-inflammatory drugs[12] and also inhibited nitric oxide and tumor necrosis factor-α production in lipopolysaccharide-stimulated mouse peritoneal macrophages.[13] Regarding anti-inflammatory effects of HCP, this study showed that eHCP has the anti-inflammatory effect to inhibit IL-8 and CCL20 productions from *S. aureus* LTA-stimulated human keratinocytes in a concentration-dependent manner without cytotoxic effect (Fig. 7). These findings are in agreement with the previous report showing that water extract of HC leaves by boiling for 1 h had an anti-inflammatory activity against LTA-induced inflammation in human dermal fibroblast.[10] These studies indicate that active constituents of HC, which has anti-inflammatory effects for TLR-2-mediated inflammation, probably exist in fresh leaves of HC and are stable against drying, smothering in hot ashes or over charcoal fire and boiling.[10–13] Our recent results also suggest that HCP has natural infection-fighting properties against *S. aureus,* which causes skin abscess, and plays important roles in a host defense against *S. aureus* infection. However, these active constituents in HCP, which plays various roles in anti-inflammatory effects, still remain unclear, and they are now under investigation in our laboratory.

S. aureus is the main pathogen of the cutaneous and systemic infections such as furuncle, furunculosis, impetigo, and staphylococcal scaled skin syndrome.[14,15,19] And impetigo is a highly cutaneous infectious disease most commonly seen in children. *S. aureus* is the most common pathogen of impetigo and *S. pyogenes* is frequently isolated with *S. aureus*.[20,21]

The detection of gentamicin resistant *S. aureus* in children with impetigo by the abuse of the gentamicin ointment becomes the serious problem in Japan.[32–35] As HCP showed anti-bacterial activity against *S. aureus* and *S. pyogenes*, it would be useful for therapeutic adjunct against impetigo.

Since the development of penicillin, antibiotics have been a valuable medicinal product. However, overuse of antibiotics often causes a serious medicinal problem for emergence of drug-resistance strains such as MRSA and multidrug-resistant *P. aeruginosa*. Patients with diabetic, chronic ulcer in hospital, and cancer or elderly people in nursing home are the high-risk population of these infectious diseases.[36–38] As many MRSA carriers among long-term hospital inpatients develop bedsore, the spread of nosocomial infection becomes an increasingly serious problem in Japan.[39] Our results that eHCP has anti-bacterial activity against bacteria including *S. epidermidis*, *S. pyogenes*, *S. mitis*, and *E. faecalis*, which is often detected in bedsore region,[23] suggesting that HCP is useful for therapeutic adjunct against cutaneous infections such as bedsore caused by normal inhabitant.

An anti-inflammatory effect of HC was documented in Chinese "Systematic Materia Medica (*BenCao-GangMu*)" *et al.*[40,41] Therefore, HC has been used for the treatment of inflammatory skin diseases for 1500 years. Moreover, before 900 years ago in these antiquarian books, similar processing methods for HCP, by which fresh leaves of HC were put in to a Henon bamboo and smothered in hot ashes, was documented for a treatment of aggravated carbuncle.[40,41] The remedy of using HCP for treatment of skin abscess including furuncle and carbuncle was the ancient tradition and widely spread in Japan. Our findings demonstrated that HCP had the anti-bacterial, anti-biofilm, and anti-inflammatory effects against *S. aureus* including MRSA with multiple activities and supported that HCP as a traditional remedy is a rational approach for skin abscess. However, the detailed mechanism of HCP is still unclear and the identification of active constituents is currently under investigation.

Conclusions

Our survey shows that HCP had been frequently used for the treatment of skin abscess with high effectiveness and widely accepted. This experimental study also demonstrated the anti-infective properties of HCP, such as the anti-bacterial, the bacteriostatic, and the anti-biofilm effects against MRSA. Moreover, we elucidated that HCP had the anti-inflammatory effect to inhibit IL-8 and CCL20 productions from *S. aureus* LTA (TLR-2 ligand)-stimulated human keratinocytes without any cytotoxic effect. These findings suggest that HCP might be therapeutically useful as both antibiotic and anti-inflammatory modulators for skin infection and inflammation, such as skin abscess including furuncle and carbuncle, with high and long-term effectiveness due to various infection-fighting properties of HCP.

Author contributions

Conceived and designed the experiments: Yasuko Sekita, Hiroyuki Mizuguchi, Satoshi Ogino, Hiroyuki Fukui, Takashi Matsuo, Yoichiro Miyake, and Yoshiki Kashiwada. Performed the experiments: Yasuko Sekita, Keiji Murakami, Hiromichi Yumoto, and Takashi Amoh. Analyzed the data: Yasuko Sekita, Keiji Murakami, and Hiromichi Yumoto. Wrote the paper: Yasuko Sekita, Keiji Murakami, Hiromichi Yumoto, Yoichiro Miyake, and Yoshiki Kashiwada.

Disclosure statement

No potential conflict of interest was reported by the authors.

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