Antimicrobial Effect of \textit{trans}-Cinnamaldehyde, \textit{(-)}-Perillaldehyde, \textit{(-)}-Citronellal, Citral, Eugenol and Carvacrol on Airborne Microbes Using an Airwasher

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Citral, \textit{trans}-cinnamaldehyde, \textit{(-)}-perillaldehyde, \textit{(-)}-citronellal, eugenol and carvacrol were tested for their influence on microbial count in air by vaporizing with an air washer. The highest antibacterial activity was observed when \textit{(-)}-perillaldehyde was sprayed. The average reduce of germ count was 53%. On the other hand, the antimicrobial activity of eugenol was the lowest of these six compounds. The average reduction of germ count was 13%. When water without volatile compounds was sprayed, the colony forming units increased. These results suggest the utility of selected aroma-compounds for the control of bacteria in the room.

Key words antibacterial activity; airborne microbe; air washer; volatile compound

We are exposed to air bacteria in our daily life. Many microbes are floating in the air. For example, when microbes drop down on foods, the microbes will grow up there and foods will be spoiled. Therefore it is expected to develop an easy and safety way to decrease airborne microbes at places where we treat foods such as kitchen and convenience store.

Antimicrobial effects of aroma compounds have been described in several studies. In the majority of the studies, direct contact between the aroma compounds in a culture medium such as the disc diffusion, well diffusion, agar dilution and broth dilution method is mainly used for evaluating antimicrobial activity of aroma compounds. However, studies on vapours of aroma compounds on microbes are rare, and beyond that they are performed only using small scale methods.1–7

Therefore, we focused our attention on the antimicrobial activity of vapours of aroma compounds and attempted to evaluate it in a large scale, namely, using a room. It is considered that to use a room for measurement is enough to estimate the antimicrobial activity of vapours of aroma compounds. Furthermore, it seems that this large scale of evaluation is necessary and useful for practical applications.

For spreading the aroma compounds in the room, an air washer is used. Although an airwasher removes floating dusts and imparts moisture into the room, concerning airborne microbes we assumed that by spreading water into the room, it promotes an increase of airborne microbes.

In the present study six volatile compounds, namely \textit{trans}-cinnamaldehyde, \textit{(-)}-perillaldehyde, citral, \textit{(-)}-citronellal, eugenol and carvacrol were chosen and examined for their antimicrobial activity of their vapours spread with an air washer which is a convenient way to spread volatile compounds.

The antimicrobial activity of volatile compounds \textit{(-)}-citronellal and citral, which is a mixture of geranial and neral, were reported.6,8–10 \textit{(-)}-Citronellal and citral are main components of lemon scented tea tree oil and this oil has been used for foods as flavor.\textit{(-)}-Perillaldehyde is also known as an antimicrobial agent.6,11 It is the major compound of the green leaves of Japanese shiso which is used for traditional Chinese herb medicines. In Japan, the high humidity causes food poisoning, particularly at rainy season. Furthermore, as known well, Japanese are customed towards eating raw sea foods. Therefore people have used some plants for inhibiting foodborne disease since long ago and Japanese shiso have used as one of inhibiting foodborne agents.

The strong antimicrobial activity of \textit{trans}-cinnamaldehyde was also reported.12–14 Cinnamon is used for variety of foods or beverage as flavour and to inhibit food poisoning. Several studies have shown the antimicrobial activity of eugenol and carvacrol.13–16 These phenolic compounds have also been used as spice on account of their strong antimicrobial activity for long time.

MATERIALS AND METHODS

General The air samples were taken with a RCS Air Sampler, purchased from Biotest AG, Dreieich, Germany. The RCS Air Sampler uses inertial impaction to collect the airborne microbes. The microbes are impacted on commercially available agar strips, which are incubated after sampling in an incubator. According to the manufacturer’s specifications the sampling volume of the RCS Air Sampler is 280 l/min and the separation volume is 40 l/min for particles with a diameter of 4 \mu m. The volatile compounds were spread with an air washer. The air washer (Type LW24, with a capacity of 7 l water) was purchased from Venta, Germany. The experiments were carried out in a lecture room at the University-Centre Althanstrasse 14 (UZA II), Centre of Pharmacy, Vienna, Austria. The air volume of lecture room was 168 m$^3$. During testing the doors and windows were kept closed.

Culture Medium Agar strips TC (Art. No. 941105050) for determination of total microbial counts, obtained from Biotest AG, Dreieich, Germany, were used as culture media. Colony forming units (CFU)/m$^3$ were calculated after incubation at 30 °C for 48 h. Then the average reductions of germ count were calculated.

Aroma Compounds \textit{trans}-Cinnamaldehyde (CAS-No. 14371-10-9), \textit{(-)}-perillaldehyde (CAS-No. 18031-40-8), citral (CAS-No. 499-75-2), \textit{(-)}-citronellal (CAS-No. 5949-05-3), eugenol (CAS-No. 97-53-0), carvacrol (CAS-No. 499-75-2) were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany.

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Measuring Procedure The air washer was fixed on the desk at centre of the lecture room (Fig. 1, point 1). At first, the air samples in this room were taken with the RCS Air Sampler for 8 min at each five well-defined measuring points (Fig. 1, measuring points 1—5) in order to get the blank values. Then 0.84 g of volatile compounds were added to 7 l water in the air washer and vaporized. The concentration of aroma compounds was 5.0 mg/m³. One hour later, the air samples were collected again by the same procedure. The measurements were performed ten times with each aroma compound and the blanks.

Statistical Analysis The statistical comparisons between control and each volatile compound as well as value comparison prior and after fragrance diffusion were performed using Student’s t-test. \( p<0.05 \) was considered to be significant.

RESULTS AND DISCUSSION

The first data were taken after pure water was spread. The germ count increased in comparison to blank values, taken before. As shown in Fig. 2, the colony forming units increased approximately 35%.

Next, the antibacterial activities of four aldehydes were evaluated. The highest antibacterial activity was obtained by \((-\)-perillaldehyde, which reduced the germ count by 53% \( p<0.001 \)) on average as shown in Fig. 2. \( trans \)-Cinnamaldehyde also yielded good antimicrobial results on airborne microbes. As demonstrated in Fig. 2, it decreased the germ count by 45% \( p<0.001 \). \( (-\)-Citronellal exhibited moderate antimicrobial activity, the average germ count reduction was 30% \( p<0.001 \), results see Fig. 2). Citral, another aldehyde (mixture of geranial and neral) however, did not furnish acceptable results. The average reduction of germ count was only 13% \( p<0.001 \), results see Fig. 2).

It can be stated that according to the above results, \((-\)-perillaldehyde and \( trans \)-cinnamaldehyde are demonstrably effective against airborne microbes. Although the antimicrobial activity of \((-\)-citronellal and carvacrol was not high, these compounds performed acceptable. As described above, these chosen six volatile compounds are recognized as strong antimicrobial agents. However, surprisingly phenolic compounds showed lower activity than it was expected in this research. Although the reason for these poor results is not clear, this might be on account of their rather moderate solubility in water and low volatility.

Seven liters of water was used for the air washer, therefore the compounds which have hydroxyl group might be solved to a certain extent and thus remain more in the water phase.

In this research 5.0 mg/m³ of aroma compounds were used, respecting that this amount of aroma compounds is not great. Generally glutaraldehyde, formaldehyde, and phenol derivatives such as cresol are used as disinfectants because of their high disinfection capacity.

However, glutaraldehyde shows a high toxicity and its vapour irritates eyes, nose and throat. Formaldehyde also stimulates mucosa and furthermore it is a carcinogen. The toxicity of cresol is lower and it is used as a disinfectant, but spreading this compound is also harmful. Thus, with regard to their high toxicity and ugly smell, common disinfectants are not useful to decrease airborne microbes. On the other hand, the toxicity upon inhalation of the six aroma compounds tested in the present study is generally not recognized as being harmful in the chosen concentration. Therefore, people could stay in the testing room during measurement after spreading aroma compounds. Spreading volatiles with an air washer is an easy and safety way to decrease the germ count in rooms, such as places where we treat foods. Furthermore, this method is also considered to improve environmental health at public places, such as theatres, stations and airports. Particularly, old people or patients show generally weak resistance against microbes. Therefore, it is useful to decrease airborne microbes also at retirement homes and hospitals, too. The convenient new safety method, presented in our investigation, could contribute to improve environmental health.
REFERENCES