

Decreased immunoreactivity of hepatitis E virus antigen following treatment with Sakhalin spruce (*Picea glehnii*) essential oil

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Objectives: The hepatitis E virus (HEV) has become a problem in the meat processing field in recent years, and it is known to cause an acute hepatitis called hepatitis E in humans. The 20 million cases that occur every year include more than 3 million symptomatic cases and 60,000 fatalities. Hepatitis E virus is a known zoonotic causative virus and is distributed worldwide. HEV of non-enveloped is highly resistant to inactivation by alcohols and commercially available alcohol-based disinfectants. The hepatitis E virus (HEV) causes a common infectious disease that infects pigs, wild boars, deer, and humans. In most cases, humans are infected by eating raw meat. Some essential oils have been reported to exhibit antiviral activities. In this study, in order to investigate the anti-HEV properties of essential oils, the immunoreactivities of HEV antigen proteins against the relevant antibodies were analyzed after the HEV antigens underwent treatment with various essential oils.

Materials and Methods: The branches and leaves of Sakhalin spruce growing in Teshikaga, Hokkaido (Japan), were collected and crushed. Subsequently, water were added to crushed branches and leaves, and the mixture thus obtained was heated. The steam generated during heating was cooled in a cooling tube and recovered together with the essential oil. In the present study, only the essential oil layer was collected and used. A partially improved enzyme immunoassay test kit (IgG/IgM anti-HEV antibody determination kit by EIA, Institute of immunology, Tokyo, Japan) was used. The absorbance at 450 nm was measured using an absorption microplate reader. The sakhalin spruce essential oil was analyzed using a GC/MS. The identification of each compound was based on the computer matching of the mass spectrometry pattern obtained for the essential oil with the mass spectra included in standard reference databases.

Results and Discussion: Sakhalin spruce essential oil, lemon essential oil, tea tree essential oil, eucalyptus essential oil, and lavender essential oil affected reactivity to HEV antigens. The antigen reactivity of different concentrations (10-100%) of sakhalin spruce essential oil was analyzed by EIA. Comparison of the EIA absorbance of the reaction mixtures obtained revealed that sakhalin spruce essential oil inhibited the reactivity of the antibodies to the HEV antigen at concentrations above 30%. As part of the effort to determine the composition of Sakhalin spruce essential oil, GC/MS analyses were conducted on the said substance. The results of a similarity search conducted to compare the experimental MS pattern with the MS peaks included in the standard reference databases allowed the identification in the Sakhalin spruce essential oil sample of monoterpenes like bornyl acetate, borneol, α -pinene, camphene, camphor, and limonene. A total of 12 different compounds representing 95.69% of the contents of the Sakhalin spruce essential oil were identified. The main volatile constituents in the Sakhalin spruce essential oil were bornyl acetate (32.30%), α -pinene (16.66%), camphene (11.14%), β -phellandrene (9.09%), camphor (5.52%), borneol (4.77%), and limonene (4.57%). Bornyl acetate and α -pinene made up the majority of the components in the Sakhalin spruce essential oil. The five most prominent components of Sakhalin spruce essential oil identified by GC/MS were examined by EIA for IgM reactivity to HEV antigen. Bornyl acetate, α -pinene, camphene and limonene significantly inhibited binding to the HEV antigen compared to the positive control. Changes in binding inhibition to HEV antigens at different concentrations (10-100%) of bornyl acetate were investigated by EIA. Significant inhibitory binding of this reagent to HEV antigens was revealed at the concentrations of 30% (1.07 mol/L) and more. Evidence from the present study indicates that bornyl acetate has an antiviral effect on HEV; notably, this monoterpene may cause the capsid structure of HEV to mutate. However, it is unclear what mechanism is effective. We will use a recombinant baculovirus expression system to use virus-like particles (VLP) with a diameter of about 23 to 24 nm, which has a structure close to that of native virus particles, antigenicity, and immunogenicity. Indeed, the direct involvement of bornyl acetate in the capsid structure of HEV, the elucidation of its site of action, as well as its adsorption and infectivity on cells, will need to be clarified.

Conclusion: The results of this study show that bornyl acetate, the main component of Sakhalin spruce essential oil, suppressed antibody reactivity due to its denaturing effect on the capsid structure, the antibody-binding part of HEV. Furthermore, several essential oil components of Sakhalin spruce, including α -pinene, camphene and limonene, which exhibit similar antibody-binding inhibitory effects, were found to have a denaturing effect on HEV antigens. This essential oil is expected to be applied as an inactivating agent for meat processing and cooking utensils, such as knives and chopping boards, as a formulation with the potential to eliminate the infectivity of HEV in food-borne infections.

Key words: Hepatitis E virus, Sakhalin spruce, Essential oil, Bornyl acetate, Enzyme immunoassay