Assessment of the peaks responsible for antimicrobial activity of *Satureja hortensis* L. essential oil using gas chromatography-mass spectrometry technique

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Abstract

Satureja hortensis L. is used in traditional medicine in the world due to its antifungal, antibacterial and antioxidant properties. In this study, the antimicrobial activities in *Satureja hortensis* L. essential oils against three microbial strains were evaluated by two different methods, including: disc diffusion method, micro-broth dilution assay and qualitative analysis was performed by gas chromatography-mass spectrometry technique. The peaks potentially responsible for the antimicrobial activity in essential oils samples were indicated by some linear multivariate calibration techniques with different preprocessing methods. From the studied techniques, independent component regression (ICR) was preferred to exhibit the potential antimicrobial active compounds in *Satureja hortensis* L. essential oils because of its high repeatability, simplicity and interpretability of the regression coefficients.

Keywords: Chromatographic fingerprints, *Satureja hortensis* L., Potentially antimicrobial compounds, Linear multivariate calibration, Independent component regression

Introduction

Essential oils from medicinal herbs have good antimicrobial and antioxidant activities. There is a complex mixture of volatile compounds in various concentrations, which their chemical compositions are different in different species or subspecies [1]. *Satureja hortensis* L. (Summer Savory) belongs to the Lamiaceae family. *Satureja hortensis* L. is used in the pharmaceutical industries as stomachic, stimulant, expectorant, carminative and aphrodisiac as well as in treatment of different types of infectious diseases. Phenolic compounds are the most active natural antimicrobials in plants and *Satureja hortensis* L. essential oil is particularly rich in phenolic compounds [2].

In this study, the antimicrobial activities of *Satureja hortensis* L. essential oils against different kinds of microorganisms were determined and by gas chromatography-mass spectrometry (GC-MS) and linear multivariate calibration techniques, important compounds that contribute in the antimicrobial property of these essential oils samples were indicated. Several linear multivariate calibration techniques were used and compared, including principal component regression (PCR), partial least squares (PLS), orthogonal signal correction-partial least squares (OSC-PLS), orthogonal projections to latent structures (OPLS) and independent component regression (ICR) [3-5].

**Methodology**

Aerial parts of flowering *S. hortensis* L. were collected in Auguest and May 2014 from different regions of Iran. Air-drying of the plant was performed in a shady place for seven days at room temperature. A hundred grams of dried plant material was hydrodistilled for 4 hours using Clevenger apparatus. The essential oils were dried over anhydrous sodium sulphate and stored at low temperature (2 °C). The oil analysis was carried out using GC and GC-MS. The GC apparatus was Agilent technology 7890A system equipped with a spilt/ spiltless (ratio 1:20) injector, a flame ionization detector (both operating at 250 °C) and capillary column of HP-5 (19091J-413 USA) (30 m length, 0.32 mm id, film thickness 0.25µm). The antimicrobial activities of the essential oils were evaluated against Escherichia coli, Staphylococcus aureus and Candida albicans.

Results and discussion

The antimicrobial activities of 10 samples of *S. hortensis* L. essential oils were evaluated against different kind of microorganisms by disc diffusion and micro broth dilution assays. The antibacterial activity evaluation by disc diffusion assay showed *S. hortensis* L. had high inhibition zone diameters against C. albicans (17.10 to 26.10 mm), followed by S. aureus (6.80 to 17.00 mm) and E. coli (9.20 to 15.05 mm). The antimicrobial activity of *S. hortensis* L. was depended on the type of pathogen. In according to the inhibition zone diameter, the minimal inhibitory concentration **(**MIC) values (µl/ml) of *S. hortensis* L. were 0.0625-0.25 for C. albicans, 0.125-0.5 for S. aureus and 0.25-0.50 for E. coli. A data matrix with dimension of 10 × 7500 was obtained from the chromatogram of *S. hortensis* L.. Response vector indicates the antimicrobial activities of *S. hortensis* L. samples. Different preprocessing methods such as mean centering, normalization and scaling were performed. Several multivariate calibration models for the antimicrobial activity were developed using PCR, PLS, OSC-PLS, OPLS and ICR techniques, on the data matrix **X** containing fingerprints of 10 samples of *S. hortensis* L. and response vectors y_1, y_2, y_3 containing MIC values. In this study, we compared the fingerprints of each samples with the obtained regression coefficients from the models including PCR, PLS, OSC-PLS, OPLS and ICR for three microorganisms (see Figure 1). Our main purpose was to determine the peaks in the fingerprints that are potentially responsible for the antimicrobial activity of the measured samples. In the regression plots, compounds, which are irrelevant to antimicrobial activity, have small coefficients while the potentially important compounds have large coefficients. Comparison of regression coefficients shows that for PCR and PLS, negative and positive peaks are more than the OSC-PLS, OPLS and ICR. For this case study, OPLS and ICR regression coefficients are very similar but the ICR was preferred. In probability theory, independence is a high-order statistic and it is a much stronger condition than orthogonality. Therefore, ICR is considered more powerful in analyzing multivariate data sets and by ICR method, irrelevant and

minor peaks were completely eliminated from the regression plots. Based on the ICR plots compound that appear in 27.71 min in *S. hortensis* L. is responsible for the antimicrobial activity against each three microorganisms. According to the GC- MS spectrum, Carvacrol was found in the area that is corresponding to the negative peaks in the regression plot and this compound suggested as the *S. hortensis* L. potentially antimicrobial compound.
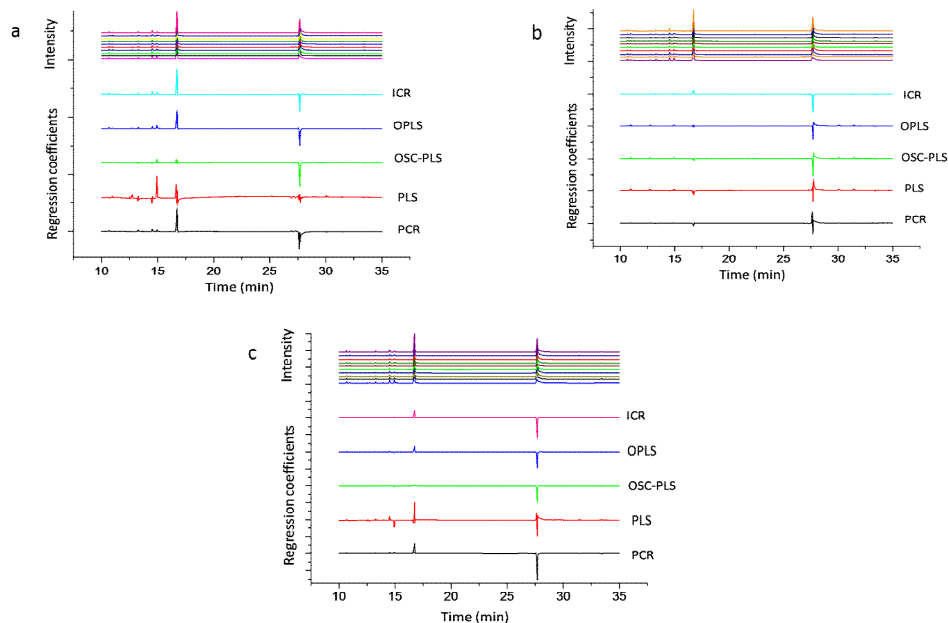
Figure 1. Fingerprints plot along with the obtained regression coefficients from some linear multivariate calibration methods for a) *Escherichia coli* b) *Staphylococcus aureus* c) *Candida albicans*

Conclusion

This study suggests ICR as preferred method for communicating chromatographic data and antimicrobial activity of *S. hortensis* L. essential oil samples. Its better performance for indication of potential antimicrobial active compounds in *S. hortensis* L. samples is due to its repeatability and simplicity

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