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Application of Mid-infrared spectroscopy and Partial Least-Squares Regression to predict antioxidant activity on herbal Mediterranean infusions

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The application of infrared spectroscopy to quantify phenolic content and antioxidant activity is a new area in herbs. Recently, we demonstrated the feasibility of mid-infrared spectroscopy to predict the phenolic content in lyophilised infusions of aromatic and medicinal herbs [1].

Sample preparation. 2g of Chamomile, Dittany, Lemon Balm, Mint, Rosemary and Sage were steeped in 200 ml (1 cup) of hot water (85 ^{0}C, 15 min) and cold water (room temperature, 15 min) either without stirring or with the use of ultrasound (35 MHz). The herbal infusions were filtered though a Whatman filter No. 1. Then 100 ml of infusions were extracted three times with petroleum ether. Part of the infusions was used as such and the rest was freeze-dried and kept in -21 ^{0}C until further analysis.

Determination of antioxidant activity and total phenolic content. Antioxidant activity was determined in herbal infusions before and after the extraction with the solvent by 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)- ABTS assay [2] and 2, 2-diphenyl-1-picrylhydrazyl radical- DPPH assay [3]. Results were expressed as μmole TROLOX per cup. Total phenolic content (in terms of caffeic acid) was also determined using a Folin-Ciocalteu assay [4].

Indeed, a good correlation between the phenolic content and antioxidant activity was found for both methods (r= 0.99 for ABTS and r= 0.98 for DPPH assay), indicating that the antioxidant properties of these compounds are related to their reducing power.

FT-IR spectroscopy. The spectra of freeze-dried infusions were recorded in the Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) mode using the FT6700 (Thermo Electron Corporation) with the micro-sampling cup of a Spectra-Tech diffuse reflectance accessory (2 mg of sample) against a KBr background. Spectra were collected and manipulated using the OMNIC (ver. 7.3) software supplied from the manufacturer of the spectrometer.

Statistics. Partial least squares (PLS) regression was used to construct calibration models. PLS calibration models were carried out with the TQ Analyst software (ver. 7.2.0.161, Thermo Electron Corporation).

Partial least-squares –PLS regression models were constructed with 45 spectra from lyophilised infusions and their corresponding ABTS and DPPH values. The best calibration model was achieved in the spectral region 1800-1500 cm^{-1} and 1320-1180 cm^{-1}. The correlation coefficient (r) was 0.99 for both models while the root mean squared of calibration (RMSEC) was 42.3 μmole TROLOX/cup for ABTS and 40.7 μmole TROLOX/cup for DPPH. The root mean square error of cross validation (RMSECV) and correlation coefficient (r) were also determined as shown in table 1.

Table 1*. The comparison of calibration models of total phenolic content of herbal infusions developed in different spectral regions*

ASSAY	PLS factors	Calibration		Cross-validation		Prediction
		r	RMSEC	r	RMSECV	RMSEP
ABTS	10	0.99	42.3	0.97	94.6	52.9
DPPH	10	0.99	40.7	0.97	83.6	41.9

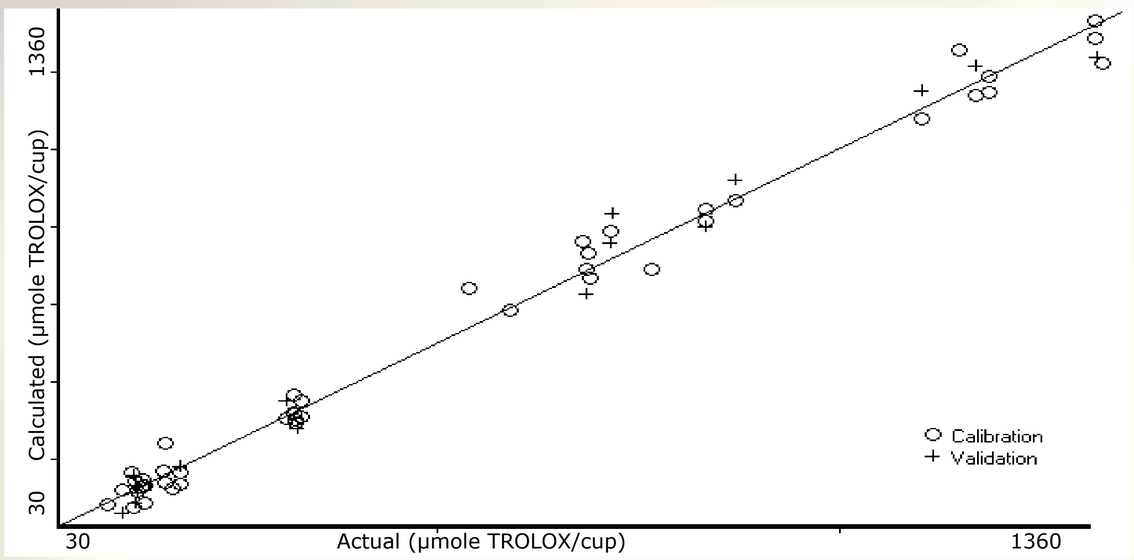

Figure 1. *Partial least square-PLS model calibration for the prediction of antioxidant activity by ABTS assay in herbal infusions using FT-IR spectroscopy*

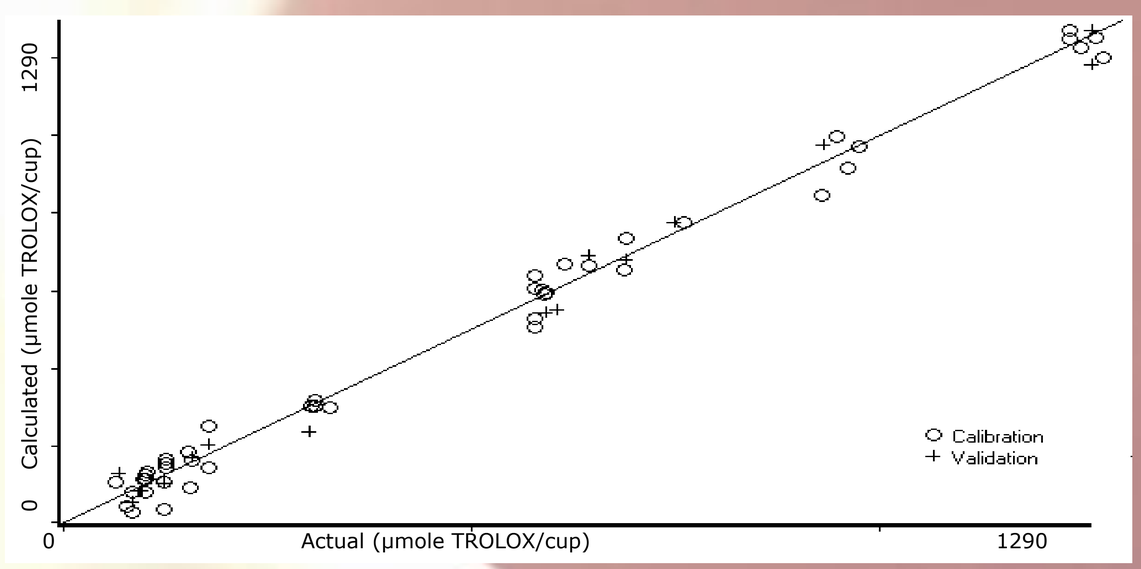

Figure 2. *Partial least square-PLS model calibration for the prediction of antioxidant activity by DPPH assay in herbal infusions using FT-IR spectroscopy*

Additional 15 lyophilised infusions were used for prediction. The root mean squared of prediction (RMSEP) 52.9 μmole TROLOX/cup and 41.9 μmole TROLOX/cup, respectively.

The antioxidant activity as determined using the ABTS reference assay were fluctuated between 86.2 and 1321.0 μmole TROLOX/cup. The corresponding values as calculated using the FT-IR model ranged from 62.8 to 1237.2 μmole TROLOX/cup.

The antioxidant activity as determined using the DPPH reference assay were fluctuated between 69.2 and 1264.0 μmole TROLOX/cup. The corresponding values as calculated using the FT-IR model ranged from 54.9 to 1269.9 μmole TROLOX/cup.

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