Near infrared spectroscopy supported by multivariate data analysis and gas chromatography-mass spectrometry for discrimination and classification of different species in *Achillea* Genus

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Introduction

This study evaluated the use of near infrared (NIR) spectroscopy for discriminating and classifying traditional medicinal plants.[1–4] *Achillea millefolium* and three of its related species, namely, *A. clypeolata*, *A. collina* and *A. nobilis* were chosen as sample material because they are well known in the field of traditional medicine. The present study was subdivided into following sections: (1) Discrimination of *A. millefolium* flowers and leaves by using NIR spectroscopy and gas chromatography-mass spectrometry (GC-MS) as reference method. (2) Classification of differently treated *A millefolium* samples by principal component analysis (PCA). (3) Classification of four *Achillea* species by PCA. The results showed that NIR spectroscopy is suitable to discriminate between different *A. millefolium* parts (or example, flowers and leaves), as well as between different sample preparation techniques (for example, air-dried, oven-dried). Furthermore, the established NIR spectroscopy method proved great potential for classification of related *Achillea* species.

Materials and methods

Achillea genus samples

Achillea clypeolata, *nobilis* and *collina* were supplied by the Institute of Pharmacognosy, University of Vienna. One third of the samples were dried in an oven at 40 °C, and the remaining two thirds were dried at room temperature. After the drying process the flower heads were cut off and ground by a roll cut machine to a particle size of about 1 mm.

Near-infrared spectroscopy

NIR spectra were recorded by a Fourier transform (FT-NIR) instrument (Büchi, Flawil, Switzerland). NirCal 4.21 (Büchi) was used for creating a model. Spectra were randomly divided into a learning-set (67%, c-set) and a test-set (33%, v-set). The reflection spectra were transposed to the log (1/*R*) absorbance spectra followed by various data pretreatments. PCA was implemented to build the models.

Gas chromatography – mass spectrometry (GC-MS) analysis

The dried flower heads were extracted three times with CH_2Cl_2 (1:10 w/v) and ultrasonicated for 10 min.

Results and discussion

Prior to GC-MS and NIR spectroscopy analysis the plant samples were air-dried or oven-dried and the relevant analytes of *A. millefolium s.l.* were extracted (**Figure 1**).

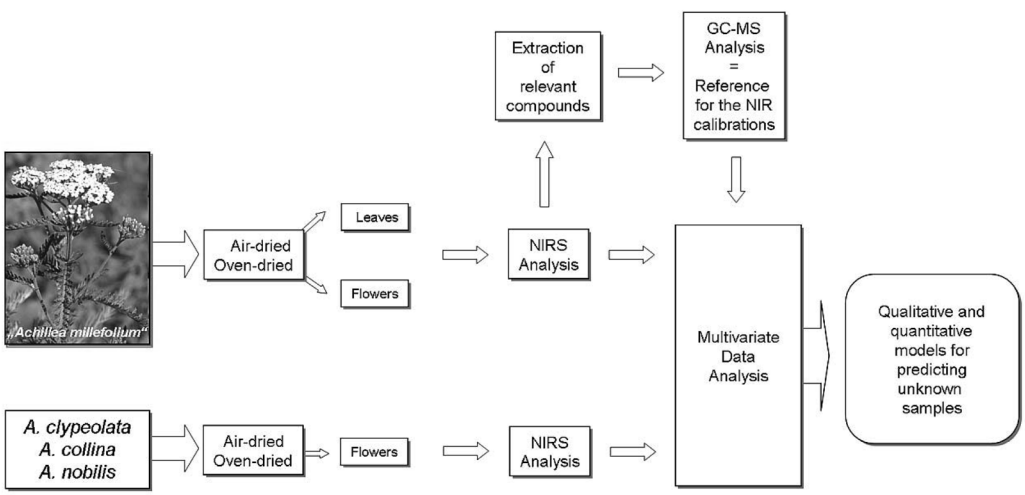

Figure 1. Workflow of the conducted experiments on *Achillea* genus.

Principal component analysis for discriminating *A. Millefolium* flowers and leaves

There were 240 spectra (75 spectra for air-dried flowers, 75 for air-dried leaves and 90 for mixture of air-dried flowers and leaves) recorded for the discrimination between *A. millefolium* flowers and leaves. The averaged spectra were used to work out the spectral differences in the NIR spectra based on visual interpretation [Figures 2(a) and 2(b)].

The 3-dimensional cluster plot in Figure 3, representing principal components (PC) one, two and three, shows the classification of the samples into the three groups.

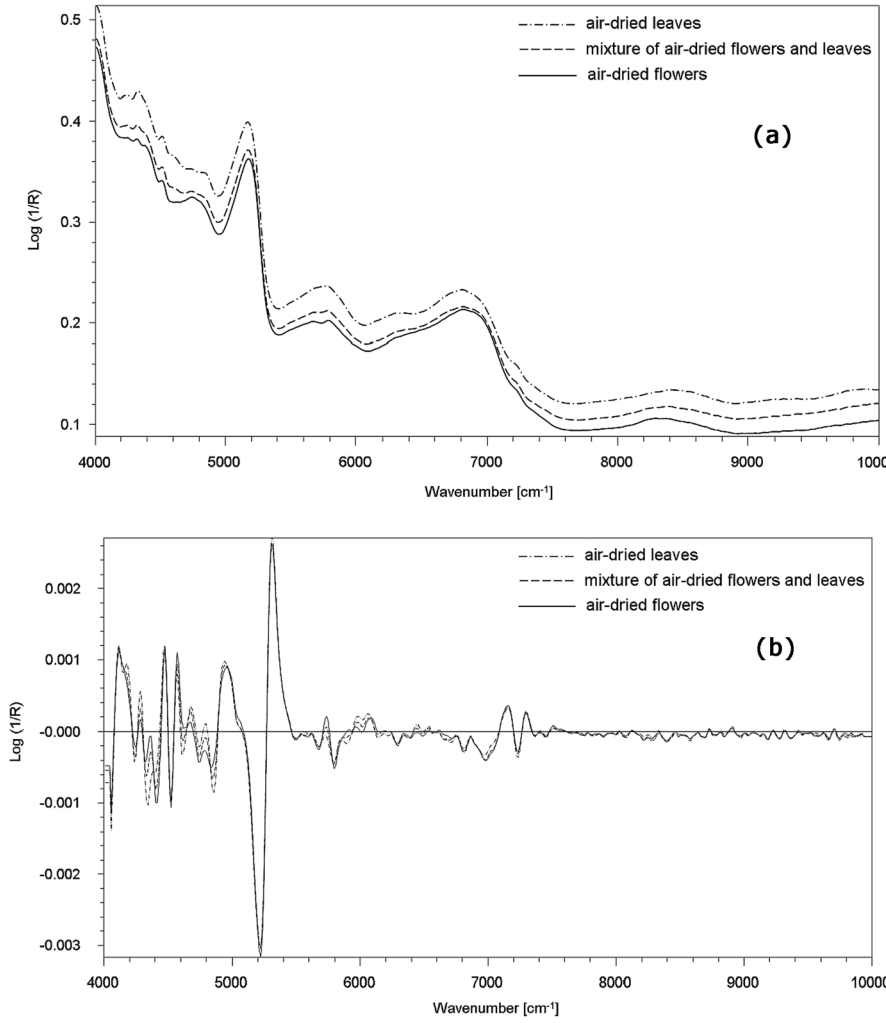

Figure 2. (a) Averaged and (b) pretreated (2nd derivative; Savitzky-Golay, 9 points) NIR absorbance spectra of air-dried *Achillea millefolium s.l.* flowers, leaves and a mixture of both.

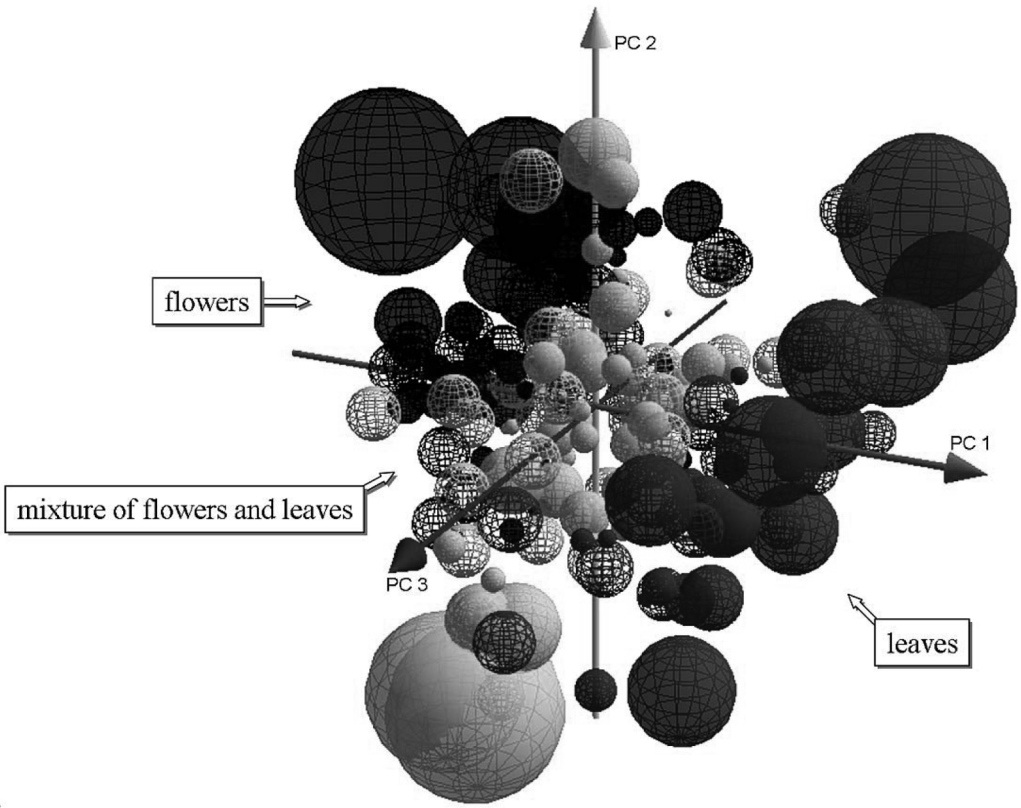

Figure 3. 3-dimensional factor plot, representing principal components (PC) one, two and three, for classifying the aerial parts of *A. millefolium s.l.*

In total there were 44 compounds detected in the flowers, leaves and the mixture. 34 were found in flowers and 23 in leaves, whereas 15 of these compounds were found in both flowers and leaves, as can be gathered from Table 1.

Discrimination of the air-dried and oven-dried *A. millefolium* flower samples

171 spectra (75 spectra for air-dried flowers and 96 for oven-dried flowers) were recorded and subjected to PCA for the discrimination among different *A. millefolium* preparation procedures. Ten compounds showed significant differences in the air-dried and oven dried-flowers. Oven-dried flowers showed a decreasing amount of compound (1) $p<=0.01$ and (2) $p<=0.05$ and an increasing amount of (3) $p<=0.01$, (4) $p<=0.01$, (5) $p<=0.01$, (6) $p<=0.01$, (7) $p<=0.01$, (8) $p<=0.01$, (9) $p<=0.05$ and (10) $p<=0.05$ (Table 1). 2nd derivative spectra showed that the ν (OH) + δ (OH) combination band around 5200 cm^{-1} showed a decrease of absorbance intensity on drying. The cluster model showed two separate clusters for each sample treatment procedure

Table 1. Compounds found in *A. millefolium* by GC-MS in air-dried flowers and leaves and oven-dried flowers.

Compound number	Name of compounds	Found in‡
1	1,3-dimethyl-Benzene	FA
2	1,2,3,4-tetrahydro-1,1,6-trimethyl-Naphthalene	LA
3	n-Decanoic acid	FA, FO, LA
4	2,5-bis(1,1-dimethylethyl)-Phenol	FA, LA
5	7-ethyl-1,4-dimethyl-Azulene	FA, FO
6	(Z)-3-Tetradecene	FA, LA
7	cis,cis-7,10,-Hexadecadienal	FA, FO
8	cis-11-Hexadecenal	FO
9	(Z)-9-Octadecenal	FA
10	cis-11-Tetradecen-1-ol	FO
11	(Z)-13-Octadecenal	FA, LA
12	12-Methyl-E,E-2,13-octadecadien-1-ol	LA
13	Alpha-Calacorene	FA
14	Neophytadiene	FA, FO, LA
15	[R-[R*,R*-(E)]]-3,7,11,15-tetramethyl-2-Hexadecene	FA, FO, LA
16	n-Hexadecanoic acid	FA, FO, LA
17	Tetradecanal	FA
18	Octadecanal	FA, FO
19	3,6,7,8-tetrahydro-3,3,6,6-tetramethyl-As-Indacen-1(2H)-one	FA
20	3a,5,5a,9,9a,9b-hexahydro-9-hydroxy-3,5a, 9-trimethyl-Naphtho[1,2-b]furan-2,6(3H,4H)-dione	FA, FO
21	3a,5,5a,9,9a,9b-hexahydro-9-hydroxy-5a, 9-dimethyl-3-methylene-Naphtho[1,2-b]furan-2,6(3H,4H)-dione	FA, FO, LA
22	(Z,Z)-9,12-Octadecadienoic acid	FA, FO, LA
23	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	FA, FO, LA
24	Oleic Acid	FA, FO, LA
25	2-Methyl-Z,Z-3,13-octadecadienol	FA, FO
26	[2R-(2.alpha.,4a.alpha.,8a.beta.)]-1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)- Naphthalene,	LA
27	Z,E-2,13-Octadecadien-1-ol	FA, FO

(*continued on next page*)

Table 1. (*continued*)

Compound number	Name of compounds	Found in‡
28	Octadecanoic acid	FA, FO, LA
29	Z-10-Octadecen-1-ol acetate	FA, FO
30	Octadecanal	FA
31	.beta.-methyl-2'-butenate ester 4-Benzyloxyphenol	FA
32	Octadecanal	FA, FO
33	Bicyclo[10.1.0]tridec-1-ene	FA
34	(Z,E)-9,12-Tetradecadien-1-ol, acetate	FA, FO
35	gamma-Sitosterol	FA, FO, LA
36	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	LA
37	beta-Amyrin	LA
38	Octacosane	FA
39	(3.beta.,21.beta.)-a'-Neogammacer-22(29)-en-3-ol	FA
40	Lupeol	FO
41	Taraxasterol	LA
42	Stigmastan-3,5-dien	FA, FO, LA
43	1S-(1.alpha.,3a.alpha.,4.beta.,6a.alpha.)]-5,5'-(tetrahydro-1H,3H furo[3,4-c]furan-1,4-diyl)bis-1,3-Benzodioxole	LA
44	Vitamin E acetate	FA, FO, LA

† Rt = Retention time [min].
‡ FA = air-dried flowers; FO = oven-dried flowers; LA = air-dried leaves.

by PC 1 and PC 2. ANOVA analysis of the 14 compounds showed that there were six compounds that were significantly different in the air-dried flowers and the air-dried leaves.

Classification and discrimination of four *Achillea* species

224 spectra (75 for *A. millefolium*, 60 for *A. clypeolata,* 39 for *A. collina* and 60 for *A. nobilis*) were recorded to classify and discriminate these four different *Achillea* species. Spectra were averaged and normalised (between 0 and 1) to calculate the final PCA model. All of these four species were found to be very similar in their composition (GC-MS) and therefore the classification ability by a multiple compound model (MCM, one model for classifying more than two species) is limited. However, two compound models (one model for classifying only two species) offered higher prediction abilities, but have to be specified for each single analysis. The two compound models

showed more precise clustering of the samples than MCM by employing a KM spectral transformation. In other words, the classification ability decreases with increasing numbers of different species. A decision has to be made if a rough but simultaneous analysis by employing MCM or a more precise but limited analysis by using a two compound model is preferred.

Conclusion

Our study indicated that NIR spectroscopy is an efficient technique for the discrimination of different aerial parts, sample preparation procedures and related *Achillea* species.

Acknowledgements

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