Volatiles Profiling in Medicinal Licorice Roots Using Steam Distillation and Solid-Phase Microextraction (SPME) Coupled to Chemometrics

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Abstract: Licorice (*Glycyrrhiza glabra* L.) is a plant of considerable commercial importance in traditional medicine and for the flavor and sweets industry. Although *Glycyrrhiza* species are very competitive targets for phytochemical studies, very little is known about the volatiles composition within that genus, although such knowledge can be suspected to be relevant for understanding the olfactory and taste properties. To provide insight into *Glycyrrhiza* species aroma composition and for its use in food and pharmaceutical industry, volatile constituents from *G. glabra*, *G. inflata*, and *G. echinata* roots were profiled using steam distillation and solid-phase microextraction. Two phenols, thymol and carvacrol, were found exclusively in essential oil and headspace samples of *G. glabra,* and with highest amounts for samples that originated from Egypt. In *G. echinata* oil, (2*E*, 4*E*)-decadienal (21%) and β-caryophyllene oxide (24%) were found as main constituents, whereas 1α, 10α-epoxyamorpha-4-ene (13%) and β-dihydroionone (8%) predominated *G. inflata*. Principal component and hierarchical cluster analyses clearly separated *G. echinata* and *G. inflata* from *G. glabra;* with phenolics and aliphatic aldehydes contributing mostly for species segregation.

Keywords: essential oil, GC/MS, *Glycyrrhiza glabra* L., *Glycyrrhiza inflata* L., *Glycyrrhiza* echinata L.

Practical Application: Licorice (*Glycyrrhiza glabra*) has large economic, nutritional, and medicinal values. The data presented in this article help in licorice quality control analysis to identify *G. glabra* from its closely allied species. The presence of thymol and carvacrol exclusively in *G. glabra* suggests that these volatiles could serve as chemotaxonomic markers and also might be considered as potentially relevant for taste.

Introduction

Licorice root, specifically of the *Glycyrrhiza* species of the Leguminoseae family (Kitagawa 2002), is one of the most important medicinal herbs and special food ingredients since antiquity that have been used worldwide (Fenwick and others 1990). Numerous medicinal activities and potential health benefits were reported for licorice including antibacterial, antiulcer, anticancer, hepatoprotective, antiatherosclerotic, anti-inflammatory, and expectorant effects (Wang and Nixon 2001; Kobayashi and others 2002; Sasaki and others 2002; Krausse and others 2004; Lau and others 2010). Owing to these vast medicinal effects, licorice is a competitive target for phytochemical studies. For example, glycyrrhizin, which is the main components of licorice root, has been clinically used in the treatment of hyperlipemia, allergic inflammation, and ulcer (Sasaki and others 2002; Krausse and others 2004). The flavonoid licochalcone A has been shown to inhibit melanogenesis and prostate cancer growth (Wang and Nixon 2001; Lee and others 2008).

Chemical studies have mostly focused on the nonvolatile constituents of licorice root. However, licorice contains numerous volatile chemicals of potential use in medicine, aromatherapy and of relevance for flavoring. *G. glabra* is used as a natural sweetener, and as flavor additive in the preparation of candies and specialty foods (Montoro and others 2011). The sweet taste found in licorice is ascribed to glycyrrhizin, with a sweetening power 50 times greater than sugar. Nevertheless, few studies have focused on examining flavor components in *Glycyrrhiza* and with most studies focused on *G. glabra*. Frattini and others (1977) reported a change in *G. glabra* volatiles composition in response to heating, compared to unheated licorice. Most abundant compounds were acetol, propionic acid, 2-acetyl-pyrrole, 2-acetyl-furan, and with furan derivatives most common due to pyrolysis of sugars enriched in licorice. Several phenolics and terpenoids were also characterized as aroma volatiles in licorice juice that were not detected in heated samples (Frattini and others 1977). Attempts to characterize *G. uralensis* essential oil composition revealed the presence of 127 chemicals belonging to several classes and with aliphatic aldehydes and ketones present as major components (Fu and others 2009). Recently, the effect of γ-irradiation on *G. uralensis* volatiles composition has been examined whereby no major qualitative

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Disclosure Statement: The authors declare no actual or potential conflict of interest including any financial, personal, or other relationships with other people or organizations.

doi: 10.1111/j.1750-3841.2012.02927.x

and quantitative loss in volatile compounds was found (Gyawali and others 2008). Typical licorice aroma is likely to be mediated by a large collection of volatiles, rather than by the odor of 1 or 2 components (Frattini and others 1977). Nevertheless, whether relevant variation in the volatile composition among *Glycyrrhiza* species and or species grown in different regions exists, has yet to be investigated.

Continuing our studies on *Glycyrrhiza* species bioactive secondary metabolism and to provide further insight into licorice aroma, we now report on volatiles analyses from 6 licorice samples representing different geographic and or genetic origins (Farag and others 2012). One of the most popular methods used for plants volatile analysis is based either on steam distillation or headspace volatile analysis. Compared with steam distillation, headspace (HS) gives more realistic and clean picture of the volatiles profile (Tholl and others 2006). Although headspace solid-phase microextraction (HS-SPME) is also dependent on the type of solid phase and the conditions used, it is an excellent if not the best currently available technique for the extraction of volatile compounds from dried or living (fresh) plants. HS-SPME has been successfully used as a technique for screening complex volatile mixtures and for complementing information obtained from other methods (Rohloff and others 2000; Zini and others 2002). Taking advantage of the differences between both techniques, our aim was to apply both approaches for volatiles profiling in *Glycyrrhiza*. *G. glabra* is the species widely employed in the flavor industry. However, nothing is known about variations of volatiles composition of this commercially most relevant species as influenced by plants grown in different regions and habitats. Multivariate analyses were also performed to define both similarities and differences among samples.

Materials and Methods

Plant material

We selected 6 well-characterized *Glycyrrhiza* samples representing broad geographic and genetic sampling species including: G. *inflata,* China, Xinjiang (GI), *G. glabra,* Egypt (GG1), *G. glabra,* Afghanistan (GG2), *G. glabra,* Syria (GG3), *G. echinata*, Bonn, Germany (GE1), and *G. echinata,* Kiel, Germany (GE2). Information on collected samples origin is cited in Farag and others (2012).

SPME material and chemicals

SPME holder and fiber coated with 100 μm polydimethyl siloxane (PDMS) were supplied by Supelco (Oakville, ON, Canada). Before use, fibers were conditioned according to the supplier's instructions. All other chemicals and terpene standards were provided from Sigma Aldrich (St. Louis, Mo., U.S.A.).

Extraction of essential oil

Fresh ground root samples (250 g) were distilled for 3 h in a modified Clevenger apparatus with distilled water. The distillate was extracted with chloroform (GC grade) and concentrated under nitrogen gas (Farag 2009) to give brownish yellow oil. The oil was dried over anhydrous Na_2SO_4 and stored at −20 °C until further analysis.

SPME volatiles isolation

Headspace volatiles analysis using SPME was adopted from Zini and others (2002) with few modifications. Briefly, licorice roots were ground, and 100 mg was placed inside 1.5 mL clear glass vials. The 0.1 M sodium phosphate buffer solution (pH 7.2 mL) was added to each vial, which was then immediately capped and placed on a temperature controlled tray for 1 h at 50 °C with the SPME fiber inserted into the headspace above the root sample. Adsorption was timed for 30 min. SPME fibers were desorbed at 210 °C for 1 min in the injection port of an HP 6890A GC (Hewlett-Packard, Palo Alto, Calif., U.S.A.). A system blank containing no plant material was run as a control.

GC/MS analysis and GC/FID quantification

Essential oil and SPME-trapped volatile components were identified by gas chromatography (Agilent Hewlett-Packard 6890, Agilent Technologies, Palo Alto) coupled to a mass spectrometer (Agilent Hewlett-Packard 5973, Agilent Technologies) or a flame ionization detector (FID). Volatiles were separated on a DB5-MS column (30 m length, 0.25 mm inner diameter, and 0.25 μm film (J&W Scientific, Santa Clara, Calif.). Injections were made in the splitless mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220 °C, column oven 40 °C for 3 min, then programmed at a rate of 12 °C/min to 180 °C, kept at 180 °C for 5 min, and finally ramped at a rate of 40 °C/min to 250 °C and kept for 2 min, He carrier gas at 1 mL/min. The transfer line and ion–source temperatures were adjusted at 230 and 190 °C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV and a source temperature of 180 °C. Volatile components were identified using the procedure described in Farag (2008) and peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to n-alkanes (C_6-C_{20}), mass spectrum matching to NIST, WILEY library database (>90% match) and with authentic standards (when available). Similar column-type and ramping program was used for GC-FID analysis, with hydrogen used as a carrier gas at 2 mL/min. The relative amounts of the individual volatiles were determined by GC-FID from 3 independent roots for each sample to assess for biological variance.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) analysis of volatiles

PCA and HCA analyses were performed using 3 R packages, PCA methods, Heatplus, and gplots, which can be downloaded freely as an R package from the Metlin Metabolite Database under R 2.9.2 environment from GC/FID quantification peak list for a total of 38 volatiles.

Results and Discussion

Essential oil analysis in *Glycyrrhiza* species

The major goal of this study was to investigate *Glycyrrhiza* volatiles metabolism in the context of its genetic diversity and or geographical origin so as to set a framework for taxonomy, and to help identify *G. glabra*, most widely used medicinally from its allied species. The complete list of identified volatiles in *Glycyrrhiza* species essential oils is presented in Table 1, and values presented are averages of readings recorded from 3 independent samples obtained in experiments carried out under identical conditions. A representative gas chromatogram is shown in Figure 1 and indicates the differences in essential oil composition among species. A total of 38 compounds were detected of which only 8 were previously reported from roots of *G. glabra* (Frattini and others 1977; Tanaka and others 2008). Most of the compounds were produced from *G. glabra* with the various samples producing volatiles in the highest concentrations. The qualitative

Table 1–Relative percentage of volatile compounds in *G. glabra*, *G. inflata*, and *G. echinata* root essential oils using GC-FID measurements ($n = 3$).

		Concentration (%)[a]						
RI	Compound	*G. glabra* (Egypt)	*G. glabra* (Afghanistan)	*G. glabra* (Syria)	*G. inflata* (China)	*G. Echinata* (Bonn)	*G. echinata* (Kiel)	Identification
951	(*E*)-2-heptenal	–	–	–	5.3	1.5	1.3	MS, RI, Ref[b]
963	5-Methyl-furfural	3.6	10.1	9.4	4.0	2.4	2.0	MS, RI, Ref[a]
1011	(2*E*, 4*E*)-heptadienol	–	–	–	–	4.2	3.4	MS, RI
1052	(*E*)-2-octen-1-al	–	–	–	–	7.4	5.5	MS, RI, Ref[b]
1089	*o*-Guaiacol	2.2	*tr*.	*tr*.	3.4	–	–	MS, RI, Ref[a,b]
1114	2-Phenylethanol	0.5	2.1	*tr*.	1.6	*tr*.	–	MS, RI, STD, Ref[a,b]
1131	(*Z*)-pinene hydrate	*tr*.	2.1	–	–	–	–	MS, RI
1134	Lavandulol<tetrahydro->	–	7.6	4.1	–	1.9	1.6	MS
1178	Terpinen-4-ol	*tr*.	–	3.6	–	–	–	MS, RI, Ref[b,c]
1179	(*E*)-linalool oxide	2.1	–	–	–	–	–	MS, RI, Ref[a,b]
1185	p-Cymen-8-ol	–	2.7	3.0	–	–	–	MS, RI, Ref[b]
1188	α-Terpineol	*tr*.	–	2.4	–	–	–	MS, RI, STD, Ref[a,b]
1199	Methyl chavicol		–	2.4	–	–	–	MS, RI
1201	(4*E*)-decenal	5.3	2.8	5.4	3.3	–	0.5	MS, RI
1205	Decanal	–	–	–	–	1.6	1.4	MS, RI, STD, Ref[b]
1208	(2*E*, 4*E*)-nonadienal	–	–	–	–	2.1	1.0	MS, RI, Ref[b]
1237	Cumin aldehyde	4.7	1.8	3.3	1.5	–	–	MS, RI, STD
1241	Carvone	2.1	0.2	3.1	–	–	–	MS, RI, STD
1255	Piperitone	9.4	13.1	7.2	–	–	–	MS, RI, Ref[b]
1272	(*E*)-cinnamaldehyde	3.6	4.5	6.2	–	–	–	MS, RI, STD
1285	(*E*)-anethole	1.3	–	–	2.9	–	–	MS, RI, Ref[b]
1287	(2*E*, 4*Z*)-decadienal	–	–	–	–	6.4	5.6	MS, RI
1289	Thymol	27.2	6.0	5.5	–	–	–	MS, RI, STD, Ref[a,b]
1294	Indole	–	7.4	1.8	4.5	–	–	MS, RI, STD, Ref[a,b,c]
1301	Carvacrol	11.1	1.4	5.8	0.5*tr*.	–	–	MS, RI, STD, Ref[a,b]
1311	(2*E*, 4*E*)-decadienal	–	–	–	–	20.8	18.0	MS, RI, Ref[b]
1313	*p*-Vinyl-guaiacol	8.5	8.5	9.5	10.3	–	–	MS, RI, Ref[b]
1345	Unknown aldehyde	–	3.7	5.7	2.1	6.1	5.4	MS, RI
1360	Eugenol	9.4	7.5	8.8	2.8	*tr*	–	MS, RI, STD
1363	γ-Nonalactone	1.8	2.5	7.4	6.2	–	1.2	MS, RI, Ref[a,b]
1405	Methyl eugenol	3.5	0.2	3.8	6.1	–	–	MS, RI, Ref[b]
1418	β-Caryophyllene	0.5	0.5	1.1	2.1	1.5	–	MS, RI, STD, Ref[d]
1440	β-Dihydro-ionone	–	–	–	7.7	–	–	MS, RI
1523	Himachalene epoxide	–	8.8	1.6	–	–	–	MS
1534	Spathulenol	–	–	–	–	4.3	3.7	MS, RI
1567	(1α, 10α)-Epoxy-amorph-4-ene				12.7			MS, RI
1589	β-Caryophyllene oxide	1.1		1.1	0.3	24.5	21.5	MS, RI, Ref[d]
1615	Humulene epoxide II	–	–		–	11.7	10.3	MS, RI
Total alcohols		0.5	9.2	9.1	1.6	8.5	7.1	
Total aldehydes		17.2	22.9	30.0	16.1	48.3	40.7	
Total ethers/epoxides		8.0	11.4	6.5	22.0	36.2	31.8	
Total ketones		11.5	13.3	10.3	7.7	–		–
Total phenols		58.5	23.4	29.7	16.9	0.0		

Relative concentration based on triplicate samples measurements;—, not detected; tr, trace; RI, retention index on DB5-MS column;
MS, identification was based on comparison of mass spectra; STD, coanalysis relative to a pure compound and or previous citing reference.
[a]Frattini and others 1977; [b]Tanaka and others 2008; [c]Gyawali and others 2008; [d]Fu and others 2009.

composition of *G. glabra* essential oils from different origins was relatively similar with phenols and ketones, that is, thymol and piperitone, as characteristic constituents.

Aldehydes constitute the most dominant chemical group present in the 3 *Glycyrrhiza* species: *G. glabra* (17% to 30% aldehydes in volatile fraction), *G. inflata* (16%), and *G. echinata* (40% to 48%). Predominant aldehydes found exclusively in *G. echinata* were (2*E*, 4*E*)-decadienal and (*E*)-2-octen-1-al, measured at *ca*. 21% and 7%, respectively. Other aldehydes found in *G. echinata* include decanal, (2*E*,4*E*)-nonadienal, and (2*E*,4*Z*)-decadienal. *G. echinata* shows a similar volatiles profile to that of *G. uralensis*, with aliphatic aldehydes being the most abundant class of volatiles. Interestingly, furfurals that dominate heated licorice aroma were found at much lower levels (2% to 10%) in this study, represented by 5-methyl-furfural, common in all species. Furfurals are likely to be formed during the licorice drying process (Frattini and others 1977; Tanaka and others 2008). This compositional variation of aldehydes among *Glycyrrhiza* species might be used as taxonomic marker to help differentiate between the 3 species. Second to aldehydes, alcohols are the most common class in *Glycyrrhiza* essential oil (3% to 10%). Pinane derived alcohols predominate *G. glabra* essential oil (*ca*. 9.0%), whereas aliphatic derivatives dominate *G. echinata* (*ca*. 8.5%) essential oil. Predominant alcohols found in *G. glabra* from Syria are terpinen-4-ol and α-terpineol measured at 3.6% and 2.4%, respectively, whereas *p*-cymen-9-ol is the most abundant form in *G. glabra* from Afghanistan at 2.7%. In *G. echinata*, alcohol peaks show the same regimented pattern as found for aliphatic aldehydes, with that of (2*E*, 4*E*)-heptadienol being the most prominent form.

Phenols including isoprenoid-derived phenols constitute the most dominant chemical group among *G. glabra* volatiles with qualitative differences observed among species from different regions: Egypt (58% relative content of phenols), Afghanistan (23%), and Syria (30%). The exclusive detection of thymol and its

isomer carvacrol in *G. glabra* samples suggests that the terpenoid biosynthetic machinery is the dominant or even exclusive generator for these volatiles, which could serve as chemotaxonomic markers for *G. glabra* species. Highest levels of thymol and carvacrol were found in *G. glabra* from Egypt at 27% and 11%, respectively (Figure 1). Thymol and carvacrol were previously identified in *G. uralensis*, though at much lower levels (Tanaka and others 2008). Other phenols found commonly within *G. inflata* include *p*-vinyl-guaiacol and eugenol; no phenols were identified in *G. echinata* essential oil.

Except for *G. echinata*, epoxides are present at low levels in all other samples. β-Caryophyllene oxide and humulene epoxide account for *ca.* 24% and 12% of *G. echinata* essential oil composition, respectively, present at trace levels in other samples. These data suggest that volatiles composition in *G. echinata* is particularly distant from other samples. It should be noted that *G. echinata* ranked the lowest for all other terpene classes. Other volatiles of miscellaneous origin identified in *Glycyrrhiza* include the heterocycles indole and γ-nonalactone, both previously reported from *G. glabra* and *G. uralensis* essential oil (Frattini and others 1977; Tanaka and others 2008). Roasted odor characteristic for licorice is likely to be mediated by such heterocycles, including furfurals formed during the drying process (Tanaka and others 2008; Fu and others 2009). As can be gathered from Table 1, volatiles from the roots of *G. echinata* and to a lesser extent *G. inflata* are more similar to *G. uralensis*, which is also characterized by a remarkable amount of aliphatic aldehydes, alcohols, and a low content of terpenes and (terpene derived) phenolics. Carvone and piperitone were identified in *G. glabra* essential oil at (2% to 3%) and (7.2 to 13%), respectively; whereas β-dihydroionone is present exclusively in *G. inflata* at 7.7%. Carvone and piperitone were previously identified as major volatile component in the water distillate of *G. uralensis* (Tanaka and others 2008).

PCA and HCA multivariate data analysis of volatiles

To identify species differences regarding volatiles production, PCA was performed on the essential oil data (relative abundances of a total of 38 identified compounds). PCA is an unsupervised clustering method requiring no knowledge of the dataset and acts to reduce the dimensionality of multivariate data (Goodacre and others 2000). The PC1/PC2 scores plot (Figure 2A) shows that 3 major distinct clusters are formed corresponding to the 3 different species studied mostly along PC1 and PC2 overall explaining 57% of the variance. Most of the samples were sorted to the left side of the vertical line representing PC1, whereas *G. echinata* samples were placed on the right side. It should be noted that separation among *G. glabra* species based on geographical regions could be observed to some extent from PCA along PC2 with *G. glabra* grown in Egypt plotted apart from *G. glabra* grown in Syria and Afghanistan, with the later samples both clustering together. The metabolite loading plot for PC1 (Figure 2B), which exposes the most important components with respect to scattering behavior, reveals that (2*E*, 4*E*)-decadienal and β-caryophyllene oxide contributed the most, positively along PC1. The second

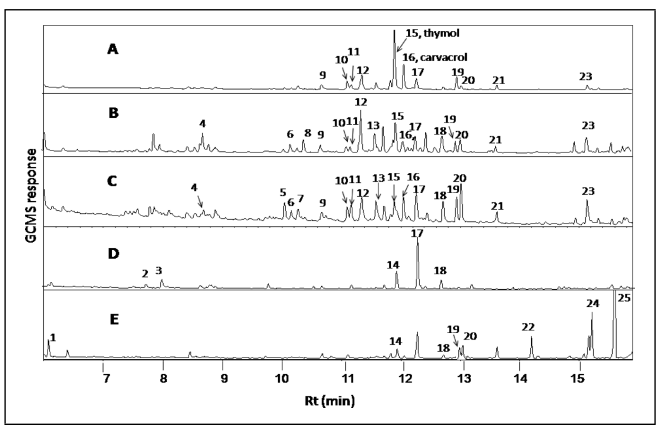

Figure 1–GC-MS chromatograms of essential oils prepared using steam distillation from *Glycyrrhiza* species: *G. glabra,* GG1 (A); *G. glabra,* GG2 (B); *G. glabra,* GG3 (C); *G. echinata,* GE1 (D); and *G. inflata,* GI (E); detail for *Glycyrrhiza* sample codes is described in Materials and Method section. Assigned peaks: 1, (*E*)-2-heptenal; 2, (2*E*, 4*E*)-heptadienol; 3, (*E*)-2-octen-1-al; 4, lavandulol<tetrahydro->; 5, terpinen-4-ol; 6, p-cymen-9-ol; 7, α-terpineol; 8, methyl chavicol; 9, (4*E*)-decenal; 10, cumin aldehyde; 11, carvone; 12, piperitone; 13, (*E*)-cinnamaldehyde;14, (2*E*, 4*Z*)-decadienal; 15, thymol; 16, carvacrol; 17, *p*-vinyl-guaiacol; 18, unknown aldehyde; 19, eugenol; 20, γ-nonalactone; 21, methyl eugenol; 22, β-dihydro-ionone; 23, himachalene epoxide; 24, (1α, 10α)-epoxy-amorph-4-ene; 25, unknown.

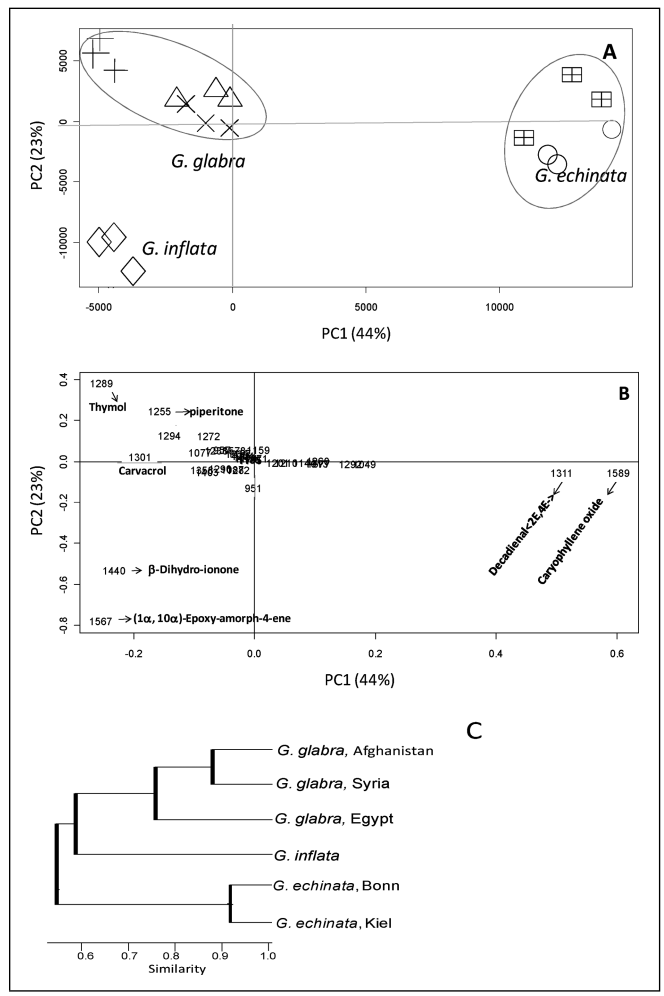

Figure 2–PCA and HCA analyses of different *Glycyrrhiza* species volatiles prepared using steam distillation. (A) PCA scores plot of PC1 and PC2 scores ($n = 3$). (B) PCA loading plot for PC1/PC2 contributing peaks and their assignments, with each volatile denoted by its RI value. GE1 (○), GE2 (⊞), GI (◇), GG1(+), GG2 (Δ), GG3 (x), details for *Glycyrrhiza* sample codes are described in Materials and Methods section. (C) HCA of *Glycyrrhiza* species based on group average cluster analysis of volatiles (mean value).

group had a negative effect on PC1, mostly from phenolics *viz*. thymol, carvacrol, and 1α, 10α-epoxyamorpha-4-ene.

Like PCA, HCA is an unsupervised data analysis method, meaning that prior knowledge of the sample is not required. As compared to PCA, HCA allows interpretation of the results in a fairly intuitive graphical way. Cluster analysis of the different *Glycyrrhiza* samples, according to their volatile profiles, was used as an additional exploratory tool to assess the heterogeneity between different genotypes (Figure 2C). HCA shows that *G. echinata* was the most distant species in comparison to the other ones, standing out as a separate group. Inspection of the dendogram also showed that *G. glabra* from Syria is more closely related to *G. glabra* from Afghanistan than toward that from Egypt. Most interestingly, PCA and HCA results derived from *Glycyrrhiza* volatiles composition mirrored those plotted from LCMS and NMR datasets (nonvolatile secondary metabolites), both revealing *G. echinata* as being most distant from other *Glycyrrhiza* species (Farag and others 2012). The fact that PCA results were generally independent of whether NMR chemical shifts or different retention time/mass signal pairs (GC-MS) suggest that classes for secondary metabolites in *Glycyrrhiza* show similar patterns among species accounting for such comparable species segregation.

With the effective differentiation of samples from different genetic origin, we tested whether multivariate statistical analysis can also differentiate the cultivation sources within a single species. Therefore, we attempted to analyze *G. glabra* samples separately with multivariate data analysis. The score plot revealed that samples could be differentiated without overlap. Most notably, *G. glabra* sample from Egypt was plotted on the left side (negative score values), whereas samples from *G. glabra* from Afghanistan and Syria were placed on the right side with positive score values (Figure 3). This group can still be separated along PC2. Examination of the loadings plot suggested that thymol and carvacrol contributed the most to the discrimination of samples. Relative quantification of both phenols pointed to its high abundance in *G. glabra* sample originating from Egypt (Figure 1, Table 1). We are aware that more samples from more accessions need to be studied eventually to provide a more significant and generalized picture of regional and genotype distributions, but our study clearly shows that simple GC/MS and PCA analysis of volatiles in principle can be used to distinguish between samples of different origin.

Comparison of SPME and steam distillation in *Glycyrrhiza* species

Comparing results of the SPME headspace volatiles analysis (Table S1) to those of distilled essential oil (Table 1) revealed that steam distilled oil exhibited a higher number of compounds compared to headspace trapped analysis for the 3 species. Both techniques also showed notable differences in volatile composition with SPME enriched in several minor monoterpene compounds like α-pinene, β-pinene, and myrcene; with the latter not detected in the steam distillation sample. The high performance of PDMS fiber in the extraction of low molecular weight nonpolar compounds has previously been reported (Zini and others 2002; Rout and others 2006). In total, of the 38 identified volatiles from steam distillation, 15 were detected using SPME technique. Quantitative analysis of the volatiles detected using SPME was not intended in this work, considering that fiber adsorption capacity (that is, response) for individual volatiles was not measured, and thus the method does not allow for accurate comparison with amounts detected by conventional essential oil analysis. In addition, both techniques are based on fundamentally different principles and extract different matrices (Tholl and others 2006). Nevertheless, relative comparison for presence and/or absence of volatiles is still possible and indeed with such disparity between both techniques, qualitative differences in results could lead to interesting findings (Table S1). Peaks for thymol and carvacrol associated with *G. glabra*, show the same regimented pattern using SPME, confirming their potential as chemotaxonomic marker for *G. glabra* species. Figure 4 shows peaks corresponding to these compounds in parts of the GC chromatogram of the SPME volatiles obtained from *G. glabra* roots. Among ketones, piperitone and carvone were both found in *G. glabra* volatile samples in agreement with steam distillation, whereas β−dihydro-ionone, a major constituent in *G. inflata* volatiles, was not detected using SPME. Epoxides and furans represent another group of volatiles that were identified only in essential oil samples. Some of these compounds may have formed through abiotic chemical conversions like peroxidation and subsequent reactions that might have taken place during hydrodistillation, compared to the mild SPME technique, which does not involve vigorous processes (Fernando and Grun 2001).

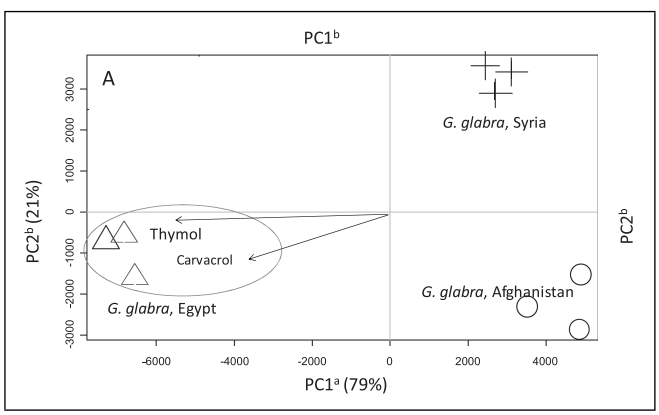

Figure 3–Principal component analyses biplot of *G. glabra* samples volatiles prepared using steam distillation, GG1 (Δ), GG2 (∘), and GG3 (+). Detail for *Glycyrrhiza* sample codes is described in Table 1, $n = 3$. [a]Axes refer to scores from the samples. [b]Axes refer to loadings from thymol and carvacrol represented as vectors from the origin. The model explains 100% of total variation, revealing separation of *G. glabra* grown in Egypt from other samples from Syria and Afghanistan along PC1.

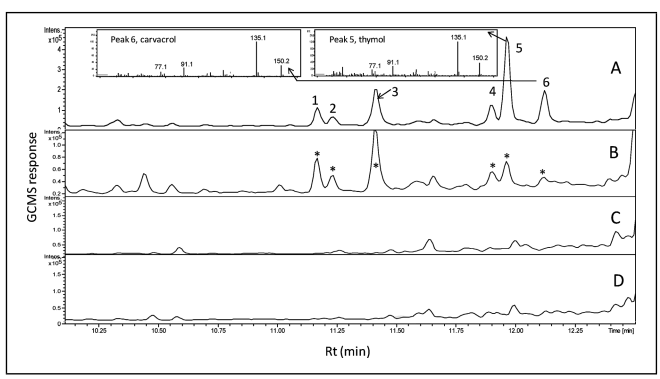

Figure 4–SPME gas chromatogram sections from *Glycyrrhiza* species: *G. glabra,* GG1 (A); *G. glabra,* GG2 (B); *G. inflata,* GI (C); and *G. echinata,* GE1 (D). Assigned peaks: 1, cumin aldehyde; 2, carvone; 3, piperitone; 4, (*E*)-anethole; 5, thymol; and 6, carvacrol. Asterisks in the lower chromatogram indicate compounds that align with numbered peaks above. Insets in panel A shows the mass spectra for (thymol, peak 5) and (carvacrol, peak 6).

Conclusions

Volatiles were extracted from the root of 3 *Glycyrrhiza* species from different geographic origins by 2 methods and then analyzed by GC/MS and GC/FID. A total of 38 compounds were detected of which only 8 were previously reported from roots of *G. glabra*. *G. glabra* ranked the highest for all terpene volatile classes. The most notable difference in composition was the high percentage of phenols in *G. glabra*, compared with high percentage of aliphatic aldehydes in *G. echinata*. The presence of thymol and carvacrol exclusively in *G. glabra* suggests that these volatiles could serve as chemotaxonomic markers and also might be considered as potentially relevant for taste. SPME in parallel to essential oil analyses provided the first comprehensive volatiles profile in *Glycyrrhiza* species which can be further applied for investigating other factors on volatiles composition, for example, growing habitats, seasonal variation, growth stage, and or storage conditions.

Acknowledgments

Dr. M. A. Farag thanks the Alexander von Humboldt-foundation, Germany for financial support. We are grateful to Prof. Tadato Tani, Otani Univ., Osaka, Japan, for providing *G. inflata* samples. We also thank Dr. Tilo Lübken for providing R scripts for PCA and HCA data analysis.

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Supporting Information

The following supporting information is available for this article.

Table S1. Volatiles detected in SPME headspace from *G. glabra*, *G. inflata,* and *G. echinata* and their relative abundances using GC-FID. Compounds are listed in order of elution from DB5-MS column. +++ = peak area usually 10% to 100% or more of the total area of all detected compounds; ++ = peak area usually between 1.0% and 10% of the total area of all detected compounds; and + = peak area usually <1.0% of the total area of all detected compounds. Empty cells indicate that the compound was not detected. Volatiles identified in the essential oil analysis are highlighted in bold.

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