Analysis of Antioxidant Polyphenolics in Medicinal Plants by GC/MS and LC/EI/MS

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INTRODUCTION

Monarda fistulosa is a prairie plant used widely by North American Indian tribes, especially the Plains Indians, to serve a variety of medicinal purposes. The plant is most closely related to ailments associated with the digestive system. The Monarda Fistulosa leaves were commonly used to relieve gas or alleviate nausea and vomiting. Furthermore, the leaves were used to induce sweating and urination, as well as act as a stimulate. It was a common practice of Plains Indian tribes to prepare tea extractions to treat colds, catarrh, headaches, aching kidneys, ease fevers and appease sore throats. Monarda fistulosa leaves were also used externally to treat a wide variety of skin eruptions, including pimples and cuts. Native tribes used the leaves fresh or dried, finding results with both. The leaves have also been used as an insect repellant. Essential oils of the Monarda Fistulosa leaves include bergamot oil, which can be inhaled to nurse bronchial ailments, and thymol, which can be used to relieve gas from the digestive track.

SAMPLE PREPARATION FOR MASS SPECTROSCOPY

Two separate samples of Monarda fistulosa leaves were treated by two separate methods. One sample was dried seven years before experimentation began; the other sample was analyzed a few months after harvest. Organic constituents were extracted with hexane/dichloromethane (60:40), and were constantly stirred for 19 hours at 150 rpms. The leaves were removed by suction filtration; the organic solvent was dried over sodium sulfate and rotary evaporated at 35º to obtain a viscous dark oil. In addition, the leaves were steam distilled (11 g) in order to acquire volatile constituents. The distillate underwent two 100 mL ether extractions, the ether was washed 1:1 with saturated sodium chloride, dried with sodium sulfate and rotary evaporated at 35ºC, resulting in a golden-colored oil. Samples were dissolved in acetonitrile for LC/EI/MS and either acetone or dichloromethane for GC/MS.

INSTRUMENTATION – PARTICLE BEAM LCMS INTERFACE

For particle beam LCMS, the system included the following components. The liquid chromatograph used was an Agilent Model 1100 modular system with quaternary pump, vacuum degasser, 100 vial autosampler and variable wavelength detector. The HPLC column used was a Zorbax SB-C18 (Agilent pn 830990-902), narrow bore 2.1 x 150 mm 3.5 micron. The Genesis II particle beam interface (CSS Analytical Co. Inc.) was attached to an Agilent 5973 MSD so that samples can be analyzed by LC/MS with electron impact and chemical ionization. The Genesis II is an improved particle beam interface, which delivers a higher amount of analyte to the ion source, when compared to previous commercial interfaces. The mass spectrometer used was an unmodified Agilent 5973 Mass Selective Detector (Agilent Technologies, Inc., Palo Alto California) with turbo molecular pump. The Agilent 5973 is a benchtop quadrupole mass spectrometer with mass range of 1.6 to 800 mass units, 10,000 volt HED, and is available with EI or EI/CI capabilities.

INSTRUMENTATION – GCMS

The GC utilized for the analysis was a Agilent 5890 equipped with a Zebron ZB-1 column (15m x 0.25mm x o.25um) (Phenomenex Torrance, CA). The mass spectrometer used was an unmodified benchtop quadrupole Agilent 5971 Mass Selective Detector (Agilent Technologies, Inc., Palo Alto California). The 5971 was controlled by ATX-EIGHTY-X data system (CSS Analytical Co. Inc.) with Agilent G1701BA Chemstation running on Microsoft Windows 2000. The conditions for the GC were initial oven temperature of 40ºC, injector 250ºC, transfer line 280ºC, a solvent delay of 2.00 min, the temperature was ramped at 10ºC/min to a final temperature of 230ºC and held for 1.00 min.

LIQUID CHROMATOGRAPHIC SEPARATION

The constituents were separated using a water (A) and acetonitrile (B) gradient. Initial conditions were 5% acetonitrile (0-3min) increasing to 95% acetonitrile at 50 minutes holding to 65 minutes and returning to starting conditions at 70 min. The detection wavelength was 254nm.

MASS SPECTRAL ANALYSIS

The data collected from the GC-MS and the Particle Beam EI Ionization of the chromatographic separation was analyzed with AMDIS (Automated Mass Spectral Deconvolution and Identification System), version 2.1, DTRA/NIST, 2002. The Total Ion Chromatograph (FIGURE 1) is delayed by approximately 0.2 minutes relative to the HPLC trace as determined by comparison of the two respective data sets.
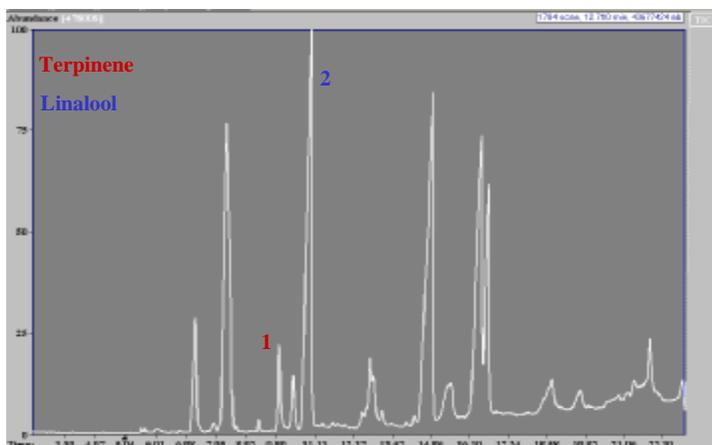
FIGURE 1. TOTAL ION CHROMATOGRAPH FROM GCMS

FIGURE 2. LIBRARY MATCH FOR THYMOQUINONE

(Text File) Scan 2158 (14.917 min) in H:\050221-2.D\DATA.MS
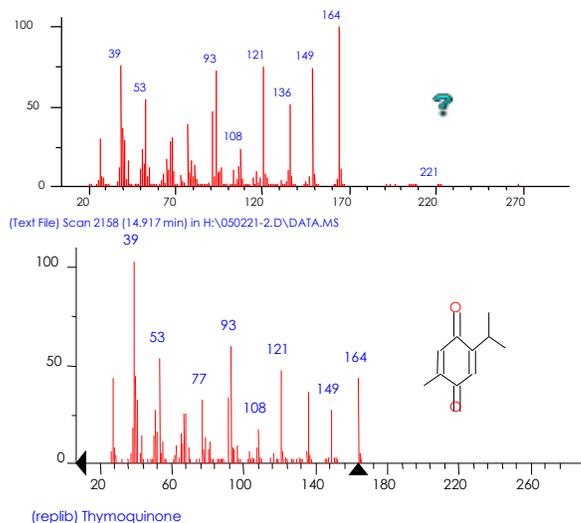
(replib) Thymoquinone

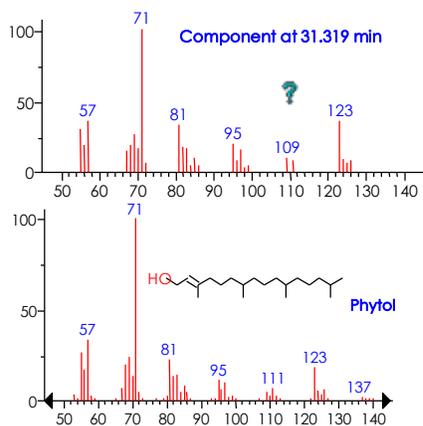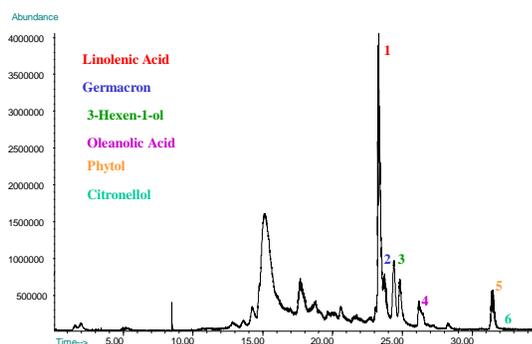**FIGURE 3. LIBRARY MATCH FOR PHYTOL**

FIGURE 4. TOTAL ION CHROMATOGRAPH FROM PB-LCMS

RESULTS

The separation techniques coupled with GCMS allowed successful separation of constituents as shown in the TIC Trace in FIGURE 1. This separation coupled with the Particle Beam mass spectrometer obtained the Total Ion Chromatograph shown in FIGURE 4. The collected data for the GCMS and Particle Beam LCMS was submitted for deconvolution and extracted ion analysis using the AMDIS program. The deconvolution program found 120 components in the GCMS TIC for the organic extract and 93 components in the Particle Beam TIC for the organic extract of the leaves. Comparisons of unidentified constituents and AMDIS library matches is shown in FIGUES 2 and 3. Using this information we were able to identify 34 constituents from the GCMS and 17 constituents from the PB-LCMS conclusively. The presence of these constituents were confirmed by comparison to previously published accounts of the constituents as shown in TABLE 1 and 2. However, many constituents found were absent in previous studies; thus, more proof is needed to confirm the constituents' absolute identity.

We have identified several, but not all of the chemical constituents of the Monarda fistulosa leaves. Many of the previously unidentified constituents have been linked to medicinal properties. The majority of the medicinally active constituents were obtained from the PB-LCMS data, some of them labeled in FIGURE 4. Oleanolic acid is reported to be used as an anti-inflammatory, while linolenic acid has been used to treat heart disease and rheumatism. The newly identified constituent phytol, used in making vitamins E and K, has been researched in connection with preventing various illnesses. The sesquiterpene germacron was identified and has been shown to work against bronchitis, and has also been known to benefit skin. 3-hexen-1-ol (leaf alcohol) and citronellol both possess aromatic properties. Bergamot oil, previously identified in Monarda fistulosa, composed of several constituents obtained from the GCMS including limonene, linalool, and terpinene, has many medicinal uses including the treatment of depression, tension, insomnia, and skin problems.

TABLE 1. IDENTIFIED CONSTITUENTS GC/MS

α-Thujene (AE,SD)	Cineole* (AE,SD)	Bornyl Acetate* (SD)	N,N-dibutylformamide (SD)
α-Pinene* (AE,SD)	Camphor (AE)	1-Terpinen-4-ol (AE,SD)	α-Cubebene (AE,SD)
Camphene* (AE,SD)	Terpinene* (AE,SD)	Cis-Terpineol* (SD)	Caryophyllene (AE,SD)
β-Pinene (AE,SD)	Cis-Linalool Oxide (AE,SD)	2,6-Dimethyl-3,7-octadiene-2,6-diol (SD)	Dodecanoic Acid (SD)
1-Ocetene-3-ol (AE,SD)	4-Carene (SD)	2-Methyl-5-(1-methylethyl)-2,5-cyclohexadiene-1,4-dione (SD)	D-Limonene* (AE)
α-Myrcene* (AE,SD)	Trans-Linalool Oxide (AE,SD)	ButylatedHydroxyTolulene (SD)	Thymoquinone (AE)
α-Phellandrene (AE,SD)	Linalool* (SD)	Asarone (SD)	Phytol (AE)
3-Octanol* (AE,SD)	Borneal (AE)	Carvacrol* (AE,SD)	
β-Cymene (AE,SD)	Cis-1-Methyl-4-(1-methylethyl)-2-cyclohexen-1-ol (SD)	Thymol* (AE,SD)	

AE – Separated by Alcohol Extraction Method   *Previously identified in Monarda fistulosa

SD – Separated by Steam Distillation Method

TABLE 2. IDENTIFIED CONSTITUENTS PB-LC/EI/MS

Camphene* (AE)	α-Linolenic Acid (AE)	Leaf Alcohol (AE)	Pantolactone (AE)
β-Cymene (SD)	6-Camphenol (AE)	p-tert-octylphenol (SD)	Palmitic Acid (SD)
Carvacrol* (AE)	Oleanolic Acid (AE)	Ursolic Acid (AE)	
Thymol* (SD)	Phytol (AE)	α-Limonene (AE)	
Dodecanoic Acid (AE)	Germacron (AE)	Citronellol (AE)	

CONCLUSIONS

Almost all new commercial LC/MS systems today are atmospheric pressure ionization instruments. Despite this, it is important to remember that the analysis of a true unknown is an extremely difficult task and may require the utilization of more than one technique. Nothing helps more than the direction that can be obtained from a standardized database of mass spectra with which to match. In summary, an evaluation of our experiments showed the following:

1. The Particle Beam LCMS works well for the organic extract obtained from the leaves and eliminates the need for any preparatory pre-purification or derivatization steps.

2. The ability to search spectral libraries makes the identification of the constituents much less tedious.

3. The use of GCMS proved to be a successful means of identification for multiple constituents from both the organic extraction and the steam distillate.

4. LCMS with Particle Beam ionization is capable of providing fragmentation information on molecules inaccessible to other techniques.

FUTURE WORK

Our intentions are to use Electrospray Ionization (ESI) LCMS to further identify unknown constituents. Through this method, compiled with other techniques, we hope to isolate and identify unknown peaks. We plan to obtain standards of several of the identified constituents, especially those with indicated medicinal purposes, to verify their location in the observed spectra. Future research would including studying the effects of location and environmental conditions on the production of these constituents in Monarda fistulosa around Missouri and Kansas.