Analysis of Smoke Plants using Gas Chromatography-Mass Spectrometry in Archaeological Residue Analysis

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Introduction:

Smoking as a human activity dates back at least four millennia, but the time period during which tobacco became the most commonly smoked plant remains unknown. Gas chromatography-mass spectrometry (GC-MS) is particularly useful in identifying compounds persisting within the matrix of archaeological smoking pipe fragments. Rafferty (2002) outlined a technique for detection of nicotine, a biomarker for tobacco, in pipe fragments by extracting it into an organic solvent, but protocol for detecting biomarkers in other smoke plants is less developed. Mint and mugwort were utilized by Native Americans for medicinal purposes. Mint leaves and stems were boiled in water to produce a tea used to treat stomach pain, while mugwort-based tea was utilized as a diaphoretic and emmenagogue (Hutchens, 1991. pp.197-200). However, little is known about these plants' potential use as smoke plants. By analyzing smoke plants and their combustion products, this study aimed to better define plants' chemical footprints so that more plant species can be identified in archaeological pipe residues.

Methods:

Dried and crushed plant samples were immersed in 2:1 chloroform:methanol solvent, ultrasonicated for one hour, and centrifuged for 15 minutes to separate the sample from the solvent containing the plant extract. For the mint, a 1.0 mL aliquot of the extract solution was evaporated to dryness under nitrogen and then reconstituted in 1 mL of hexane to concentrate the sample. The solvent was filtered through sodium sulfate to remove residual water from the sample and analyzed using a Varian 3900/Saturn 2100T GC-MS system. To simulate smoking, dried and crushed plant samples were placed within granite pipes and combusted using a Welch dry vacuum pump. Ash was removed, and pipes were immersed in 2:1 chloroform:methanol solvent and ultrasonicated for one hour. The solvent, containing the combusted plant extract, was evaporated to 2 mL under nitrogen and filtered through sodium sulfate for GC-MS analysis.

Background image downloaded from https://images.fineartamerica.com/ images-medium-large/swirling-smoke-on-white-background-luis-a-pena.jpg.

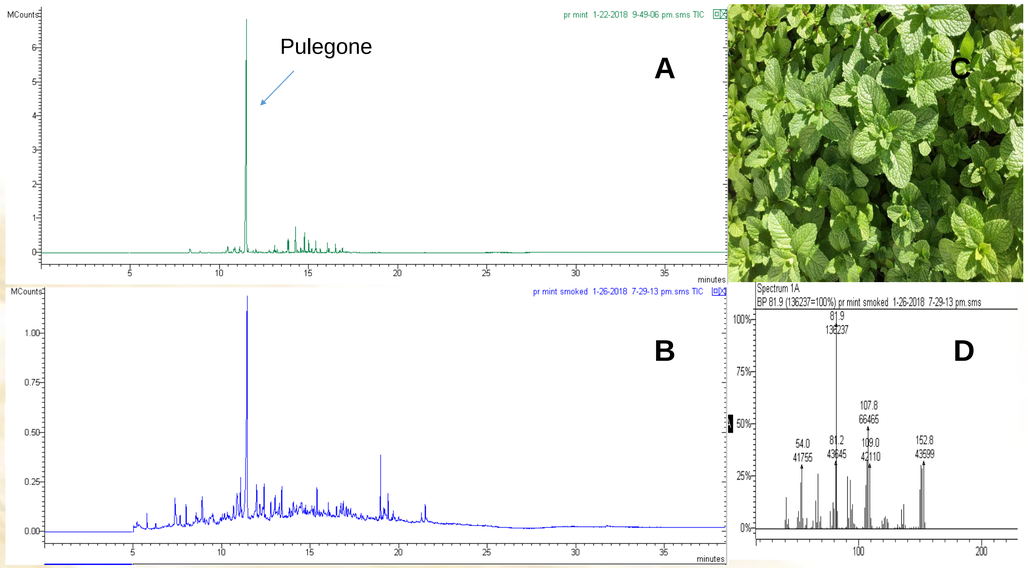

Figure 1. (A) is the chromatogram from the mint extract showing the prominent pulegone peak detected at a retention time of 11.451 minutes; (B) is the chromatogram from the pipe residue after combustion of the mint plant where pulegone was detected at a retention time of 11.437 minutes; (C) shows the mint plant (Image downloaded from https://www.almanac.com/blog/ natural-health-home-tips/magnificent-medicinal-and-sometimes-maleficent-mints); (D) shows the mass spectrum for the pulegone peak at 11.437 minutes in the residue sample extracted from the pipe with combusted mint characterized by *m/z* fragments of 82 and 152 in the mass spectrum.

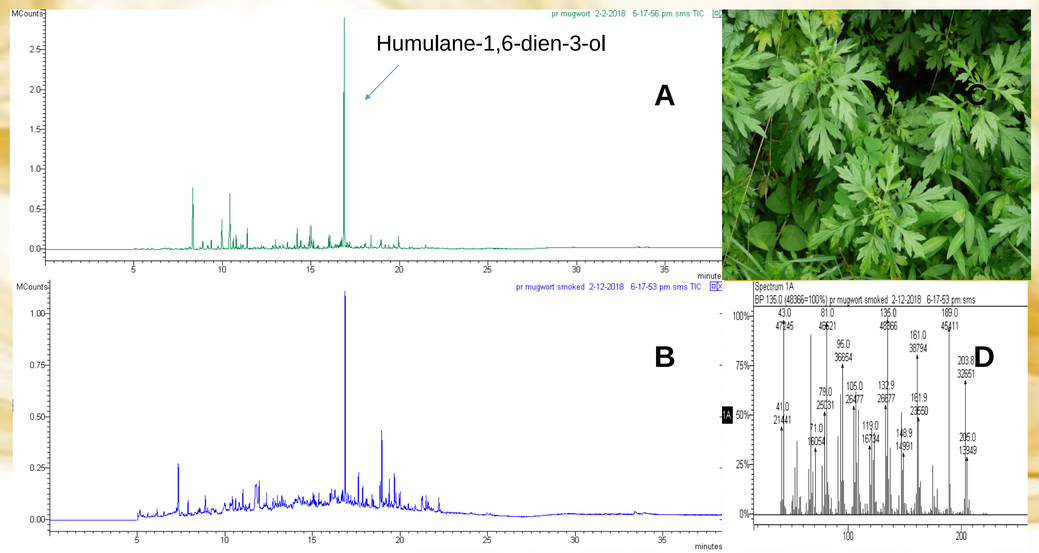

Figure 2. (A) is the chromatogram from the mugwort extract showing the prominent humulane-1,6-dien-3-ol peak detected at a retention time of 16.868 minutes; (B) is the chromatogram from the pipe residue after combustion of the mugwort plant where humulane-1,6-dien-3-ol was detected at a retention time of 16.869 minutes; (C) shows the mugwort plant (Image downloaded from https://www.crimson-sage.com/images/stories/virtuemart/product/100613-mugwort-closer-1024x768.jpg); (D) shows the mass spectrum for the humulane-1,6-dien-3-ol peak at 16.869 minutes in residue sample extracted from the pipe with combusted mugwort characterized by *m/z* fragments of 81, 95, 161, 189, and 204 in the mass spectrum.

Results:

Pulegone was the primary compound identified in the mint extract (Fig.1). Other compounds of interest were caryophyllene and (+)-Epi-bicyclosesquiphellandrene. The pulegone peak was still most prominent in the smoked sample, but caryophyllene, (+)-Epi-bicyclosesquip-hellandrene, and 7,11,15-Tetramethyl-2-hexadecen-1-ol were also detected (Fig. 1). Humulane-1,6-dien-3-ol was the primary compound detected in mugwort (Fig. 2). Eucalyptol and borneol were also detected. In the smoked mugwort sample, humulane-1,6-dien-3-ol peak was still most prominent (Fig.2). Eucalyptol and borneol were again detected, along with phosphoric acid and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol.

Discussion:

Both the mint and mugwort extracts were characterized by compounds that persisted in the pipe matrix after combustion. Pulegone, the dominant compound in the mint sample, is a major volatile component in mint essential oils (Hajlaoui et al., 2009; Hawrył et al., 2015). Recent research has shown that pulegone has potential depressant effects in the central nervous system, aligning it with other recreational depressants utilized throughout history (de Sousa, 2011). The primary compound identified in the mugwort extracts was humulane-1,6-dien-3-ol. Chen-Zing et al. (2014) confirmed the presence of this compound in extracts in other plants in the *Artemisia* genus. This study shows that the major volatile compounds detected in mint and mugwort persist after combustion and can be detected through analysis of the pipe matrix after the plant has been smoked. While the chemical composition of pipe residues should be more thoroughly described, these results suggest that knowledge of these characteristic volatile compounds can be helpful in identifying smoke plants in archaeological residue analysis.

Literature Cited:

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