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Speciation of Benzophenanthridine Alkaloids in Commercially Available Black Salve Products

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Abstract

Black salve products are marketed to consumers as cures for cancer and other medical conditions. These products are readily available for purchase online even though they are based on outdated research from the 19th century. The chemical compositions of black salve products are not provided by the manufacturers and minimal data are available regarding safety measures. They are believed to contain bloodroot (the rhizome of *Sanguinaria canadensis*) and additional plant extracts. Bloodroot extracts have been previously studied for their anticancer properties. The studies have shown that they are not effective cancer treatments and are associated with severe side effects. Sanguinarine, a compound known to be biologically active, is the major benzophenanthridine constituent that can be obtained from bloodroot. Other benzophenanthridine constituents present in bloodroot include chelerythrine, sanguilutine, chelilutine, chelilutine, sanguirubine, and chelirubine. Although some constituents are known, a comprehensive chemical analysis of black salve products has never been conducted. Liquid chromatography-mass spectrometry (LC-MS) is a powerful analytical technique that has been used to detect and quantify sanguinarine and numerous other benzophenanthridine compounds from a variety of sample matrices. Data will be presented showing detection and quantification of sanguinarine using an external standard calibration. A method will be developed to extract sanguinarine from black salve creams. Black salve extracts will be analyzed via LC-MS. Along with detecting and quantifying sanguinarine, mass spectral data will be monitored for the presence of other benzophenanthridine constituents of bloodroot. These species will also be quantified if detected in the extracts.

Keywords: Sanguinarine, Black Salve, Liquid Chromatography-Mass Spectrometry

1. Introduction

Black salve products are advertised online to customers as a cure for certain types of cancers and other conditions. These products are becoming more popular among cancer patients looking for alternative treatment rather than the traditional routes of therapy, like chemotherapy and radiation. This alternative product is classified as an escharotic agent, which are caustic and corrosive[1-2]. Escharotic agents are claimed to have the ability to destroy skin and cancer cells. The name escharotic comes from the dry, dark scar (eschar) that is produced after using these products. An eschar is pictured in Figure 1. The most common ingredients of escharotic agents are zinc chloride and bloodroot[3]. In this study, black salve products with bloodroot constituents were focused on. Black salve products are based on the research done by Harry Hoxsey's family which began in the 19th century. The research and formulas for these cancer treatments were passed down to Hoxsey by his father, whom is claimed to use the first successful preparation on humans. Hoxsey began treating patients in 1921 by using a concoction of herbs and bloodroot in either a paste or tonic form. By 1960, the Food & Drug Administration had banned the sale of Hoxsey's products in the United States since the research conducted had shown that these products had no health benefits[4].

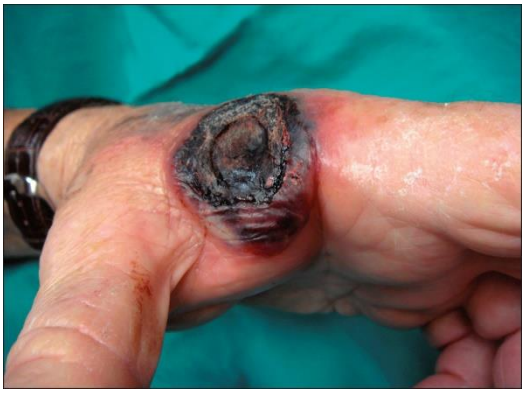

Figure 1. An eschar

The main active ingredient in black salve products is the rhizome from the plant bloodroot (*Sanguinaria canadensis*)[5]. The rhizome contains six quaternary benzophenanthridine alkaloids which attribute to the plant's purposed anticancer properties. The benzophenanthridine constituents of bloodroot are sanguinarine, chelerythrine, sanguilutine, chelilutine, sanguirubine, and chelirubine[6]. The most abundant and studied constituent is sanguinarine (Figure 2). Sanguinarine (M.W. of 332.33 g/mol) has two naturally occurring forms: a charged iminium or an uncharged alkanolamine. The iminium is the biologically active form of sanguinarine, which can easily move into cells and accumulate near the nucleus[7]. Once inside the cell, sanguinarine acts as a toxin and acts on the sodium-potassium-ATPase transmembrane protein effectively killing the cell[8]. Sanguinarine is not regulated in black salve products which can lead to uneducated, dangerous use of the escharotic compound[9].

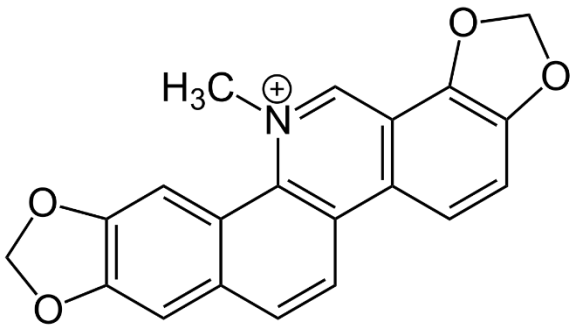

Figure 2. Chemical composition of sanguinarine

While black salve products are readily increasing popularity, the exact chemical composition of these products remain unknown. The primary goal of this project is to quantify sanguinarine and additional quaternary ammonium benzophenanthridines in black salve products, using liquid chromatography-mass spectrometry (LC-MS). LC-MS is a powerful analytical technique that has been used to detect and quantify sanguinarine and numerous other benzophenanthridine compounds from a variety of sample matrices. This research seeks to develop an LC-MS method for quantifying sanguinarine and then apply the method to extracts from various black salve products.

2. Methodology

2.1. Preparation of Laboratory Standards

Sanguinarine chloride powder was obtained from Tocris Biosciences (Bristol, UK). A 100 ppm stock solution was prepared by dissolving solid sanguinarine chloride in ethanol. This solution was used to prepare six laboratory standards (0.1, 1, 10, 20, 30, and 40 ppm). The standards were prepared by diluting various amounts of the sanguinarine stock solution with ethanol. All solutions were stored in the dark at 4 ℃.

2.2. Liquid Chromatography

The LC system was an Agilent 1100 Series (Santa Clara, CA) equipped with a degasser and an autosampler. The LC separations were conducted on a Restek Raptor C18 reversed-phase column (Bellfonte, PA) with dimensions of 2.1 mm x 50 mm and a 2.7 μm particle size.

Autosampler injections for each standard were 20 μL. An isocratic mobile phase consisting of 50 % deionized water with 0.1% formic acid and 50 % methanol with 0.1 % formic acid was used for the analyses. Analysis time for each standard was 3.0 minutes and each standard was run in triplicate.

2.3. Mass Spectrometry

The MS system used for these experiments was a ThermoFinnigan LCQ Advantage Ion Trap MS (Waltham, MA) equipped with a heated capillary interface and electrospray ionization. The MS was operated in positive ion mode. Thermo scientific XCalibur software was used to control and monitor all experimental conditions as well as for data analysis.

3. Data

Sanguinarine was detected in less than one minute using the isocratic LC method developed for this study. Electrospray ionization allowed sanguinarine to be primarily detected as a molecular ion (m/z =332). Selected ion monitoring was used to detect this specific ion, thus reducing noise when compared to a total ion chromatogram. The ion of interest was scanned as a function of time resulting in a selected ion mass chromatogram. Figure 3 displays an example of such a chromatogram for sanguinarine. Figure 4 displays a corresponding mass spectrum, with the sanguinarine signal at m/z = 332.

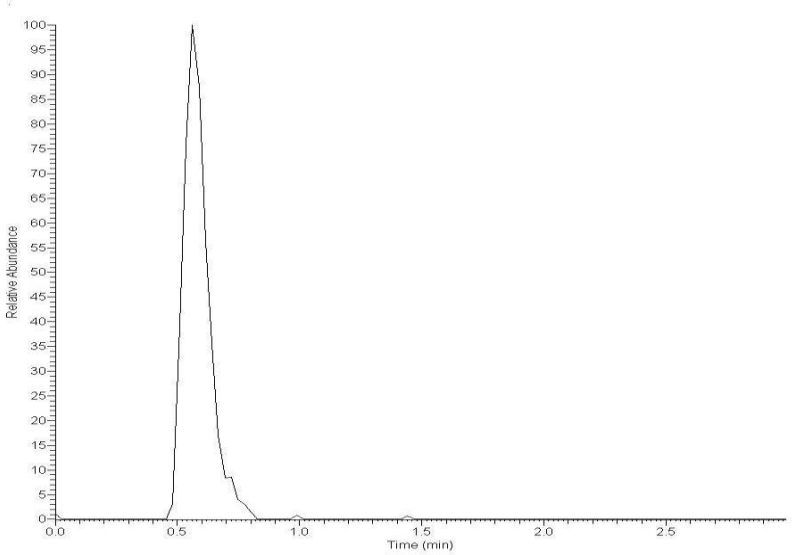

Figure 3. Selected ion mass chromatogram of sanguinarine

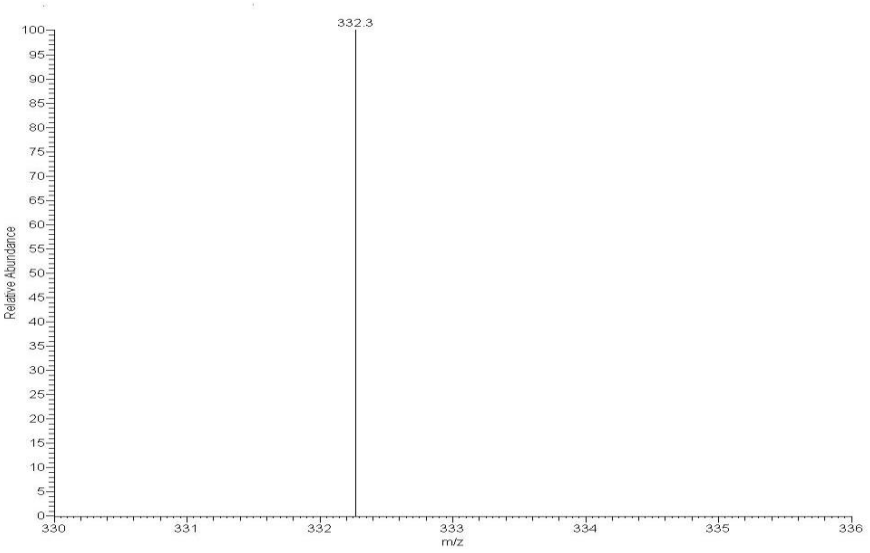

Figure 4. Mass spectrum of sanguinarine

After it was established that the LC-MS method could efficiently detect a signal from the sanguinarine ion, an external standard calibration was developed over a concentration range of 0.1-40 ppm. Specifically, sanguinarine standards with concentrations of 0.1, 1, 10, 20, 30, and 40 ppm were prepared. Each standard was run in triplicate. For each standard experimental run, the chromatographic peak area was obtained. The peak areas for each set of replicates was averaged and plotted versus concentration. A linear relationship was obtained with a coefficient of determination (R^2) value of 0.9869. The calibration curve is displayed in Figure 5.

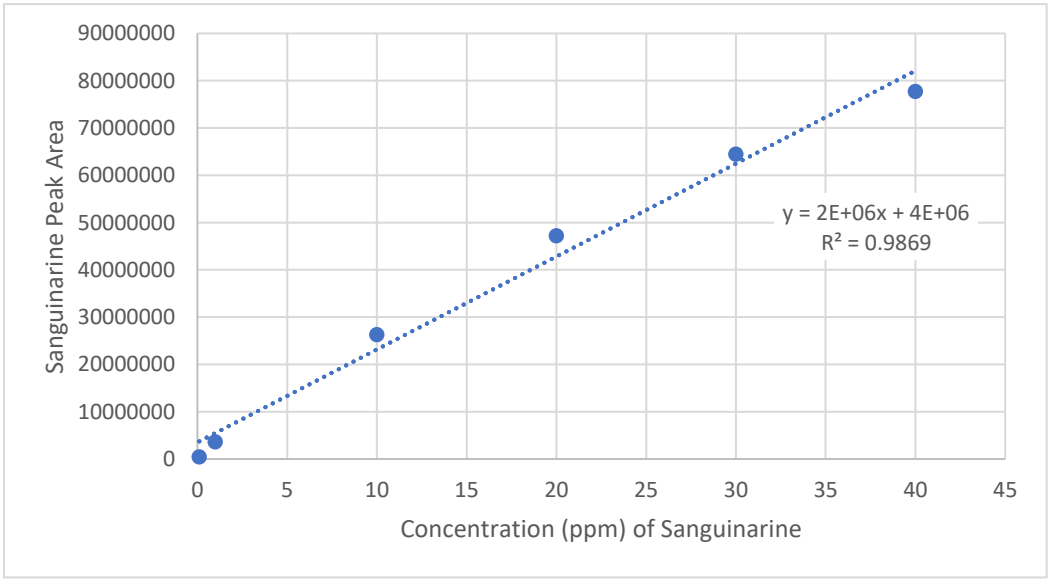

Figure 5. External standard calibration curve for sanguinarine

4. Conclusion

By plotting the peak area to the varying sanguinarine concentrations, a calibration curve was obtained with a linear relationship. This linear relationship had a 0.9869 coefficient of determination which indicates that the stated method

can quantify sanguinarine in a concentration range spanning three orders of magnitude. This method can now be applied to detection of sanguinarine in extracts from black salve products.

5. Future Work

Future work will be done with extracts from black salve creams to test for the presence of sanguinarine as well as other benzophenanthridine compounds. An extraction method will be developed in order to prepare samples for introduction to the LC-MS. Future experiments will also aim to develop an internal standard calibration for sanguinarine, which will help negate the effects of signal fluctuation in the LC-MS instrumentation.

6. Acknowledgments

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7. References

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