

Chemical and Biological Investigation of the Effects of Vinegar Baking *Sambucus canadensis* and *Salvia officinalis* Anthocyanins

Elizabeth Martin, Chiraz-Soumia M. Amrine, Ph.D.
Department of Physical and Earth Sciences, Arkansas Tech University, Russellville, AR 72802, USA

Abstract

New treatments for cancer and bacterial infections are needed, especially because most patients acquire resistance to conventional first-line treatments. Many natural product metabolites exhibit potent activity. *Sambucus sp.*, elderberry, and *Salvia sp.*, or sage, are well-known shrubs for their therapeutic benefits. This study aims to analyze how secondary metabolites are extracted from the native American Elderberry, *Sambucus canadensis*, and Sage, *Salvia officinalis*. It also aims to investigate the process of vinegar baking of medicinal plants to enhance the chemical space and increase the biological properties. Vinegar baked and non-vinegar baked elderberries and sage were utilized to test this theory. A Soxhlet extractor was used to extract the medicinal molecules. A rotary evaporator and a separatory funnel were required for many liquid-liquid extraction steps. The samples are subjected to Gas chromatography coupled to a mass spectroscopy GC-MS to identify the secondary metabolites. Our preliminary results show an improvement in the antibacterial activity of both the elderberries and sage baked in vinegar extract compared to the raw organic extract.

Introduction

Salvia officinalis, or sage, is also native to Arkansas, and has been traditionally used in Native American and Chinese medicine.

Sambucus canadensis, more commonly known as the American Elderberry, is a plant native to Arkansas and is becoming well researched due to the medicinal properties of the secondary metabolites of the elderberry. The secondary metabolites studied are anthocyanins. Anthocyanins, which are abundant in the elderberry, are flavonoid pigments that have a variety of applications. The berries were stored in a -80°C freezer to preserve the anthocyanins. Vinegar baking is common practice in traditional Chinese medicine to change the pharmacological effect of medicinal herbs. The elderberries were baked in vinegar for 8 days at RT to examine the changes in the therapeutic effects. To extract the anthocyanins various steps were performed: extraction, rotary evaporation, and separatory processes. These steps produced a sample of anthocyanins. These anthocyanins undergone a minimum inhibitory concentration test against both, *Escherichia coli* and *Staphylococcus epidermidis*, as well as tests using the Kirby-Bauer disk diffusion method.

References

Xing J, Sun HM, Li ZY, Qin XM. Comparison of Volatile Components between Raw and Vinegar Baked Radix Bupleuri by GC-MS Based Metabolic Fingerprinting Approach. Evid Based Complement Alternat Med. 2015;2015:653791. doi: 10.1155/2015/653791. Epub 2015 Jul 21. PMID: 26265925; PMCID: PMC4523648.



Concentration (mg/mL)	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
100	NI	I	NT
33.3	NI	I	NT
11.1	NI	I	NT
3.7	NI	I	NT
1.23	NI	I	NT
0.411	NI	NI	NT
0.137	NI	NI	NT

Table 1. Antibacterial inhibition when tested against the SC extracts. Concentrations at which no inhibition was found is represented as NI. Concentrations in which there was inhibition is represented as I. If a bacterium was not tested it is represented as NT.

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100	I	NT	I
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11.1	NI	NT	NI
3.7	NI	NT	NI
1.23	NI	NT	NI
0.411	NI	NT	NI
0.137	NI	NT	NI

Table 2. Antibacterial inhibition when tested against the A-VBSC extracts. Concentrations at which no inhibition was found is represented as NI. Concentrations in which there was inhibition is represented as I. If a bacterium was not tested it is represented as NT.

Conclusions

This study shows the promising potential that vinegar baking medicinal herbs have on their antibacterial capabilities against *E. coli* and *S. epidermidis*. Further study would ensure the reproducibility of the study, and further study of *E. coli* and other bacterial species. Additionally, HPLC-MS and gas chromatography will be performed to determine the chemical composition that occurred during the vinegar baking process.

Results

This study shows promise for the continuation of the research leading to testing of the cytotoxic and antimicrobial effects of anthocyanins and the effects of vinegar baking on the medicinal herbs. The results of minimum inhibitory concentration tests indicated that vinegar baking the anthocyanins had a positive effect on the inhibition of growth of *Escherichia coli*. At a concentration of 33µg/mL, 23 colonies were able to grow on the agar; this growth was not present at a concentration of 100µg/mL. The raw elderberry anthocyanin extract was shown to have no inhibitory effect of the growth on *Escherichia coli*. This is in comparison to the vinegar baked elderberry extracts that showed inhibition at 100µg/mL, but no inhibition at any other concentrations. *Staphylococcus epidermidis* showed growth of a total of 12 colonies, but no growth at a concentration of 33.3µg/mL. This bacteria was not tested on the raw elderberry extract, so a direct comparison is unavailable. *Staphylococcus aureus* was tested on the raw elderberry extract and showed an MIC of 3.7µg/mL, a much lower concentration. The results of the Kirby-Bauer tests showed that there is a significant ZOI for the grape vinegar and the raw sage extract, at higher concentrations.

Both gram positive bacteria had inhibitory effects, but the effect of vinegar baking can not be determined since we were not able to directly test against the raw and vinegar baked elderberry and the raw and vinegar baked sage. HPLC-MS was not able to be performed and will be added to this study when results are available.

