

Optimizing eugenol extraction conditions from fresh and dried samples of holy basil (*Ocimum sanctum*)

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ABSTRACT

*The study of herbal medicine is simultaneously an ancient practice and a currently growing and evolving field. A staggering variety of therapeutic agents have already been isolated from essential oils and their beneficial medical properties demonstrated. Holy basil (*O. sanctum*) has been the subject of many studies with the active ingredient eugenol proving to be a potent adaptogenic agent, with healing potential across a wide array of ailments. For continuing research, as well as therapeutic use, determining and understanding the ideal eugenol extraction conditions for different preparations of the herb is essential. This study investigates the optimal conditions under which eugenol can be extracted from freshly grown and commercially prepared samples of holy basil (*O. sanctum*).*

Keywords: Holy basil, eugenol, extraction, optimization

INTRODUCTION

Holy Basil (*Ocimum sanctum*) is one of the most widely grown herbs for therapeutic use [1]. Native to India, the heavily branched and tropical plant can grow up to 18 inches in height [2]. Three distinct varieties of holy basil exist: Rama, which is the most commonly found white-green variety, Krishna, which is the stronger flavored red-purple variety, and Vana, which grows wild in the forests of South Asia and Malaysia [3-5]. With its roots in the ayurvedic system of medicine, holy basil has been utilized for thousands of years [2]. The herb is used as a remedy for a variety of conditions including the common cold, headaches, stomach disorders, heart disease, inflammation, malaria, various forms of poisoning, as well as spiritual and flavoring purposes [6-7]. Previous studies on holy basil have proven the herb to be an adaptogen—it assists the body with stress and helps to normalize body functions [6]. The essential oil of holy basil, eugenol, is thought to be responsible for such properties [1].

Herbal essential oils contain hundreds of phyto-chemicals that help to promote general health and boost the body's immune system [6,8-10]. The essential oil of any herb can be extracted using a variety of methods including distillation, solvent extraction, cold pressing, maceration, or supercritical carbon dioxide extraction [1]. Essential oils are extracted from the dried or fresh herbs because they are much more concentrated (75 to 100 times) [3]. An increasing interest in herbal medicine for therapeutic use has sparked a surge in the study of the essential oils from a variety of herbs [11-15] for their pharmacological uses since it is the oils that demonstrate the maximum benefits [16]. The objective of the current study is to determine the optimal eugenol extraction conditions for fresh and dried samples of holy basil. By determining these optimal conditions, industrial production of these therapeutic agents, as well as general household use, could be greatly improved.

MATERIALS AND METHODS

Preparation of Plant Samples:

Organic Rama *Ocimum sanctum* seeds were purchased from Horizon Herbs LLC in Williams, Oregon. Seeds were allowed to germinate on a damp paper towel inside a re-sealable plastic bag in direct sunlight. After germination, (approximately 7 days) samples were planted in soil containing 0.12% ammonical nitrogen, 0.09% nitrate nitrogen, 0.07% phosphate, 0.14% soluble potash, and 0.10% iron fertilizers by weight. Planting took place in July of 2011. Plants were maintained at 20°C with 10-12 hours of indirect sunlight per day. Samples were watered as needed. Flower buds were removed to promote continual growth and to keep the plant from dying prematurely. The harvesting of holy basil leaves began in late August and continued until March of 2012. To harvest, leaves were cut from the stems and placed onto a flat surface to dry, free from heat and light. Complete dryness was achieved in five days. Fresh samples were then stored in an airtight container. For comparison, additional samples of pre-grown, organic Rama holy basil were purchased from Mountain Rose Herbs in Eugene, Oregon and stored in a separate airtight container.

Isolation of Essential Oil:

The essential oil was isolated from both types of holy basil samples using a micro-scale distillation apparatus. A sand bath was heated to 100°C using a hot plate before the reflux and distillation were performed. Exactly 1.0 g of the dried holy basil along with 12 mL of distilled water was added to a round bottom flask to begin reflux. Samples were refluxed for 10, 20, 30, 40, or 55 minutes. After the appropriate time had elapsed, the flask was immediately removed from reflux apparatus and quickly cooled to room temperature in an ice bath. The dregs were removed by gravity filtration and the filtrate was stored in a clean glass vial. A separate micro-distillation apparatus was assembled to isolate the essential oils from the samples. Five milliliters of the filtrate along with a boiling chip were added to the round bottom flask. A Hickman column was secured to the flask and the entire apparatus was lowered into a heated sand bath. The essential oil distillate was collected between 88°C-95°C. These samples were stored in a refrigerator at 2°C until needed for further analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis:

Analysis of the essential oils was carried out using a Shimadzu GC/MS-QP2010. The instrument was equipped with a SHR5XLB fused silica capillary column (30m × 0.25mm). For each sample type and reflux time, separate GC-MS samples were prepared by adding 500 µL of essential oil and 1000 µL of Hexane (spectrometer grade). The method parameters for sample analysis consisted of an injection port temperature of 230°C, a column temperature starting at 35°C, and ramping at a rate of 15°C/min to a final temperature of 300 °C. The analysis ended with a 30-minute hold at 300°C.

Identification and Qualification of Essential Oil Components:

The chemical constituents of the essential oil were qualitatively identified using the Shimadzu software for the GC/MS. Since each sample was prepared in the same manner, quantification of eugenol in each sample was determined by peak area, using TIC peak integration. Area normalization was then used to calculate the percent concentration using the equation; % concentration = $\frac{A_i}{\sum A_i} \times 100$ (where A_i is peak area).

RESULTS AND DISCUSSION

Eugenol was found to be the primary component in the extractions from each sample series. In general, subjecting the samples to increasing extraction times resulted in increasing concentrations of eugenol (Table 1).

It was noted that for both sample series, extractions lasting longer than 55 minutes resulted in steadily decreasing eugenol concentrations. The fresh basil samples displayed an optimal eugenol extraction (highest concentration per sample) time of 55 minutes while the commercially grown and dried sample series showed an optimal extraction time of 40 minutes with eugenol concentration beginning to decrease when subjected to a 55 minute extraction time (Figure 1).

Table 1: Eugenol concentrations as a function of extraction times

Fresh Holy Basil Samples

Extraction Time (min)	Peak Area (counts)	Peak Height (counts)	A/H	Concentration %	Peak Retention Time (min)
10	85429	57454	1.48	0.36	12.964
20	197250	134335	1.46	1.35	12.974
30	303536	197780	1.53	2.26	12.974
40	557014	409714	1.35	3.82	12.976
55	1297786	931459	1.39	6.01	12.966

Dried Holy Basil Samples

Extraction Time (min)	Peak Area (counts)	Peak Height (counts)	A/H	Concentration %	Peak Retention Time (min)
10	1451464	985727	1.47	11.33	12.983
20	1976157	1327988	1.48	15.67	12.982
30	2170666	1454148	1.49	10.99	12.984
40	5496507	3586915	1.53	28.77	12.990
55	3105189	2104361	1.47	23.74	12.986

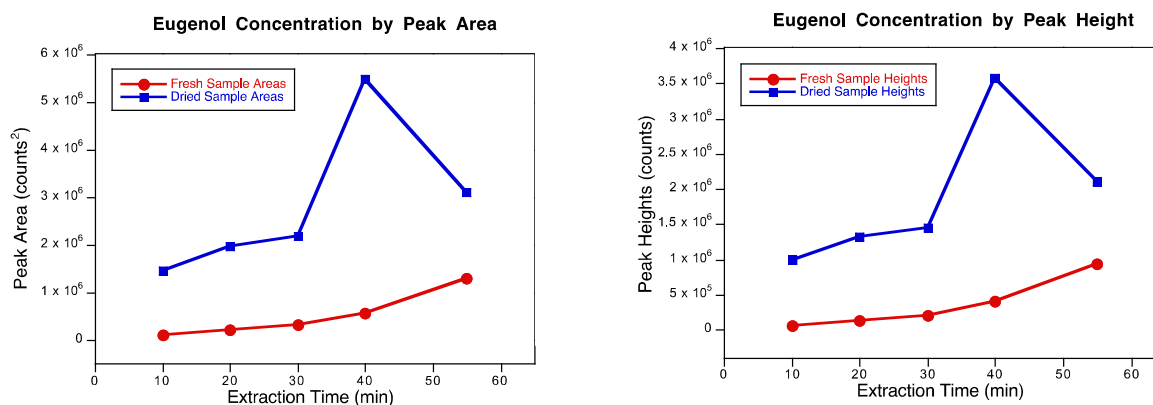


Figure 1: Eugenol concentrations as a function of extraction times

When comparing the eugenol concentrations of one series to the other, it is important to note that the commercially grown and dried samples consistently showed significantly higher concentrations of eugenol in the extracted oils under any of the extraction conditions (Table 1) even though the overall trends within the series differed.

The results of this study indicate that eugenol is much more concentrated in the essential oils of dried samples of holy basil. It is clear that increasing the extraction time for fresh samples coincided with an increase of eugenol concentration up to a maximum at 55 minutes, with a drop in concentration for extraction times beyond that (unpublished results). The dried samples of holy basil displayed a similar trend with the exception that a maximum concentration was reached after only 40 minutes.

The reason for this difference is most likely due to the large difference in baseline eugenol concentration between the fresh and dried samples. It is clear that the commercially dried samples display a much higher eugenol concentration due to a more complete absence of water in the samples. It follows that a much larger eugenol-to-sample mass ratio can be obtained when extracting from commercially dried samples as compared to the fresh samples, which still contain a small amount of water that will be included in the total mass of each sample. Thus, holding all samples to identical total masses will yield the observed differences in total eugenol concentration.

A higher concentration of eugenol will also coincide to a shorter maximum extraction time due to the relatively low volatility of the compound [17]. Fresh samples, with much lower total eugenol concentrations, can be subjected to longer extraction times without a significant loss of the compound while their commercially dried counterparts will begin to lose the component at extraction times longer than 40 minutes.

CONCLUSION

Eugenol is thought to be primarily responsible for the many medically beneficial characteristics of the herb Holy Basil [1]. A better understanding of the optimal extraction conditions of this compound can lead to simpler and more effective ways of obtaining it for continuing research. This study demonstrates the optimal eugenol extraction conditions for two different preparations of Holy Basil.

Acknowledgements

We would like to thank Dr. Chrissie Carpenter for her help and many thoughtful suggestions. We would also like to thank the University of Great Falls for the use of their facilities and funds.

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