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Quantification and characterization of *Pimenta dioica* (Allspice) essential oil extracted via hydrodistillation, solvent and super critical fluid extraction methodologies

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Abstract

Pimenta dioica (L.) Merr (family: Myrtaceae) commonly known as Allspice, is a distinguished plant recognised for its berries. For the current study, the essential oil yield and chemical composition of Jamaican Allspice berry was investigated. The oils were obtained by hydrodistillation (HD), solvent extraction using hexane and super critical fluid extraction (SFE) with 1% ethanol as co-solvent. Oil yield percentages amounted to 1.4, 6.4 and 6.8%w/w for the HD, SFE and SE oils respectively. Analysis of the oils by GC and GC-MS resulted in the identification of forty three (43) compounds representing 90.26% HD oil; 75.40% hexane oil and 82.99% SFE oil respectively. Main components identified were eugenol (61.36%), β -caryophyllene (4.58%), α -humulene (1.90%) and 1,8-cineole (1.89%); minor components included δ -cadinene (1.08%), Germacrene D(0.86%) and β -elemene (0.69%). All compounds identified were common among each extracted oil type. Fundamentally, for commercialization purposes, a SFE extracted oil is preferred as it is a modernized technique which shortens extraction time, reduces organic solvent consumption, prevents pollution and eliminates organic solvent residues whilst producing high essential oil yields.

Keywords: *Pimenta dioica*, essential oil composition, SFE, hydrodistillation, pimento, eugenol

1. Introduction

Pimenta dioica (L.) Merr. (Myrtaceae family) also known as allspice or pimento in Jamaica, is a spice recognized and utilized worldwide. Renowned for its unique flavour profile, the spice is characterized by a flavour reminiscent of a combination of cloves, nutmeg and cinnamon [1-3]. The allspice plant is a modest evergreen tree whose growth can span up to 15 meters in height. It has a pale silvery brown bark and oblong to elliptical clustered leaves, 6-20cm in length with pellucid glands, aromatic when crushed [1, 2]. The herb has a heightened flowering period between the months of March and June; after which the fruit born from the plant (known as its berry) emerges; 3-4 months post the flowering stage.

Within the food industry, dried mature allspice berries are used as a flavourant in pastries, juices and classic liqueurs such as Chartreuse and Benedictine [2]. Consequently, essential oils derived from the plant are used mainly in the food industry for meat processing [2] and as a commercial food flavouring [4-7]. It is also utilized in the tanning industry for aromatherapeutic purposes [2], within perfumery compositions [2, 4, 8] and cosmetic products [2, 8] as well as a key ingredient in the formulation of natural repellents [2, 8].

Typically, essential oil percentage yields from *P. dioica* are minimalistic, irrespective of the processing technology and plant part utilized. Overall, yield percentages range between the values of 1.5-4.5% [8, 9]. Specifically, according to Ashurst *et al.* [10] berry essential oil yield percentages is between the range of 1.0 and 4.0%. This has been further supported by Peter [1] noting yield percentages of 3.0-4.5%.

Regardless of its low percentage yields, allspice's chemical composition is extremely diverse. Compounds found within however, differ with respect to the plant's part and its geographical origin. According to Yoshimura *et al.* [9], pimento essential oil has been documented to be rich in polyphenols and in particular hydrolysable tannins. The focal phenolic compound within *P. dioica*'s essential berry oil is eugenol. Eugenol, a volatile aromatic compound can be found in appreciable amounts equivalent to 60-90% of the total essential oil content extracted from the *P. dioica* plant (fruits or leaves) [5, 8-10].

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Characteristically, *P. dioica*'s berry essential oils contains a mixture of phenols, monoterpene hydrocarbons, oxygenated hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Extensive amounts of eugenol, methyl eugenol, caryophyllene, β -caryophyllene, limonene, terpinolene, 1,8 cineole, β -selinene, isoeugenol, levophellandrene, α - and β -cubebene, α -copaene, α - and β -gurjunene, α - and β -cadinene, palmitic acid, humulene, terpinene-4-ol and 4,5-cineole are found within [1, 2, 10, 11].

The quantity and quality of compounds detected varies by virtue of the extraction method employed and detection method utilized. Recognizable essential oil extraction methodologies include: maceration, infusion, digestion, decoction, percolation, hot continuous extraction (soxhlet), aqueous alcoholic extraction by fermentation, ultrasonic extraction, supercritical fluid extraction (SFE), water distillation (hydrodistillation), steam distillation, water and steam distillation, cohobation, enfleurage and cold press.

The commercialization of pimento essential oil and its economic gains in the food and cosmetic industries is identified by virtue of its aromatic quality determined by its chemical composition. With a wide range of extraction technologies present in the modern world, selection therefore hinges on the percentage yield, chemical composition, economic feasibility and process suitability of an extraction methodology.

2. Materials and Methods

2.1 Essential Oil Extraction

Essential oil extraction was conducted using three methods. Both HD and SE oil extractions were facilitated by a 1:10 ratio of 150g of grounded berries to 1500ml of distilled water and hexane respectively. The SFE oil however was recovered with supercritical carbon dioxide by the loading of 450g of grounded pimento berries into the extraction vessel. A Clevenger distillation apparatus was used to recover the HD oil and a simple extraction apparatus for the SE oil. Allotted extraction time was 3 and 24 hours for the HD and SE oils respectively. Distillate obtained from the hydrodistillation process was dried using anhydrous sodium sulphate and refrigerated for 24 hours until further use. SE extracts recovered were concentrated via rotary evaporation. Samples were extracted in triplicates for the HD and SE methodology.

Main operational parameters for the SFE were: Co-Solvent – 1% Ethanol; Flow Rate – 89ml/minute CO₂:1ml/minute Ethanol; Heat Exchange - 45°C; Extraction Vessel - 40°C; Collection Vessel - 50°C; Pressure - 200 bars; Run Time - 60 minutes. Extract recovered was determined gravimetrically using an analytical balance.

2.2 Chemical Analyses using Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS Analysis was performed using a Hewlett Packard 6890 Gas Chromatography Apparatus, fitted with a HP-5MS column (60m x 0.25mm x 0.25 μ m) coupled to a Hewlett Packard 5973 Mass Selective Detector. A temperature program of 60 °C – 260 °C at a rate of 10 °C per min, maintained at 260 °C for 20 minutes was employed. Other Operational Parameters: Helium Flow Rate - 1.0mL/min; Injection Volume - 1 μ L; Injection Mode - split (1:100 split ratio); Acquisition Mode – scan; Scan Range, 30-550m/z.

The components of each essential oil sample was identified by the comparison of their mass spectra with those of NIST/EPA/NIH Mass Spectral Library with Search Program and Database and confirmed by the comparison of their Retention Index (RI). Experimentally, the RIs were determined using the standard method which involved the retention times the n-alkanes, which were injected after the essential oil under the same chromatographic conditions. RI's were then validated by using Adams [12].

3. Results and Discussion

The essential oil yielded from the ground pimento berries, varied with the method of extraction employed. Average yield of extract from the respective methods were 1.4%, HD oil; 6.4%, SFE oil and 6.8%, SE oil (Table 1).

Table 1: Quantification of Pimento Essential Oil Obtained from Hydrodistillation, Solvent and Supercritical Fluid Extraction

Batch	Yield Percentage (% w/w)		
	HD	SE	SFE
1	1.3	6.9	6.4
2	1.1	7.1	
3	1.9	6.5	
Avg.	1.4	6.8	

Typically, yield percentage for pimento berry essential oil is between 1.0 and 4.0% [9] with a narrowed percentage (NP) between 3.0 and 4.5 [1]. The results obtained through experimentation exceeded the upper limit for the NP range by 2.3% and 1.9% for the SE and SFE oils respectively, with average %w/w yields of 6.8% and 6.4%. Garcia-Fajardo *et al.* [13] suggested that the geographical location from which a sample is obtained can affect the yield percentage of oil obtained. In their study on *P. dioica* samples obtained from Mexico, yield percentages amounted to 2.5% and 4.0% for their HD and SFE samples respectively.

GC-MS analysis of the samples led to the identification of forty three (43) components, representing between 75 and 91% of the total oils extracted (Table 2). Components accounting for 90.26% of the total HD oil sample, 75.40% of the SE oil and 82.99% of the SFE oil were identified and accepted.

Table 2: Chemical Composition of the *P. dioica* Essential Oils Obtained by Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

Compounds	Retention Index	Relative Area (%)		
		Hydrodistillation	Hexane	SFE
Monoterpene Hydrocarbons		5.49	4.87	1.32
1. α -pinene	941	0.05	0.09	-
2. sabinene	981	-	0.06	-
3. β -myrcene	993	0.14	0.18	-
4. α -phellandrene	1012	0.71	0.20	-
5. 3-carene	1019	0.04	0.04	-
6. α -terpinene	1024	0.12	-	-
7. p -cymene	1032	0.59	0.88	0.12
8. 1,8-cineole	1040	2.14	2.48	1.04
9. (z)- β -ocimene	1050	-	0.05	-

10. γ -terpinene	1065	0.46	0.69	0.11
11. terpinolene	1097	1.24	0.20	0.05
Oxygenated Monoterpenes		1.89	1.27	1.52
12. Linalool	1103	0.11	0.11	0.07
13. cis-p-menth-2-en-1-ol	1122	0.06	-	-
14. trans-p-menth-2-en-lol	1141	0.08	-	0.08
15. terpinen-4-ol	1189	0.59	-	0.55
16. p-cymen-8-ol	1194	0.14	0.34	0.12
17. α -terpineol	1201	0.82	0.69	0.62
18. methyl salicylate	1207	0.05	-	-
19. α -phellandrene epoxide	1213	0.04	0.06	-
20. trans piperitol	1217	-	0.07	0.08
Phenolic		67.06	53.57	64.31
21. Chavicol	1260	0.26	0.23	0.37
22. Eugenol	1389	66.80	53.34	63.94
Sesquiterpene Hydrocarbon		15.68	15.69	14.41
23. α -copaene	1398	0.26	0.37	0.32
24. β -elemene	1412	0.99	0.93	0.14
25. α -gurjunene	1433	0.35	0.39	0.30
26. β -caryophyllene	1446	4.69	4.40	4.65
27. aromadendrene	1463	0.24	0.22	0.23
28. α -humulene	1478	2.12	1.74	1.83
29. allo-aromadendrene	1486	0.64	0.81	0.71
30. γ -muurolene	1496	0.56	0.47	0.56
31. Germacrene D	1504	1.24	0.74	0.59
32. β -selinene	1511	0.64	0.76	0.81
33. α -selinene	1519	1.47	1.01	1.12
34. α -cadinene	1530	0.08	0.07	0.08
35. γ -cadinene	1535	0.28	0.39	0.43
36. δ -cadinene	1542	1.22	0.96	1.06
37. trans-cadina-1,4,diene	1553	0.05	0.08	0.08
38. Caryophyllene oxide	1612	0.85	2.35	1.50
Esters, Fatty Acids and Steroids		0	0	1.10
39. ethyl palmitate	1996	-	-	0.11
40. methyl linoleate	2102	-	-	0.06
41. steric acid	2198	-	-	0.93
Miscellaneous Compounds		0.14	0	0.33
42. Vanillin	1415	-	-	0.20
43. Palustrol	1595	0.14	-	0.13
Total		90.26	75.40	82.99

Each oil presented a complex mixture of compounds. The most predominant groups of compounds identified in terms of relative area percentage were: phenols, sesquiterpene hydrocarbons, monoterpene hydrocarbons, oxygenated monoterpenes and esters, fatty acids and steroids. Sixteen (16) sesquiterpene hydrocarbon compounds in total were identified, eleven (11) monoterpene hydrocarbons, nine (9) oxygenated monoterpenes, three (3) esters, fatty acids and steroids and two (2) phenolic compounds. Volatile fractions obtained from the samples were characterized by the predominance of monoterpene hydrocarbons (5.49% in the HD oil; 4.87% in the hexane oil; 1.32% in the SFE oil), oxygenated monoterpenes (1.89% in the HD oil; 1.27% in the hexane oil; 1.52% in the SFE oil), phenols (67.06% in the HD oil; 53.57% in the hexane oil; 64.31% in the SFE oil), sesquiterpene hydrocarbons (15.68% in the HD oil; 15.69% in the hexane oil; 14.41% in the SFE oil), esters, fatty acids and steroids (1.10% in the SFE oil) and miscellaneous compounds (0.14% in the HD oil; 0.33% in the SFE oil) (Table 2). Such a variety in compounds was expected and documented in numerous studies. Support for such diversity was offered by Nabney and Robinson [11], who listed phenols, monoterpene hydrocarbons, oxygenated hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes as compounds detected in a gas chromatography – mass spectrophotometry analysis of a sample of *P. dioica* berry essential oil. The main averaged constituents were: eugenol (61.36%), β -

caryophyllene (4.58%), α -humulene (1.90%) and 1,8-cineole (1.89%) whilst minor averaged constituents were δ -cadinene (1.08%), Germacrene D(0.86%), β -elemene (0.69%). Comparatively, the major and minor compounds detected were similar in most regards to data published. *P. dioica* essential oil content collectively comprises of eugenol, β -caryophyllene, β -selinene and methyl eugenol as major compounds [2, 10]. All compound with the exception of methyl eugenol were detected in all three (3) samples, with average percentages of 61.36, 4.58 and 0.74 for eugenol, β -caryophyllene and β -selinene respectively.

The eugenol content identified equated to relative area percentages of 66.80%, 53.34% and 63.94% with respect to the HD, SE and SFE oil samples. Its equivalent amount in overall percentage of the total essential oil content is 73.5% for the HD, 70.7% for the SE and 77.0% for the SFE oil samples respectively. The percentages concentrating around 70.0% is identical to inferences made on previous research conducted by Zhang and Lokeshwar [5], Gayle [3], Yoshimura *et al.* [9] and Ashurst *et al.* [10]. All four (4) researches highlighted percentages between the range of 60 and 90. Similarly, Padmakumari *et al.* [14] delineated the identification of eugenol (74.71% and 73.35%) in two samples of *P. dioica* berry essential oil obtained on Jamaican soil.

Methyl eugenol was, however, not detected in any of the three samples. Methyl eugenol by nature is insoluble in water, glycol

and propylene glycol but soluble in ethanol, ethyl ether, chloroform and most organic solvents [15-17]. In addition, the compound is extremely volatile as it evaporates readily at room temperature, but is somewhat stable at the same temperature when covered. A combination and/or either of the above conditions may be the reason why methyl eugenol was not detected as was predicted. The presence of excessive heat throughout the HD process and the inability of the compound to solubilize in water may be the main contributors to its non-detection in the HD sample. Furthermore, though methyl eugenol is soluble in organic solvents such as hexane, minimal exposure to the air may have resulted in its non-detection. Subsequently, the GC-MS technology used compares the components within each sample with that of the NIST Library associated with the instrument. It is possible that methyl eugenol was present in the sample but was not detected in the library search report as a result of the equipment being programmed to generate library reports for compounds relative to the largest peak (auto integration). As a result, depending on the percentage methyl eugenol relative to the other components, the library may not have identified it.

Of the forty three (43) compounds identified, twenty five (25) were common among all three samples. Of the remaining eighteen (18) identified compounds, five (5) were detected in both the HD and SE extracted oils, four (4) in the SFE oil only, three (3) in the HD oil only, three (3) in both the HD and SFE samples, two (2) in the SE oil only and one (1) in both the SE and SFE oils. In spite of majority of the compounds being detected in the HD oil; its yield percentage (1.4%) was inferior to that of the SFE oil (6.4%) by approximately 4.5 times.

4. Conclusion

Pimenta dioica's essential oil utilization tree stems across numerous activities within the food and perfumery industry. For the present study, extraction methodology utilized, influenced overall yield percentage but had minimalistic to no weight on chemical composition. Oil yield percentages amounted to 1.4%w/w HD oil; 6.8%w/w hexane oil and 6.4%w/w SFE oil. Major compound identified within the oils were eugenol (61.36%), β -caryophyllene (4.58%), α -humulene (1.90%) and 1,8-cineole (1.89%) whilst minor compounds included δ -cadinene (1.08%), Germacrene D (0.86%) and β -elemene (0.69%). Majority (58%) of the compounds identified were common among each extracted oil type. Of the remaining forty two percent (42%), eleven (11) were common to the HD oil, eight (8) in the SE oil and eight (8) in the SFE oil. Though majority of the compounds identified were detected in the HD oil, its yield percentage was abysmal in comparison to that of the SFE oil. As such, for commercialization purposes (percentage yield, chemical composition and economic feasibility) a SFE extracted oil is preferred as it is a modernized technique which shortens extraction time, reduces organic solvent consumption, prevents pollution and eliminates organic solvent residues. Though it is an expensive technology with a high capital investment, the returns on the initial investment (yield percentage, compound composition and environmental safety) will pay for itself in a matter of time.

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6. References

- Peter KV. Handbook of Herbs and Spices, Volume 2. Woodhead Publishing Ltd and CRC Press, United Kingdom and United States of America, 2004, 7.
- Rao P, Navinchandra S, Jayaveera KN. An Important Spice, *Pimenta dioica* (Linn.) Merrill: A Review. *Int Curr Pharm J*. 2012; 1(8):221-225.
- Gayle JR. Pimento: The Jamaican Allspice Story. Phoenix Printery Limited, Jamaica. 2013, 1.
- Asha MM, Chaithra M, Yashoda K, Vivek MN, Prashith TR. Antibacterial Activity of Leaf and Bark Extracts of *Pimenta dioica* (Linn.) Merrill against Clinical Isolates of *Staphylococcus aureus* and *Streptococcus mutans*. *World J Phar and Pharm Sci*. 2013; 2(5):3207-3215.
- Zhang L, Lokeshwar BL. Medicinal Properties of the Jamaican Pepper Plant *Pimenta dioica* and Allspice. *Curr Drug Targets*. 2012; 13(14):1900-1906.
- Sharma R. Pimenta. *Medicinal Plants of India –An Encyclopedia*. Data Publishing House Delhi, New Delhi, 2003, 229.
- Seidemann J. Pimenta Lindl-Allspice Myrtaceae. *World Spice Plants*. Springer-Verlag, Heidelberg Germany, 2005, 286.
- Jirovetz L, Buchbauer G, Stoilova I, Krastanov A, Stoyanova A, Schmidt E. Spice Plants: Chemical Composition and Antioxidant Properties of Pimenta Lindl. *Essential Oils, Part 1: Pimenta dioica* (L.) Merr. Leaf oil from Jamaica. *Ernähr*, 2007; 31:55-63.
- Yoshimura M, Amakura Y, Yoshida T. Polyphenolic Compounds in Clove and Pimento and their Antioxidative Activities. *Biosci Biotech and Biochem J*. 2011; 75(11):2207-2212.
- Ashurt PR, Firth AR, Lewis OMA. *New Approach to Spice Processing 1972*, Proceedings of the Conference on Spices, Tropical Products Institute, London.
- Nabney J, Robinson EV. Constituents of Pimento Berry Oil *Pimenta dioica*. *Flavour Frangr J*. 1947; 3:50-51.
- Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry 4th Ed*. Carol Stream, Allured Publishing Corp.
- Garcia-Fajardo J, Martinez-Sosa M, Estarrh-Esphosa M. Comparative Study of the Oil and Supercritical CO₂ Extract of Mexican Pimento (*Pimenta dioica* Merrill). *J Essent Oil Res*. 1997; 9(2):181-185.
- Padmakumari KP, Sasidharan I, Sreekumar MM. Composition and Antioxidant Activity of Essential Oil of Pimento (*Pimenta dioica* (L) Merr.) from Jamaica. *Nat Prod Res*. 2011; 25(2):152-160.
- Methyl Eugenol; MSDS No. 93-15-2 Chemical Book China. http://www.chemicalbook.com/ChemicalProductProperty_US_CB8198972.aspx. 17 May, 2015.
- Methyl Eugenol; MSDS No. 93-15-2 Chemical Land. <http://www.chemicaland21.com/specialtychem/perchem/METHYL%20EUGENOL.htm>. 17 May, 2015.
- IARC Monographs Methyl Eugenol. <http://monographs.iarc.fr/ENG/Monographs/vol101/mono101-013.pdf>. 17 May, 2015.