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# Determination of the Effect of Seasonal Variation on the Composition of Essential Oil and the Antioxidant Potential of *Mentha Piperita* L

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**Abstract**

This study investigates seasonal changes in the essential oil yield, chemical composition, and antioxidant capacity of *Mentha piperita* L. Two samples (500g each) of the pulverised fresh leaves of the plant collected during dry and wet seasons of the year 2024 were separately subjected to hydrodistillation for three hours in a Clevenger-type apparatus. The oils obtained was characterized using Gas Chromatography-Mass Spectrometry (GC-MS) and also evaluated for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching assays. The oil afforded a yield of 0.72 and 0.65 % (w/w) for dry and wet season leaves respectively. A total of 36 compounds representing 98.42% of the total oil were detected in the dry season sample, whereas a total of 28 compounds representing 97.73% of the total oil were identified in the wet season sample. Carvone increased from 31.5 to 44.97% and limonene from 15.3% to 18.51% in the dry and wet seasons respectively. However, 1,8-cineole decreased from 3.25 to 2.93, germacrene D from 1.63 to 1.18%. Qualitative variation was also observed in the oils, menthone and levomenthol detected in the oil of dry season were not identified in the oil of wet season. In addition, cis-dihydrocarvone and (-)-carvone identified in the oil of wet season were not detected in the oil of dry season. The antioxidant activity of the essential oils of the plant showed higher activity during the wet season (62.3-90.1 and 59.2-89.97%) inhibition than in the dry season (60-85.53 and 59.11-81.12%) inhibition for DPPH and β-carotene bleaching assays respectively. These results showed that *M. piperita* is a natural source of active compounds, with strong antioxidant properties. The oil content and its chemical composition were also influenced by seasonality.

**Keywords:** Antioxidant; Essential oil; GC-MS; Hydrodistillation; *Mentha piperita*; Season.

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## Introduction

Peppermint (*Mentha piperita* L.) is a medicinal, aromatic species, of the Lamiaceae family. It is a hybrid mint between watermint (*Mentha aquatica* L.) and spearmint (*Mentha spicata* L.) [1]. *Mentha piperita* is a perennial herb native to

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Europe but cultivated in the northern USA, Canada, Asia, North Africa, and many other parts of the world known for its distinct flavour and aromatic fragrance [2]. The plant is regarded as one of the best potential sources of biologically active substances used in the food, cosmetics, and pharmaceutical industries. The biological activities of its extracts and essential oils include antioxidant, antimicrobial, insecticidal, larvicidal, anticancer, antiviral, anti-allergic, anti-inflammatory, antihypertensive and urease inhibitory activity [3].

*Mentha piperita* has been one of the most broadly used aromatic herbs for medicinal purposes since its discovery, peppermint leaf is used mainly to treat upper gastro-intestinal tract diseases, respiratory tract problems, irritable bowel syndrome (IBS), nausea, hepatotoxicity, diabetes etc [4]. In addition, *M. piperita* constitutes one of the most widely consumed single-ingredient herbal teas [3]. *Mentha piperita* was used as a room deodorizer for spreading over floors with the purpose of reducing the unpleasant smell of hard-packed soils [1].

Free radicals collectively known as reactive oxygen species (ROS) are highly reactive and unstable molecules with a significant impact on the human biological system. A disproportionate amount of ROSs and the lack of their scavenging systems in cells lead to oxidative stress, which increases the risk of the development of various diseases such as hypertension, diabetes, cancer neurodegenerative disorder and also speeding up the aging process [5]. Nowadays, the use of synthetic antioxidants in food, such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBQ), propyl gallate(PG), has raised social concern, these compounds though effective and relatively cheap to produce, can generate allergies and serious health problems in the long term(Gulcin,2020). Numerous studies have shown the ability of herbal preparations and essential oils to scavenge free radicals, donate electrons or hydrogen, and inhibit lipid peroxidation both *in vitro* and *in vivo* [3;5;6]. Several studies reported that *M. piperita* extracts and essential oil possess significant antioxidant potential [7;3;8].

Although, many studies have investigated the chemical composition and antioxidant activity of leaf essential oil of *M. piperita* [9;7;3;5;10], no information is available regarding how seasonal variation affect the yield, chemical composition and antioxidant potential of the leaves. Therefore, it is on this basis that this study is aimed at investigating the effect of seasonal variation on the yield, chemical composition and antioxidant potential of leaf essential oil of *M. piperita.*

## Materials and Methods

### Sample Collection, Preparation and Authetification

Fresh *Mentha piperita* leaves were collected at a plant garden in Tudun Wada near Bachama Cemetery, Kaduna South, Kaduna in the month of

February and August, 2024, representing dry and rainy seasons harvests respectively. The sample was taken to the laboratory where the leaves were picked, weighed and pulverized using pestle and mortar. The plant was identified at the Department of Botany, Kaduna State University, Kaduna Nigeria, where voucher specimen was deposited and voucher number KASU/BSH/162 was issued.

**Oil Isolation**

Pulverized leaves (500g each) of *M. piperita* were separately subjected to hydro-distillation for 3 hours in a Clevenger-type apparatus, according to the British Pharmacopoeia Specification, (1980). The oil obtained from each sample was dehydrated over anhydrous sodium sulphate and stored in sealed glass vials in a refrigerator prior to analyses.

**Gas Chromatography-Mass Spectrometry Analysis**

The chemical composition of the oils was determined by using Agilent 8860 gas chromatograph coupled to 5977C inert mass spectrometer with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). Oven was initially programmed at 80°C (2min) then ramped at 30 °C/min to 320 °C (2 min). Run time was 15 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 300 °C.

Identification of the components was done by comparison of retention indices (determined relative to the retention times of $C_5$-$C_{24}$ n-alkanes series obtained on a nonpolar HP-5 column) and mass spectra with those of authentic samples and with data from literature [11]. The constituents were identified based on their retention times relative to those of authentic samples and by comparison of the kovats retention index and matching mass spectra peaks with a mass spectral library and with literature data [11;12].

**Antioxidant Activity Using (DPPH) Assay**

The free radical scavenging (antioxidant) activity of the oils was measured in vitro by 1,1 diphenyl-2-picrylhydrazyl (DPPH) assay according to the method reported by Silva *et al* [13] with slight modification. Stock solution of DPPH (0.1mM) was prepared in 80% ethanol and 100µL of varying amounts of essential oils (0.6-0.12µg) was added into 900µL of DPPH stock solution. The reaction mixture was incubated in the dark at ambient temperature for 30 minutes and the absorbance was taken at 517nm in UV-Visible spectrophotometer against a blank/control solution. The experiments were performed in triplicates. The DPPH radical scavenging activity by the oils were calculated thus:

$$\text{Scavenging Activity}(\%) = \frac{Ac - As}{Ac} \times 100$$

Where Ac and As are the absorbance of the control and sample, respectively.

**Antioxidant Activity Using β-carotene Bleaching Assay**

The β-carotene-linoleic acid antioxidant assay was determined according to the method reported by Ozer [14] with slight modification.

**Preparation of β-carotene/linoleic acid emulsion**

A solution of β-carotene was prepared by dissolving 0.5 mg in 1 mL of chloroform, was added to 25 µL of linoleic acid was then added followed by 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen was added and the mixture is stirred vigorously to ensure the beta-carotene is uniformly emulsified in the solution.

**Assay procedure**

A mixture of 2.5mL of the emulsion was transferred into different test tubes containing different concentrations of the sample (10, 20, 30, 40 and 50 µg /mL). Butylated hydroxyanisole (BHA) was used as positive control. The mixtures were incubated at 50ºC for 60 minutes and the absorbance was taken at 470nm in UV-Visible spectrophotometer against a blank/control solution. The experiments were performed in triplicates. $IC_{50}$ values of all samples were estimated.

The % inhibition of oxidation is calculated thus:

$$\text{Inhibition \%} = \frac{Ac(t) - As(t)}{Ac(t=0) - Ac(t)} \times 100$$

Where Ac = Absorbance of the control sample without antioxidant; As = Absorbance of the sample with the antioxidant; $A_{c(t=0)}$ = Absorbance at time 0.

**Statistical analysis**

The experiments were performed in triplicate, and the results were presented as the mean ± standard deviation (SD). Statistical analysis and mean comparisons were conducted using GraphPad Prism v8 software, utilizing one-way analysis of variance(ANOVA) followed by the Tukey test. Statistical significance was considered for p-values less than 0.05.

**Results and Discussion:**

The oil obtained from dry season harvest afforded a yield of 0.72%(w/w) whereas that of wet season harvest recorded a yield of 0.65%(w/w) indicating that the yield was affected by seasonal changes. [15] reported a similar trend where the oil yield of *O. basilicum* with the highest yield of 0.8% in winter reduced to 0.5% in summer. In a related study conducted by Parodi *et al* [16], which evaluated the effect of seasonality on the composition and yields of essential oils of *Aloysia triphylla* leaves, cultivated and collected in Brazil, different oil yields were reported during the summer (0.42%), autumn (0.31%), winter (0.19%), and spring (0.30%) seasons. The season with the lowest rainfall presented the best yield during the studied period.

The factors influencing the yield, chemical variability and biological activity of essential oils can be grouped into exogenous and endogenous factors. The endogenous factors are genetic (species, genotype, age), that is, factors related to the anatomy and physiology of the plants. On the contrary, exogenous factors are factors related to the environment such as light, temperature, rainfall and soil type, seasonal variations.

**Table 1: Chemical Composition of Leaf Essential Oils of *M piperita* Harvested during the Dry and Rainy Seasons**

| | | | | Composition (%) | | |
|---|---|---|---|---|---|---|
| **S/N** | **Compound[a]** | **$R_T$[b]** | **RI[c]** | **DS** | **RS** | **Mass Spectral Data[d]** |
| 1 | β-pinene | 3.14 | 981 | - | 0.35 | 136 121 **93** 79 |
| 2 | 1,4-hexadiene | 3.15 | 618 | 0.23 | - | 82 **67** 41 39 |
| 3 | D-limonene | 3.38 | 1031 | 15.3 | 18.51 | 136 121 93 **68** |
| 4 | 1,8-cineole | 3.44 | 1033 | 3.25 | 2.93 | 154 139 **81** 71 |
| 5 | γ-Terpinene | 3.57 | 1062 | 0.25 | - | 136 121 **93** 77 |
| 6 | (E)-Sabinene hydrate | 3.64 | 1070 | 0.23 | - | 154 136 121 **93** |
| 7 | Terpinolene | 3.78 | 1096 | 0.23 | - | 136 121 **93** 79 |
| 8 | Butanoic acid | 3.86 | 1068 | - | 0.25 | 88 73 **60** 43 |
| 9 | Carbonic acid | 3.86 | 2579 | 0.3 | - | 86 58 **44** 43 |
| 10 | Trans-limonene oxide | 4.12 | 1138 | 0.19 | 0.37 | 137 108 **94** 67 |
| 11 | Menthone | 4.27 | 1137 | 6.29 | - | 138 123 81 **72** |
| 12 | Endo-Borneol | 4.33 | 1165 | - | 0.51 | 139 110 **95** 41 |
| 13 | Citronellal | 4.39 | 1161 | - | 1.09 | 121 95 79 **41** |
| 14 | Levomenthol | 4.40 | 1182 | 13.93 | - | 138 95 81 **71** |
| 15 | Cis-Dihydrocarvone | 4.51 | 1195 | - | 5.47 | 150 95 68 **67** |
| 16 | D-Carvone | 4.84 | 1242 | 31.65 | 44.97 | 150 108 **82** 54 |
| 17 | (-)-Carvone | 5.14 | 1253 | - | 6.41 | 150 108 **82** 54 |
| 18 | Cis-carvone oxide | 5.2 | 1268 | - | 0.6 | 123 95 67 **43** |
| 19 | Trans-carveyl acetate | 5.45 | 1337 | 3.18 | 0.8 | 152 119 109 **84** |
| 20 | Methylacetate | 5.19 | 1168 | 3.63 | - | 74 59 **43** 28 |
| 21 | Trans-Carane | 5.25 | 978 | 0.25 | - | 138 123 **95** 81 |
| 22 | (R)-Dihydrocarveyl acetate | 5.3 | 1305 | 0.8 | - | 136 121 93 **43** |
| 23 | (-)-Cis-Carveyl acetate | 5.33 | 1364 | 0.18 | - | 152 119 109 **84** |
| 24 | Propan-2-one | 5.53 | 477 | 3.08 | - | 58 **43** 39 27 |
| 25 | (-)-β-Elemene | 5.62 | 1391 | 3.37 | 3.52 | 204 189 107 **93** |
| 26 | Aromadendrene | 5.69 | 1439 | 0.22 | - | 204 161 105 **41** |
| 27 | β-Isocomene | 5.7 | 1403 | - | 0.38 | 136 **93** 77 69 |
| 28 | Caryophyllene | 5.78 | 1418 | 2.8 | 3.05 | 204 161 133 **93** |
| 29 | (-)-β-Sesquiphellandrene | 5.82 | 1149 | 0.22 | - | 204 161 93 **69** |
| 30 | Germacrene D | 5.86 | 1480 | 1.63 | 1.18 | 204 **161** 105 91 |
| 31 | Humulene | 5.91 | 1456 | 0.18 | - | 204 147 121 **9** |
| 32 | Bicyclogermacrene | 6.08 | 1494 | 0.57 | 0.25 | 204 161 121 **95** |
| 33 | γ-Muurolene | 6.14 | 1477 | 0.3 | 0.26 | 204 **161** 119 105 |
| 34 | Cis-Muurola-4(15), 5-diene | | 1463 | 1.65 | 1.6 | 204 **161** 119 105 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35 | Trans-Calamenene | 6.19 | 1522 | 1.1 | 1.43 | 202 **159** 144 129 |
| 36 | α-Cadinene | 6.33 | 1538 | 0.3 | 0.32 | 204 **133** 119 93 |
| 37 | (-)-Spathulenol | 6.43 | 1578 | 0.24 | 0.35 | 220 205 **159** 91 |
| 38 | Caryophyllene oxide | 6.47 | 1581 | 0.58 | 1.04 | 220 205 **79** 69 |
| 39 | Carotol | 6.51 | 1595 | 0.29 | - | 204 **161** 119 69 |
| 40 | Epicubenol | 6.57 | 1627 | 1.33 | 0.57 | **179** 161 121 117 |
| 41 | α-Cadinol | 6.72 | 1653 | 0.92 | 0.97 | 222 204 **95** 81 |
| 42 | Muurol-5-en-4-one | 6.88 | 1689 | 0.16 | 0.36 | 222 207 195 179 |
| 43 | Isolongifolene epoxide | 6.98 | 1544 | - | 0.39 | 121 108 93 **41** |
| 44 | Isoaromadendrene epoxide | 6.98 | 1589 | 0.33 | 0.3 | 220 121 107 **41** |
| 45 | Copaen-15-ol | 7.08 | 1578 | 0.26 | - | 162 105 80 **59** |
| | **Total(%)** | | | **98.42** | **97.73** | |

**Key**: **a**-compounds listed in order of elution from HP-5 column; **b** - retention time; **c**-retention indices from the literature;
DS-Dry season; RS- Rainy season; - = Not detected; **d**-mass spectral data, base peak in bold.

Other factors include agricultural practices (harvesting time, fertilization, irrigation, planting density), and post-harvest handling (drying methods, storage, processing conditions, etc)

The chemical compositions of leaf essential oils of *M. piperita* harvested during the dry and rainy seasons is shown in Table 1. Dry season harvest was done in the month of February, whereas rainy season harvest was done in the month of August, 2024. From the Table a total of 36 compounds representing 98.42% of the total oil were detected in the dry season sample, whereas a total of 28 compounds representing 97.73% of the total oil were identified in the rainy season sample.

The total percentage of the various class of compounds detected in the oils were Hydrogenated monoterpenoids (16.03 and 18.36%), oxygenated monoterpenoids (59.7 and 63.15%), hydrocarbon sesquiterpenoids (12.34 and 11.99%), oxygenated sesquiterpenoids (4.11 and 3.98%) and others (7.24 and 0.25%) for dry and rainy season samples respectively.

The oils exhibited a preponderance of oxygenated monoterpenoids. This is in line with the findings of Olayemi *et al*; Mahboubi & Kazempour [17; 18]. The most abundant compound in the oils was D-carvone accounting for 31.65 and 44.97% for dry and rainy season samples respectively.

This is also in agreement with the findings of Arrahmounl *et al* [19], who determined the chemical composition of *M.piperita* essential oils from three different geographical areas in Morocco. Their findings revealed that D-carvone (5.75%) was the most abundant compound detected in the oil from Sidi Ayach area, whereas linalool (59.34%) was the most abundant compound in the oil from Akkari area. This is an indication that the chemical composition of *M. piperita* leaf essential oil varies with geographical location. However, the major compounds of *M. piperita* oil reported in the literature were α-pinene, β-pinene, sabinene, myrcene, cis ocimene, β-caryophyllene,γ-terpinene, germacrene D,carvone (3.5%), L–menthol (38–

70%), L–menthone (0.4–35%); isomenthone (1.5–10%), menthyl acetate (0–20%), 1,8 cineole (0.4–12%), menthofuran (0.1–21%); limonene (0.6–4.5%), pulegone (traces–7%), and neoisomenthol [3;9;20].

For the dry season sample, other major compounds detected were D-limonene (15.3%), menthone (6.29%), levomenthone (13.93%). This is in agreement with the findings of Arrahmouni *et al* [21], they reported menthone and limonene as the second component of peppermint oil. Compounds present in appreciable quantities (between1-4%) were 1,8-cineole, trans-carveyl acetate, methyl acetate, propan-2-one, (-)-β-elemene, caryophyllene, germacrene D, *cis*-muurola-4(5), 5- diene, *trans*-calamenene and epicubenol. The remaining compounds were present in minute (< 1%) quantities.

For the rainy season sample, the principal compounds identified were D-limonene (18.51%), *cis*-dihydrocarvone (5.47%) and (-)-carvone (6.41%). Compounds found in appreciable amounts (1-4%) were 1,8-cineole, citronellal, (-)-β-elemene, caryophyllene, germacrene D, *cis*-muurola-4(5), 5- diene, *trans*-calamenene and caryophyllene oxide. Other compounds were present in minute (< 1%) quantities.

There were qualitative and quantitative variations in the chemical profile of the oils, qualitatively, β-pinene, endoborneol, citronellal, cis-dihydrocarvone, (-)-carvone, cis-carvone oxide, β-isocomene, and isolongifolene epoxide were not detected in the oil sample harvested during the dry season but these compounds were identified in the oil obtained from rainy season harvest. Also, γ-terpinene, (E)-sabinene hydrate, terpinolene, menthone, levomenthol, trans-carane, (R)-dihydrocarvyl acetate, (-)-cis-carveyl acetate, aromadendrene, (-)-β-sesquiphellandrene, humulene, carotol and copaen-15-ol present in the oil of dry season harvest were not detected in the oil from rainy season harvest.

Quantitatively, the concentration of the most abundant compound in the oils, D-carvone was higher (44.97%) in the oil of rainy season harvest than that of dry season (31.65%). A major compound D-limonene however recorded a lower concentration (15.3%) in the oil of dry season sample than that of rainy season (18.51%). Other compounds such as 1,8-cineole (3.25 and 2.93%), *trans*-carveyl acetate (3.18 and 0.8%), germacrene D (1.63 and 1.18%), bicyclogermacrene (0.57 and 0.25%), γ-muurolene (0.3 and 0.26%), *cis*-muurola-4(15),5-diene (1.65 and 1.6%), epicubenol (1.33 and 0.57%), and isoaromadendrene epoxide (0.33 and 0.3%) revealed higher concentrations in the dry season oil than in the rainy season one. On the other hand, trans-limonene oxide (0.19 and 0.37%), (-)-β-elemene (3.37 and 3.52%), caryophyllene (2.8 and 3.05%), *trans*-calamenene (1.1 and 1.43%), α-cadinene (0.3 and 0.32%), (-)-spathulenol (0.24 and 0.35%), caryophyllene oxide (0.58 and 1.04%), α-cadinol (0.92 and 0.97%) and muurol-5-en-4-one (0.16 and 0.36%)

showed higher concentrations in the rainy season samples than in the dry season one. [22] also reported variation in chemical composition of *M.piperita* essential oil influenced by seasonality. An increased menthol (16.31 to 41.26%), neomenthol (3.02 to 6.39%) and menthone (5.56 to 41.58%) contents were reported during spring and summer respectively.

The yield and chemical composition of essential oils from the same plants may vary from season to season and this is influenced by variation in precipitation during the dry and the rainy season and temperature changes within seasons. The results revealed that essential oil compositions were sensitive to seasonal climatic changes. Generally, the qualitative and quantitative changes in the essential oil composition of *M.piperita* could be due to its developmental stages at the time of harvest as affected by environmental conditions.

**Antioxidant Activity:**

The result of antioxidant activity of leaf essential oil of *M. piperita* is presented in table 2. From the Table, the oils exhibited a dose dependent activity for both seasons, and the activity was significant compared to that of BHT standard. Generally, the scavenging activity was higher in DPPH than β-carotene assay. For the dry season activity, β-carotene linoleic acid assay recorded % inhibition ranging from 59.11-81.12%, whereas DPPH assay ranged from 60-85.53% compared to the BHT standard of 87.47-98.92%. For the wet season the activity was higher with β-carotene assay having 59.92-89.97% inhibition, while DPPH had 62.3-90.1% inhibition compared to the standard-BHT of 88.45-99.55%.

It has been reported that overall antioxidant activity of oxygenated monoterpenoids was the strongest followed by monoterpene hydrocarbons. Hydrocarbon sesquiterpenoids and their oxygenated derivatives were reported to have very low antioxidant activities [23]. The activity of the oils could be due to abundance of oxygenated monoterpenoids in the oils. The activity could also be attributed to high percentage of the major compounds in the oils, or to the minor constituents acting as pro-oxidants. The constituents of the oils could also act synergistically to create effective defense system against free radical attack. The findings revealed that the yield and chemical composition of the oils varied significantly with the seasons.

**Table 2: Antioxidant Activity of *M. piperita* Leaf Essential Oil**

| | % Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Dry Season | | | Wet Season | | |
| Conc (µg/ml) | β-carotene | DPPH | BHT | β-carotene | DPPH | BHT |
| 5 | 59.11±0.80 | 60.0±0.32 | 87.47±0.15 | 59.92±0.13 | 62.3±0.13 | 88.45±0.11 |
| 10 | 60.71±0.04 | 62.65±0.11 | 90.01±0.13 | 62.30±0.64 | 65.59±0.13 | 92.04±0.12 |
| 15 | 75.39±0.06 | 77.76±0.18 | 93.47±0.11 | 77.87±0.03 | 80.12±0.06 | 95.45±0.18 |
| 20 | 79.97±0.04 | 80.73±0.18 | 97.18±0.09 | 83.44±0.05 | 85.19±0.03 | 98.42±0.17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 25 | 81.12±0.15 | 85.53±0.15 | 98.92±0.02 | 89.97±0.04 | 90.1±0.11 | 99.55±0.03 |

**Conclusion**

Antioxidant activity of essential oil at different seasons of the two assays showed highest radical scavenging activity during the wet season than in dry season. The results reflect the effect of seasonal changes on the growth ability of plant to adapt to climatic variations. The results of antioxidant activity are compatible to the composition of essential oil. The best time to harvest *M.piperita* leaves for best essential oil production which has the best activity is during the wet season

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