The Volatile Phytochemistry of Monarda Species Growing in South Alabama

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Abstract: The genus Monarda (family Lamiaceae) contains 22 species of which three are native to southern Alabama, M. citriodora, M. fistulosa, and M. punctata. Several species of Monarda have been used in traditional medicines of Native Americans, and this present study is part of an ongoing project to add to our understanding of Native American pharmacopeia. Plant material from M. citriodora, M. fistulosa, and M. punctata was collected in south Alabama and the essential oils obtained by hydrodistillation. The essential oils were analyzed by gas chromatographic techniques to determine the chemical compositions as well as enantiomeric distributions. The compounds thymol, carvacrol, p-cymene, and their derivatives were the primary terpenoid components found in the essential oils. The known biological activities of these compounds are consistent with the traditional uses of Monarda species to treat wounds, skin infections, colds, and fevers.

Keywords: Monarda citriodora; Monarda fistulosa; Monarda punctata; essential oil; thymol; carvacrol; p-cymene

1. Introduction

The Plant List [1] shows 22 different Monarda L. (Lamiaceae) species, 18 of which occur in the United States [2]. There are three Monarda species native to south Alabama, namely Monarda citriodora Cerv. ex Lag., Monarda fistulosa L., and Monarda punctata L. (see Figure 1) [2].

Several Monarda species have been used by Native Americans as medicinal plants [3]. For example, M. fistulosa was used by the Blackfoot, Navajo, Lakota, and Winnebago people to treat boils, cuts and wounds; the Cherokee, Chippewa, Flathead, Ojibwa, and Tewa used the plant to treat colds, fever, and influenza; the Crow, Lakota, Menominee, and Ojibwa used the plant for coughs, catarrh, and other respiratory problems. Monarda punctata was used by the Delaware, Mohegan Nanticoke, and Navajo tribes to treat colds, fever coughs, and catarrh.

Both M. citriodora and M. fistulosa are popular ornamentals and have been introduced to temperate locations around the world [4–6]. Geographical location likely plays an important role in the phytochemistry of Monarda species. To our knowledge, however, there have been no previous examinations of M. citriodora, M. fistulosa, or M. punctata growing in their native range of south Alabama. In this work, we have examined the chemical compositions and enantiomeric distributions of essential oils of the three Monarda species from south Alabama.
2. Results

2.1. Monarda Citriodora

The *M. citriodora* essential oils were obtained as clear orange oils. The essential oil yields for *M. citriodora* aerial parts essential oil were 1.59% and 1.79% for samples #1 and #2, respectively, while the root essential oil was obtained in 0.879% yield. The chemical compositions of the essential oils from the aerial parts and the roots of *M. citriodora* cultivated in south Alabama are summarized in Table 1. The essential oils were dominated by the phenolic monoterpenoids thymol (RI\(_{db}\) = 1289) and carvacrol (RI\(_{db}\) = 1296). The other major components were \( p \)-cymene (RI\(_{db}\) = 1024) and thymol methyl ether (RI\(_{db}\) = 1239).

| RI\(_{calc}\) | RI\(_{db}\) | Compound | #1, % | Aerial Parts Essential Oil | Root Essential Oil |
|----------------|-----------|----------|-------|---------------------------|-------------------|
|                |           |          |       | ED\(_1\), (+):(-) | ED\(_2\), (+):(-) | #2, % | ED\(_2\), (+):(-) |
| 923            | 925       | \( \alpha \)-Thujene | 1.0   | 69.0:31.0 | 0.8 | 66.8:33.4 | 0.5 | 64.2:35.8 |
| 930            | 932       | \( \alpha \)-Pinene | 0.3   | 84.2:15.8 | 0.3 | 62.8:37.2 | 0.2 | 74.5:25.5 |
| 947            | 950       | Camphene | tr    | tr | tr | tr |
| 970            | 971       | Sabinene | tr    | tr | — |
| 975            | 978       | \( \beta \)-Pinene | 0.1   | 64.5:35.5 | 0.1 | 64.2:35.8 | 0.7 |
| 976            | 974       | 1-Octen-3-ol | 0.7   | 0.7 | 0.7 |
| 983            | 984       | 3-Octanone | 0.1   | 0.2 | 0.2 |
| 987            | 989       | Myrcene | 0.7   | 0.4 | 0.4 |
| 995            | 996       | 3-Octanol | 0.2   | 0.3 | 0.3 |
| 1002           | 1004      | Octanal | tr    | tr | — |
| 1003           | 1004      | \( p \)-Mentha-1(7),8-diene | tr    | — | — |
| 1005           | 1006      | \( \alpha \)-Phellandrene | 0.1   | 95.1:4.9 | 0.1 | 100:0 | tr |
| 1007           | 1008      | \( \delta \)-3-Carene | 0.1   | 100:0 | 0.1 | 100:0 | 0.7 |
| 1015           | 1017      | \( \alpha \)-Terpinene | 1.8   | 100:0 | 1.1 | 100:0 | 100:0 |
| 1016           | 1022      | m-Cymene | tr    | tr | — |
| 1023           | 1024      | \( p \)-Cymene | 7.8   | 6.4 | 6.4 |
| 1027           | 1030      | Limonene | 0.5   | 26.7:73.3 | 0.4 | 63.3:36.7 | 0.3 | 57.3:42.7 |
| 1028           | 1029      | \( \beta \)-Phellandrene | 0.2   | 0.100 | 0.1 | 0.100 | 0.1 |
| 1030           | 1033      | Benzyl alcohol | —    | — | — |
| 1030           | 1030      | 1,8-Cineole | 0.2   | 0.3 | 0.3 |
| 1055           | 1057      | \( \gamma \)-Terpinene | 1.7   | 0.5 | 0.5 |
| 1067           | 1069      | \( cis \)-Sabinine hydrate | 0.8   | 95.3:4.7 | 1.0 | 89.5:10.5 | 0.9 | 90.7:9.3 |

**Figure 1.** *Monarda* species discussed in this work (photographs by S. K. L).
| R\textsubscript{calc} | R\textsubscript{db} | Compound | RI | Aerial Parts Essential Oil | Root Essential Oil |
|-----------------|-----------------|---------|-----------------|--------------------|
|                 |                 | #1, %   | #2, %           | #2, %              |
| 1083           | 1086            | Terpinolene | tr              | tr                |
| 1084           | 1086            | trans-Linalool oxide | (furanoid) | —                 |
| 1087           | 1093            | p-Cymene | —              | —                 |
| 1098           | 1099            | Linalool | 0.1 71.9:28.1 | 0.1 49.7:50.3     |
| 1099           | 1099            | trans-Sabinene hydrate | tr 76.5:23.5 | 0.3 71.1:28.9     |
| 1165           | 1167            | exo-Acetoxycamphene | —                | —                 |
| 1169           | 1170            | Borneol | 0.1 0:100      | 0.1 0:100         |
| 1178           | 1180            | Terpinen-4-ol | 0.4 60.2:39.8 | 0.4 58.5:41.5     |
| 1183           | 1186            | p-Cymen-8-ol | —                | —                 |
| 1187           | 1190            | Methyl salicylate | tr                | —                 |
| 1195           | 1195            | α-Terpinol | 0.1 100:0     | 0.1 100:0         |
| 1196           | 1197            | Methyl chavicol (= Estragole) | —                | —                 |
| 1236           | 1239            | Thymol methyl ether | 4.4 5.6       | 11.3          |
| 1252           | 1252            | Thymoquinone | 0.2 0.7      | 1.3            |
| 1253           | 1246            | Carvone | tr 39.9:60.1  | —                |
| 1290           | 1289            | Thymol | 38.2 37.0     | 29.0             |
| 1297           | 1296            | Carvacrol | 38.3 39.9     | 38.3             |
| 1305           | 1309            | 4-Vinylguaiacol | —                | —                 |
| 1306           | 1306            | iso-Ascaridole | tr                | tr               |
| 1342           | 1345            | Thymyl acetate | 0.3 0.2       | 0.3             |
| 1347           | 1356            | Eugenol | tr              | —                |
| 1361           | 1365            | Carvacyl acetate | 0.8 0.5       | 1.0            |
| 1372           | 1375            | α-Copaene | tr 100:0      | tr 0.1 100:0     |
| 1380           | 1382            | β-Bourbonone | tr               | tr 0.1          |
| 1389           | 1392            | (Z)-Jasmone | tr               | tr               |
| 1398           | 1398            | Cyperene | —              | 0.2             |
| 1404           | 1408            | Decyl acetate | tr                | —                |
| 1415           | 1417            | (E)-β-Caryophyllene | 0.3 100:0     | 0.4 100:0       |
| 1426           | 1430            | β-Copaene | tr              | tr               |
| 1451           | 1453            | α-Humulene | tr               | tr               |
| 1457           | 1457            | Rotundene | —              | 0.1             |
| 1471           | 1475            | γ-Murolene | tr 0.1        | 0.1             |
| 1473           | 1481            | (E)-β-Ionone | —                | —                |
| 1477           | 1480            | Germacrene D | 0.1 100:0    | 0.1 100:0       |
| 1481           | 1485            | γ-Thujaplicin | tr                | —                |
| 1483           | 1489            | β-Selinene | tr              | —                |
| 1487           | 1490            | γ-Amorphene | tr              | —                |
| 1491           | 1497            | α-Selinene | tr              | 0.1             |
| 1494           | 1497            | α-Murolene | tr              | tr               |
| 1509           | 1512            | γ-Cadinene | tr              | tr               |
| 1514           | 1518            | δ-Cadinene | 0.1 0.1       | 0.1 0:100       |
| 1548           | 1549            | Thymohydroquinone | 0.3 0.1      | 0.1             |
| 1577           | 1577            | Caryophyllene oxide | tr 0.1     | 0.1             |
| 1649           | 1655            | α-Cadinol | —              | 0.1             |
| 1689           | 1691            | Cyperotundone | —                | 0.2             |
| 1835           | 1841            | Phytone | —              | 0.1             |

| Monoterpane hydrocarbons | 14.3 | 10.2 | 9.6 |
| Oxygenated monoterpenoids | 84.0 | 86.3 | 86.0 |
| Sesquiterpene hydrocarbons | 0.5 | 0.7 | 1.4 |
| Oxygenated sesquiterpenoids | tr | 0.1 | 0.4 |
| Benzenoid aromatics | tr | 1.5 | 0.8 |
| Others | 1.0 | 1.2 | 1.2 |
| Total identified | 99.8 | 99.8 | 99.3 |

\( R\textsubscript{calc} = \) Retention indices determined with respect to a homologous series of \( n \)-alkanes on a ZB-5ms column. \( R\textsubscript{db} = \) Retention indices from the databases [7–10]. #1 = Plant sample #1. #2 = Plant sample #2. — = Not observed. ED = Enantiomeric distribution (dextrorotatory enantiomer: levorotatory enantiomer). tr = Trace (< 0.05%).
Chiral gas chromatography–mass spectrometry (GC-MS) analysis of the *M. citriodora* essential oils revealed the (+)-enantiomers to be the major stereoisomers for α-thujene, α-pinene, β-pinene, α-phellandrene, δ-3-carene, α-terpinene, cis-sabinene hydrate, trans-sabinene hydrate, α-terpeneol, α-copaene, (E)-β-caryophyllene, and germacrene D. On the other hand, the (−)-enantiomer was dominant for β-phellandrene, borneol, carvone, and δ-cadinene. Limonene showed variation in the enantiomeric distributions with (+)-limonene in 26.7%, 63.3%, and 57.3% for aerial parts #1, #2, and roots essential oils, respectively. Likewise, linalool also showed variation with (+)-linalool of 71.9%, 49.7%, and 50.1%. (+)-Terpinen-4-ol was the predominant enantiomer in the aerial parts essential oils (60.2% and 58.5%), but (−)-terpinen-4-ol (79.1%) was dominant in the root essential oil.

### 2.2. *Monarda Fistulosa*

*Monarda fistulosa* essential oils were obtained in 2.66–4.83% yields as bright orange oils. The chemical compositions of the essential oils from the aerial parts of *M. fistulosa* are summarized in Table 2. In samples #1 and #2, thymol (RI<sub>db</sub> = 1289) dominated the compositions (54.3% and 62.2%, respectively) with lesser quantities of p-cymene (RI<sub>db</sub> = 1024, 12.1% and 10.2%), limonene (RI<sub>db</sub> = 1030, 6.1% and 3.7%), carvacrol (RI<sub>db</sub> = 1296, 5.9% and 6.6%), and thymoquinone (RI<sub>db</sub> = 1252, 8.4% and 2.3%). Curiously, sample #3, although qualitatively similar, had a very different quantitative composition with thymoquinone as the most abundant constituent (41.3%) followed by p-cymene (21.9%), but with lower concentrations of thymol (8.9%) and carvacrol (1.6%).

| RI<sub>calc</sub> | RI<sub>db</sub> | Compound | #1, % | ED, (+):(-) | #2, % | ED, (+):(-) | #3, % | ED, (+):(-) |
|----------------|----------------|----------|-------|-------------|-------|-------------|-------|-------------|
| 923            | 925            | α-Thujene | 1.2   | 72.5:27.5   | 0.8   | 72.8:27.2   | 0.9   | 71.2:28.8   |
| 930            | 932            | α-Pinene  | 0.5   | 59.2:40.8   | 0.3   | 63.8:36.2   | 0.5   | 61.0:39.0   |
| 947            | 950            | Camphene  | 0.1   | 100.0       | 0.1   | 100.0       | 0.2   | 100.0       |
| 971            | 971            | Sabinene  | 0.2   | 58.4:41.6   | tr    | —           | 0.2   | 59.0:41.0   |
| 973            | 973            | 1-Octen-3-one |       | —           |       | —           | 0.1   | —           |
| 975            | 978            | β-Pinene  | 0.2   | 57.3:42.7   | —     | —           | 0.2   | 57.9:42.1   |
| 978            | 978            | 1-Octen-3-ol | 3.0   | —           | 3.3   | —           |
| 982            | 984            | 3-Octanone | tr    | 0.1         | 0.1   | 0.1         |
| 987            | 989            | Myrcene   | tr    | 0.3         |       | 0.3         |
| 995            | 996            | 3-Octanol  | tr    | 0.2         |       | 0.2         |
| 1004           | 1004           | p-Mentha-1(7),8-diene | tr | — | 0.1 |
| 1006           | 1006           | α-Phellandrene | 0.1 | 95.4:4.6   | 0.2   | 95.4:4.6   | 0.1   | 93.4:6.6   |
| 1008           | 1008           | δ-3-Carene | 0.1   | 100.0       | 0.1   | 100.0       | 0.1   | 100.0       |
| 1016           | 1016           | α-Terpinene | 2.1   | 100.0       | 2.3   | 100.0       | 0.8   | 100.0       |
| 1019           | 1022           | m-Cymene  | tr    | —           | 0.1   | 0.1         |
| 1024           | 1024           | p-Cymene  | 12.1  | 10.2        |       | 21.9        |
| 1025           | 1026           | 2-Acetyl-3-methylfuran | — | — | 0.5 |
| 1029           | 1030           | Limonene   | 6.1   | 0.5:99.5    | 3.7   | 2.6:97.4    | 6.3   | 1.2:98.8    |
| 1030           | 1031           | β-Phellandrene | 0.2   | 0.1:00     | 0.2   | 0.1:00     | 0.2   | 0.1:00     |
| 1031           | 1030           | 1,8-Cineole | 0.1   | 0.1         | 0.1   | 0.1         |
| 1056           | 1057           | γ-Terpinene | tr    | 0.1         |       | tr          |
| 1069           | 1069           | cis-Sabinene hydrate | 1.2 | 95.8:4.2   | 1.3   | 96.3:3.7   | 2.4   | 96.5:3.5   |
| 1078           | 1079           | 1-Nonen-3-ol | 0.1   | 0.1         | 0.1   | 0.1         |
| 1084           | 1086           | Terpinolene | tr    | 0.1         |       | tr          |
| 1089           | 1091           | p-Cymenene | tr    | —           | 0.1   | 0.1         |
| 1098           | 1099           | Linalool   | tr    | 37.8:62.2   | tr    | 37.5:62.5   | 37.9:62.1 |
| 1099           | 1099           | trans-Sabinene hydrate | 0.2 | 75.9:24.1  | 0.3   | 75.0:25.0  | 0.5   | 75.3:24.7  |
| 1103           | 1107           | Nonanal    | tr    | —           |       | tr          |
| 1115           | 1112           | (E)-2,4-Dimethylhepta-2,4-dienal | — | — | 0.2 |
| 1121           | 1121           | trans-p-Mentha-2,8-dien-1-ol | tr | — | 0.3 |
| 1123           | 1124           | cis-p-Menth-2-en-1-ol | tr | — | tr |
| 1130           | 1132           | cis-Limonene oxide | tr | — | 0.1 |
| R Lýc | R Lýb | Compound | #1, % | ED, (+):(-) | #2, % | ED, (+):(-) | #3, % | ED, (+):(-) |
|-------|-------|-----------|-------|-------------|-------|-------------|-------|-------------|
| 1133  | 1135  | 2-Vinylanisole | 0.1   | tr          |       |             |       |             |
| 1134  | 1137  | *cis*-p-Mentha-2,8-dien-1-ol | —     | —           | —     | 0.3         |       |             |
| 1135  | 1138  | *trans*-Limonene oxide | tr    | —           | —     |             |       |             |
| 1137  | 1138  | *trans*-Sabinol | —     | —           | tr    | —           | —     |             |
| 1138  | 1140  | *trans*-Pinocarveol | —     | —           | 0.1   |             |       |             |
| 1139  | 1141  | *cis*-Verbenol | —     | —           | tr    | —           | —     |             |
| 1143  | 1145  | *trans*-Verbenol | —     | —           | —     | 0.3         |       |             |
| 1144  | 1145  | Camphor | —     | —           | —     | tr          | —     |             |
| 1160  | 1164  | Pinocarvone | —     | —           | —     | tr          | —     |             |
| 1161  | 1162  | (Z)-iso-Citral | —     | —           | —     | tr          | —     |             |
| 1167  | 1168  | *trans*-Phellandrene epoxide | —     | —           | —     | 0.1         |       |             |
| 1170  | 1170  | Borneol | 0.5   | 0:100       | 0.2   | 0:100       | 0.7   | 0:100       |
| 1179  | 1180  | Terpinen-4-ol | 0.4   | 63.3:36.7   | 0.5   | 63.2:36.8   | 0.5   |             |
| 1186  | 1186  | *p*-Cymen-8-ol | 0.1   | tr          | —     | 0.6         |       |             |
| 1195  | 1195  | *α*-Terpineol | 0.3   | 100:0       | 0.2   | 100:0       | 0.3   |             |
| 1197  | 1198  | Methylchavicol (= Estragole) | —     | —           | 0.1   | 0.1         |       |             |
| 1197  | 1198  | *cis*-Piperitol | —     | —           | —     | 0.2         |       |             |
| 1217  | 1218  | *trans*-Carveol | —     | —           | —     | 0.2         |       |             |
| 1221  | 1223  | *cis*-Carveol | —     | —           | —     | 0.1         |       |             |
| 1240  | 1242  | Cuminaldehyde | —     | —           | —     | 0.1         |       |             |
| 1241  | 1242  | Carvone | —     | —           | —     | 0.3         |       |             |
| 1250  | 1241  | Pulegone | —     | 0.2        | —     | —           | —     |             |
| 1252  | 1252  | Thymoquinone | 8.4   | 2.3        | 41.3  |             |       |             |
| 1281  | 1282  | Bornyl acetate | —     | —           | —     | 0.1         |       |             |
| 1284  | 1286  | Cogeijerene | 0.1   | —           | —     | —           | —     |             |
| 1291  | 1291  | *p*-Cymen-7-ol | tr    | —           | 0.2   | —           |       |             |
| 1295  | 1293  | Thymol | 54.3   | 62.2       | 8.9   |             |       |             |
| 1300  | 1300  | Carvacrol | 5.9   | 6.6        | 1.6   |             |       |             |
| 1307  | 1306  | iso-Ascaridole | tr    | tr          | tr    | 0.1         |       |             |
| 1345  | 1346  | *α*-Cubebene | tr    | tr          | tr    | tr          |       |             |
| 1351  | 1356  | Eugenol | tr    | tr          | tr    | —           | —     |             |
| 1373  | 1375  | *α*-Copaene | 0.1   | 100:0       | 0.1   | 100:0       | 0.1   | 100:0       |
| 1382  | 1382  | *β*-Bourbonene | 0.1   | 0.1        | 0.1   | —           | —     |             |
| 1387  | 1387  | *trans*-β-Elemene | tr    | tr          | —     | 0.1         |       |             |
| 1418  | 1419  | *β*-Ylangene | tr    | —           | —     | 0.1         |       |             |
| 1419  | 1417  | *(E)-β*-Caryophyllene | 0.3   | 100:0       | 0.3   | 100:0       | 0.2   | 100:0       |
| 1427  | 1430  | *β*-Copaene | 0.1   | 0.1        | 0.1   | —           | —     |             |
| 1452  | 1453  | *α*-Humulene | tr    | tr          | tr    | —           | —     |             |
| 1473  | 1475  | γ-Muurolone | 0.1   | 0.2        | 0.1   | —           | —     |             |
| 1479  | 1479  | *α*-Amorphene | —     | tr          | —     | —           | —     |             |
| 1480  | 1483  | *trans*-β-Bergamotene | 0.1   | —           | —     | 0.1         |       |             |
| 1481  | 1480  | Germacrene D | 0.7   | 100:0       | 0.6   | 100:0       | 0.6   | 100:0       |
| 1484  | 1485  | γ-Thujaplicin | 0.4   | 0.2        | 1.3   | —           | —     |             |
| 1488  | 1490  | γ-Amorphene | tr    | —           | —     | —           | —     |             |
| 1491  | 1492  | *β*-Selinene | 0.1   | 0.1        | 0.1   | —           | —     |             |
| 1493  | 1492  | *trans*-Muurola-4(14),5-diene | —     | 0.1        | —     | —           | —     |             |
| 1496  | 1497  | *epi*-Cubebol | —     | tr          | —     | —           | —     |             |
| 1497  | 1497  | *α*-Selinene | —     | 0.1        | —     | —           | —     |             |
| 1498  | 1497  | *α*-Muurolone | tr    | 0.1        | tr    | —           | —     |             |
| 1510  | 1512  | γ-Cadinene | 0.1   | 0.2        | 0.1   | —           | —     |             |
| 1511  | 1515  | Cubebol | tr    | —           | —     | —           | —     |             |
| 1517  | 1518  | δ-Cadinene | 0.1   | 0.3        | 0.1   | —           | —     |             |
| 1518  | 1519  | *trans*-Calamenene | tr    | tr          | —     | —           | —     |             |
| 1520  | 1523  | *β*-Sesquiphellandrene | tr    | —           | —     | —           | —     |             |
| 1537  | 1538  | *α*-Cadinene | tr    | tr          | tr    | —           | —     |             |
| 1542  | 1541  | *α*-Calacorene | tr    | tr          | tr    | —           | —     |             |
| 1543  | 1546  | *α*-Elemol | tr    | —           | —     | —           | —     |             |
| 1548  | 1554  | Thymohydroquinone | 0.6   | 1.6        | 0.6   | —           | —     |             |
As was observed in *M. citriodora* essential oils, in *M. fistulosa* essential oils, the (+)-enantiomer was the major for α-thujene, α-pinene, β-pinene, α-phellandrene, δ-3-carene, α-terpinene, cis-sabinene hydrate, trans-sabinene hydrate, α-terpinol, α-copaene, (E)-β-caryophyllene, and germacrene D, while the (−)-enantiomer was predominant for β-phellandrene and bornol. (−)-Limonene (97.4–99.5%) and (−)-linalool (62.1–62.5%) dominated in all three *M. fistulosa* samples. (+)-Camphene (100%), (+)-sabinene (58.4–59.0%), and (+)-terpinen-4-ol (63.2–63.3%) were also dominant.

### 2.3. *Monarda Punctata*

Hydrodistillation of two samples of wild-growing *M. punctata* aerial parts gave bright orange essential oils in 0.781% and 0.658% yield. The most abundant components in the essential oils were thymol (RI<sub>db</sub> = 1289, 61.8% and 47.9%), p-cymene (RI<sub>db</sub> = 1024, 15.3% and 19.8%), γ-terpinene (RI<sub>db</sub> = 1057, 2.7% and 9.7%), and carvacrol (RI<sub>db</sub> = 1296, 4.5% and 4.1%) (see Table 3).

The enantiomeric distributions of terpenoids in *M. punctata* essential oils were analogous to those observed for *M. citriodora* and *M. fistulosa* oils with the exception of limonene, which was virtually racemic in sample #1, but 100% (−)-limonene in sample #2.

#### Table 3. Chemical composition of *Monarda punctata* essential oils growing wild in south Alabama.

| RI<sub>calc</sub> | RI<sub>db</sub> | Compound | #1, % ED<sub>1</sub> | #1, % ED<sub>2</sub> | #2, % ED<sub>1</sub> | #2, % ED<sub>2</sub> | #3, % ED<sub>3</sub> | #3, % ED<sub>3</sub> |
|------------------|-----------------|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 923              | 925             | α-Thujene | 0.1             | 100:0           | 0.7             | 100:0           | —               | 68.5:31.5       |
| 930              | 932             | α-Pinene | tr              |                | 0.2             | 0.2             | —               | 83.8:16.2       |
| 945              | 950             | Camphene | —               | 0.1             | —               | 0.1             | —               | 100:0           |
| 957              | 959             | Benzaldehyde | —             | 0.1             | 0.1             | 0.1             | 0.1             |
| 970              | 971             | Sabinene | tr              | 0.1             | 0.1             | 0.1             | 0.1             |
| 974              | 978             | β-Pinene | tr              | 0.1             | 0.1             | 0.1             | 0.1             |
| 975              | 974             | 1-Octen-3-ol | 1.8         | 1.8             | 1.8             | 1.8             | 1.8             |
| 980              | 983             | 3-Octanone | —             | 0.1             | 0.1             | 0.1             | 0.1             |
| 984              | 989             | Myrcene | 0.3             | 1.1             | 1.1             | 1.1             | 1.1             |
| 977              | 978             | 3-Octanol | 0.1             | 0.1             | 0.1             | 0.1             | 0.1             |
| 1001             | 1004            | p-Mentha-1(7),8-diene | —         | 0.1             | 0.1             | 0.1             | 0.1             |
| 1004             | 1006            | α-Phellandrene | 0.1         | 100:0           | 0.2             | 100:0           | 0.2             |
| 1006             | 1008            | δ-3-Carene | tr             | 0.1             | 0.1             | 0.1             | 0.1             |
| 1014             | 1017            | α-Terpinene | 1.3          | 3.0             | 3.0             | 3.0             | 3.0             |
| 1016             | 1012            | m-Cymene | —               | 0.1             | 0.1             | 0.1             | 0.1             |
| 1024             | 1024            | p-Cymene | 15.3            | 19.8            | 19.8            | 19.8            | 19.8            |
| 1025             | 1026            | 2-Acetyl-3-methylfuran | —        | tr              | tr              | tr              | tr              |
| RI<sub>calc</sub> | RI<sub>db</sub> | Compound                        | #1, % | Aerial Parts Essential Oil ED<sub>_1_</sub>(+):(-) | #2, % | ED<sub>_2_</sub>(+):(-) |
|--------------|--------------|---------------------------------|------|---------------------------------|------|------------------|
| 1026         | 1030         | Limonene                        | 0.4  | 50.2:49.8                       | 0.5  | 0:100            |
| 1027         | 1029         | β-Phellandrene                  | 0.2  | 0.2                             | 0.2  | 0:100            |
| 1028         | 1030         | 1,8-Cineole                     | 0.4  | 0.1                             |      |                  |
| 1039         | 1043         | Phenylacetaldehyde              |      |                                 |      |                  |
| 1055         | 1057         | γ-Terpinene                     | 2.0  | 9.7                             |      |                  |
| 1066         | 1069         | cis-Sabinene hydrate            | 0.6  | 100:0                           | 0.7  | 97.9:2.1         |
| 1076         | 1079         | 1-Nonen-3-ol                    |      | tr                              |      |                  |
| 1082         | 1086         | Terpinolene                     | 0.1  | 0.1                             |      |                  |
| 1087         | 1091         | p-Cymenene                      | 0.1  | 0.1                             |      |                  |
| 1095         | 1099         | Linalool                        |      |                                 |      |                  |
| 1098         | 1101         | trans-Sabinene hydrate          | 0.2  | 100:0                           | 0.1  | 83.2:16.8        |
| 1100         | 1104         | Nonanal                         |      |                                 |      |                  |
| 1104         | 1107         | 1-Octen-3-yl acetate            | 0.2  | 0.4                             |      |                  |
| 1145         | 1145         | trans-Verbenol                  |      |                                 |      |                  |
| 1161         | 1158         | Menthone                        | 0.3  | 0.1                             |      |                  |
| 1168         | 1170         | Bornol                          | 0.1  | 0:100                           | tr   | 0:100            |
| 1182         | 1183         | m-Cymen-8-ol                    | 0.8  | 58.7:41.3                       | 0.6  | 66.1:33.9        |
| 1184         | 1186         | p-Cymen-8-ol                    | 0.5  | 0.5                             |      |                  |
| 1191         | 1197         | Methyl chavicol (= Estragole)   | 0.8  |                                 |      |                  |
| 1193         | 1195         | α-Terpineol                     |      | 0.1                             |      | 100:0            |
| 1202         | 1206         | Decanal                         |      |                                 |      |                  |
| 1224         | 1224         | Thymol methyl ether             |      |                                 |      |                  |
| 1235         | 1238         | Carvacrol methyl ether          | 1.1  | 1.0                             |      |                  |
| 1239         | 1242         | Cumin aldehyde                  |      | 0.1                             |      |                  |
| 1247         | 1250         | Thymoquinone                    | 2.0  | 0.2                             |      |                  |
| 1289         | 1289         | Thymol                          | 61.8 | 47.9                            |      |                  |
| 1293         | 1291         | p-Cymen-7-ol                    |      | 0.2                             |      |                  |
| 1296         | 1296         | Carvacrol                       | 4.5  | 4.1                             |      |                  |
| 1306         | 1309         | 4-Vinylguaiacol                 |      |                                 |      |                  |
| 1347         | 1356         | Eugenol                         | 0.7  | 0.3                             |      |                  |
| 1370         | 1375         | α-Copaene                       |      |                                 |      |                  |
| 1378         | 1382         | β-Bourbonene                    |      |                                 |      |                  |
| 1384         | 1390         | trans-β-Elemene                 |      |                                 |      |                  |
| 1415         | 1417         | (E)-β-Caryophyllene             | 1.6  | 100:0                           | 1.2  | 100:0            |
| 1424         | 1430         | β-Copaene                       |      |                                 |      |                  |
| 1427         | 1430         | trans-α-Bergamotene             | 1.2  | 0.7                             |      |                  |
| 1449         | 1453         | α-Humulene                      |      |                                 |      |                  |
| 1469         | 1475         | γ-Muurolene                     |      | 0.1                             |      |                  |
| 1476         | 1480         | Germacrene D                    | 0.7  | 100:0                           | 0.4  | 100:0            |
| 1479         | 1483         | trans-β-Bergamotene             | 0.2  | 0.1                             |      |                  |
| 1480         | 1485         | γ-Thujaplicin                   |      |                                 |      |                  |
| 1482         | 1489         | β-Selinene                      |      |                                 |      |                  |
| 1489         | 1492         | α-Selinene                      |      |                                 |      |                  |
| 1492         | 1497         | α-Muurolene                     |      |                                 |      |                  |
| 1506         | 1512         | γ-Cadinen                      |      |                                 |      |                  |
| 1512         | 1518         | δ-Cadinen                      |      |                                 |      |                  |
| 1517         | 1523         | β-Sesquiphellandrene            |      |                                 |      |                  |
| 1546         | 1549         | Thymohydroquinone               | 0.3  | 2.5                             |      |                  |
| 1576         | 1577         | Caryophyllene oxide             | 0.2  | 0.2                             |      |                  |
| 1633         | 1639         | cis-Guaia-3,9-dien-11-ol        | 0.2  | 0.1                             |      |                  |
| 1648         | 1655         | α-Cadinol                      |      |                                 |      |                  |
| 1834         | 1841         | Phytone                         |      |                                 |      |                  |

|                   |               | Monoterpene hydrocarbons        | 19.9 | 35.7                            |      |                  |
|                   |               | Oxygenated monoterpeneoids      | 72.5 | 58.3                            |      |                  |
|                   |               | Sesquiterpene hydrocarbons      | 3.7  | 2.7                             |      |                  |
|                   |               | Oxygenated sesquiterpeneoids    | 0.4  | 0.4                             |      |                  |
### Table 3. Cont.

| RI<sub>calc</sub> | RI<sub>db</sub> | Compound | Aerial Parts Essential Oil #1, % | ED<sub>1</sub>, (+):(−) | Aerial Parts Essential Oil #2, % | ED<sub>2</sub>, (+):(−) |
|------------------|----------------|----------|-------------------------------|-----------------|-------------------------------|-----------------|
|                  |                | Benzenoid aromatics | 1.5                           | 0.3             | 2.1                           | 2.4             |
|                  |                | Others          | 2.1                           | 2.4             | 100.0                         | 99.8            |

RI<sub>calc</sub> = Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. RI<sub>db</sub> = Retention indices from the databases [7–10]. #1 = Plant sample #1. #2 = Plant sample #2. — = Not observed. ED = Enantiomeric distribution (dextrorotatory enantiomer: levorotatory enantiomer). tr = Trace (<0.05%).

### 3. Discussion

*Monarda citriodora* and *M. fistulosa* have been introduced throughout temperate regions of the world as popular herbal medicines as well as ornamentals [4–6]. The volatile phytochemistry has shown wide variation depending on geographical location (Table 4). The essential oils of *M. citriodora* in the present study were rich in both thymol and carvacrol, whereas essential oils from Europe and Asia were dominated by thymol with much lower concentrations of carvacrol. *Monarda fistulosa*, in particular, showed wide variation with at least three different chemotypes (carvacrol-rich, thymol-rich, and geraniol-rich, see Table 4). The essential oils of *M. fistulosa* (samples #1 and #2) in this study fit into the thymol-rich chemotype. Interestingly, there was a high concentration of thymoquinone in *M. fistulosa* sample #3, with concomitant lower concentrations of thymol and carvacrol. Thymol was reported as the major component of *M. punctata* in two old reports [11,12]. Consistent with these reports, a floral essential oil of *M. punctata* from China was rich in thymol (75.2%), which is in agreement with the aerial parts essential oils from Alabama.

### Table 4. Major essential oil components of *Monarda* species from geographical locations around the world.

| *Monarda* spp. | Plant Tissue | Collection Site | Composition (Major Components) | Ref. |
|----------------|--------------|----------------|--------------------------------|------|
| *M. citriodora* | Aerial parts | Jammu, India (cultivated) | Thymol (82.3%), carvacrol (4.8%) | [13] |
| *M. citriodora* | Aerial parts | Imola (BO) Italy (cultivated) | Thymol (19.6%), p-cymene (15.6%), γ-terpinene (13.5%), carvacrol (9.3%), α-terpinene (9.2%), myrcene (5.7%) | [14] |
| *M. citriodora* var. citriodora | Leaves | Liverpool, UK (cultivated) | Thymol (50.7%), p-cymene (22.8%), carvacrol (3.6%) | [16] |
| *M. citriodora* var. citriodora | Flowers | Liverpool, UK (cultivated) | Thymol (61.8%), γ-terpinene (13.3%), p-cymene (4.2%), carvacrol (3.8%) | [16] |
| *M. citriodora* var. citriodora | Aerial parts | Liverpool, UK (cultivated) | Thymol (56.9%), p-cymene (13.0%), α-terpinene (10.0%), carvacrol (4.3%) | [17] |
| *M. citriodora* var. citriodora | Aerial parts | Commercial (unknown) | Thymol (70.6%), p-cymene (10.6%), carvacrol (6.1%) | [18] |
| *M. fistulosa* | Aerial parts | Krasnodarsk Krai, Russia (introduced, wild) | Thymol (32.5%), carvacrol (23.9%), thymol (12.6%), carvacrol methyl ether (5.5%), unidentified aliphatic aldehyde (6.3%) | [19] |
| *M. fistulosa* | Aerial parts | Casola Valsenio, Italy (cultivated) | Thymol (26.5%), β-phellandrene (17.0%), α-phellandrene (13.7%), p-cymene (13.5%), myrcene (8.1%) | [20] |
| *M. fistulosa* | Aerial parts | Saint-Jean-sur-Richelieu, QC, Canada (cultivated) | Geraniol (61.8%), geranyl formate (16.6%), geranial (10.6%), nerol (6.6%) | [21] |
| *M. fistulosa* | Aerial parts | Poplarville, MS, USA (cultivated) | Carvacrol (39.1%), p-cymene (35.4%), (−)-1-octen-3-ol | [22] |
Table 4. Cont.

| Monarda spp. | Plant Tissue | Collection Site          | Composition (Major Components)                                      | Ref. |
|--------------|--------------|--------------------------|---------------------------------------------------------------------|------|
| M. fistulosa | Aerial parts | Imola (BO) Italy (cultivated) | Thymol (31.6%), β-phellandrene (18.1%), α-phellandrene (14.2%), p-cymene (13.1%), myrcene (8.8%) | [23] |
| M. fistulosa | Aerial parts | Imola (BO) Italy (cultivated) | Thymol (28.4%), β-phellandrene (16.9%), α-phellandrene (13.7%), p-cymene (13.3%), myrcene (8.7%) | [24] |
| M. fistulosa | Aerial parts | Imola (BO) Italy (cultivated) | Thymol (33.4%), β-phellandrene (18.0%), α-phellandrene (14.0%), p-cymene (13.2%), myrcene (8.6%) | [24] |
| M. fistulosa | Aerial parts | Ravenna, Italy (cultivated) | γ-Terpinene (25.2%), carvacrol (24.3%), γ-caryophyllene (24.3%) | [25] |
| M. fistulosa | Aerial parts | Chișinău, Republic of Moldova (cultivated) | Carvacrol (54.8%), p-cymene (23.2%), carvacrol methyl ether (10.5%) | [26] |
| M. fistulosa | Flowers      | Gallatin Valley, MT, USA (wild) | Carvacrol (45.7%), p-cymene (25.6%), γ-terpinen (6.8%), thymol (3.1%) | [27] |
| M. fistulosa | Leaves       | Gallatin Valley, MT, USA (wild) | Carvacrol (71.5%), p-cymene (13.1%), γ-terpinen (2.5%), thymol (3.3%) | [27] |
| M. fistulosa | Aerial parts | Moscow, Russia (cultivated) | α-Terpineol (37.7%), 1-octen-3-ol (10.5%), geraniol (10.4%), thymol (9.3%), p-cymene (4.9%) | [28] |
| M. fistulosa cv. Fortuna | Aerial parts | Kherson, Ukraine (cultivated) | Thymol (77.3%), carvacrol methyl ether (4.9%), carvacrol (3.8%) | [6] |
| M. fistulosa cv. Premiera | Aerial parts | Kherson, Ukraine (cultivated) | Thymol (78.3%), carvacrol methyl ether (4.8%), carvacrol (3.6%) | [6] |
| M. fistulosa var. menthifolia | Aerial parts | Morden, Manitoba, Canada (cultivated) | Geraniol (86.8%) | [29] |
| M. punctata | Flowers      | Xi’an, China (cultivated?) | Thymol (75.2%), p-cymene (6.7%), limonene (5.4%), carvacrol (3.5%) | [30] |

* Isomer not indicated.

The high concentrations of thymol, carvacrol, and p-cymene are consistent with the traditional uses of *Monarda* spp. to treat skin infections, wounds, fevers, and respiratory problems. Thymol [31], carvacrol [32], and p-cymene [33] have demonstrated antibacterial and antifungal activities [34,35], as well as wound-healing activity [36]. Thymol [37] and carvacrol [38], in addition to thymoquinone [39], have shown antitussive effects. Thymoquinone has also shown wound-healing properties [40]. Furthermore, both thymol [41] and carvacrol [32] have shown analgesic and anti-inflammatory activities [42].

As far as we are aware, this work presents the first chiral analysis of terpenoid constituents of *Monarda* species. Several investigations on the enantiomeric distributions in other members of the Lamiaceae have been reported in the literature, however. There seems to be much variation in the enantiomeric distribution of monoterpenoids across the family. Consistent with what was observed in *Monarda* essential oils, (+)-α-pinene was the major enantiomer found in *Coridothymus capitatus* [43], *Rosmarinus officinalis* [44], *Lepechinia heteromorpha* [45], *Ocimum canum,* and *Ocimum kilimandscharicum* [46]. Likewise, (+)-β-pinene predominates over (−)-β-pinene in *C. capitatus* [43] as well as the *Monarda* essential oils. On the other hand, (−)-β-pinene dominates in *R. officinalis* [44] and *Lepechinia mutica* [47]. The essential oils of peppermint (*Mentha × piperita*) and spearmint (*Mentha spicata*) have shown nearly racemic mixtures of α- and β-pinenes [48]. (+)-α-Phellandrene and (−)-β-phellandrene were the dominant enantiomers in the *Monarda* essential oils. In marked contrast, however, (−)-α-phellandrene and (+)-β-phellandrene predominated in *L. mutica* essential oil [47]. (−)-Limonene predominates in *M. fistulosa* essential oil, peppermint (*M. piperita*) and spearmint (*M. spicata*) essential oils [48] whereas (+)-limonene is the major enantiomer in *C. capitatus* [43], *O. canum,* and *O. kilimandscharicum* [46], and a nearly
racemic mixture was found in rosemary (*R. officinalis*) essential oil [44]. (+)-Linalool was the predominant enantiomer in *C. capitatus* [43], *Salvia schimperi* [49], *Pycnanthemum incanum* [50], *O. canum*, and *O. kilimandscharicum* [46], whereas (−)-linalool was the major stereoisomer in *Lavandula angustifolia* [51] and *R. officinalis* [44].

4. Materials and Methods

4.1. Plant Material

*Monarda citriodora* was cultivated in Kirkland Gardens, Newville, AL, USA (31°26’27” N, 85°21’31” W) from seeds (Outsidepride Seed Source, Independence, OR, USA). The cultivated *Monarda* spp. were grown in loamy clayey-sand and fertilized with chicken manure, kelp meal, and bone meal at planting in full sun. The aerial parts of *M. citriodora* were collected from separate plants on separate occasions (plant #1, collected on 20 June 2020; plant #2 collected on 1 August 2020). The roots of *M. citriodora* were obtained from plant #2.

*Monarda fistulosa* was cultivated in Kirkland Gardens, Newville, AL, USA (31°26’27” N, 85°21’31” W) from seedlings (Home Depot, Dothan, AL, USA) as above. The aerial parts of three different plant samples were collected on 25 June 2020.

*Monarda punctata* was collected from wild-growing plants near Newville, AL, USA (31°27’23” N, 85°22’17” W); the edge of a planted pine forest, disturbed grassland, full/partial sun, sandy-clay soil that had been intentionally burned (prescribed burn) 1.5 years before collection. The aerial parts of two different plants were collected on 1 June 2020.

Plants were identified by S.K. Lawson and a voucher specimen of each plant was deposited in the University of Alabama in Huntsville Herbarium (HALA); voucher numbers for *M. citriodora* (SKL61820), *M. fistulosa* (SKL72020), and *M. punctata* (SKL9620). The *Monarda* plant materials were allowed to dry in the shade for several days, the air-dried plant materials were pulverized and subjected to hydrodistillation using a Likens-Nickerson apparatus with continuous extraction with dichloromethane (Table 5).

Table 5. Hydrodistillation details of *Monarda* species collected or cultivated in south Alabama.

| *Monarda* spp. | Mass Plant Material | Yield Essential Oil (EO) |
|----------------|---------------------|--------------------------|
| *Monarda citriodora* #1 | 25.57 g dried aerial parts | 406.2 mg orange EO |
| *Monarda citriodora* #2 | 37.81 g dried aerial parts | 675.6 mg orange EO |
| *Monarda citriodora* #2 | 17.47 g dried roots | 153.6 mg yellow EO |
| *Monarda fistulosa* #1 | 9.60 g dried aerial parts | 364.0 mg bright orange EO |
| *Monarda fistulosa* #2 | 7.58 g dried aerial parts | 366.2 mg bright orange EO |
| *Monarda fistulosa* #3 | 8.98 g dried aerial parts | 238.9 mg bright orange EO |
| *Monarda punctata* #1 | 39.09 g dried aerial parts | 305.6 mg bright orange EO |
| *Monarda punctata* #2 | 62.62 g dried aerial parts | 411.9 mg bright orange EO |

4.2. Gas Chromatographic Analysis

The essential oils were analyzed by gas chromatography–mass spectrometry (GC-MS), gas chromatography with flame ionization detection (GC-FID), and chiral GC-MS as previously reported [52].

4.2.1. Gas Chromatography–Mass Spectrometry

Shimadzu GCMS-QP2010 Ultra, ZB-5ms GC column, GC oven temperature 50 °C–260 °C (2 °C/min), 1-µL injection of 5% solution of EO in dichloromethane (split mode, 30:1). Retention indices (RI) were determined with reference to a homologous series of *n*-alkanes. Compounds identified by comparison of the MS fragmentation and retention indices with those in the databases [7–10].

4.2.2. Gas Chromatography–Flame Ionization Detection

Shimadzu GC 2010, FID detector, ZB-5 GC column, GC oven temperature 50 °C–260 °C (2.0 °C/min). The percent compositions were determined from raw peak areas without standardization.
4.2.3. Chiral Gas Chromatography–Mass Spectrometry

Shimadzu GCMS-QP2010S, Restek B-Dex 325 column, GC oven temperature 50 °C–120 °C (1.5 °C/min) then 120 °C–200 °C (2.0 °C/min), 0.1 µL injection of 5% solution of EO in dichloromethane (split mode, 45:1). The enantiomeric distributions were determined by comparison of retention times with authentic samples obtained from Sigma-Aldrich (Milwaukee, WI, USA). Relative enantiomer percentages were calculated from peak areas.

5. Conclusions

This study presents, for the first time, analyses of the essential oils of three species of Monarda growing in south Alabama. In addition, the enantiomeric distribution of terpenoids was also carried out. This work illustrates the wide variation in essential oil compositions based on geographical location as well as variations in enantiomeric distribution. It would be interesting to compare enantiomeric distributions for Monarda essential oils from other geographical locations and for other Monarda species. Nevertheless, the phenolic monoterpenoids thymol and/or carvacrol were found to dominate the compositions of M. citriodora, M. fistulosa, and M. punctata and support the traditional medicinal uses of these plants.

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