Abstract: The differences in the composition of essential oils obtained from the aerial parts of six Ferula species viz., F. caratavica (Fc), F. kuchistanica (Fk), F. pseudoreoselinum (Fp), F. samarcandica (Fs), F. tenuisecta (Ft) and F. varia (Fv) were detected both qualitatively and quantitatively using GC-MS and GC-FID analyses. One hundred and six metabolites were identified that account for 92.1, 96.43, 87.43, 95.95, 92.90 and 89.48% of Fc, Fk, Fp, Fs, Ft and Fv whole essential oils, respectively. The data from the GC-MS analyses were subjected to unsupervised pattern recognition chemometric analysis utilizing principal component analysis (PCA) to improve the visualization of such differences among the six species. Fk and Ft are very closely related to each other and were gathered together in one cluster. The antioxidant potential was assessed in vitro using different assays including 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC), ferric reducing power (FRAP) and phosphomolybdenum (PM) assays. Ft and Fp exhibited the most notable antioxidant properties as evidenced by their pronounced activities in most of the antioxidant assays performed, followed by Fc. Fk showed the most effective tyrosinase inhibitory potential, which was estimated as 119.67 mgKAE/g oil, while Fp exhibited the most potent α-amylase inhibitory potential, which was equivalent to 2.61 mmol ACAE/g oil. Thus, it was concluded that Ferula species could serve as a promising natural antioxidant drug that could be included in different products and spices to alleviate hyperglycemia and used as a natural ingredient in pharmaceutical cosmetics to counteract hyperpigmentation.

Keywords: Ferula; GC; essential oils; chemometrics; antioxidant activity; enzyme inhibition

1. Introduction

Essential oils comprise a mixture of secondary metabolites, which are biosynthesized by aromatic plants as natural protectants [1]. The role of essential oils is not restricted to protection as they also...
offer many therapeutic benefits to humans that can exceed the benefits provided by the dried herbs on their own [2]. Recently, they have become well known as a part of traditional medicine for the treatment of a plethora of human ailments, in aromatherapy, as well as in spices with high nutritive value [3]. In addition, many essential oils as well as plant extracts have shown significant antioxidant potential [4–6]. New sources of medicinal agents that are effective and safe as well as selective has recently become the main target in drug discovery. Medicinal plants in general, and their volatile constituents in particular, act as a very important sources for the production of a huge number of biologically active agents, which are attractive chemical leads that are promising therapeutic agents for the alleviation of many ailments [7,8]. Many biological activities have been ascribed to the volatile constituents obtained from a variety of plants such as antinociceptive, anticancer, antiphlogistic, antiviral, antioxidant, antimicrobial, antimycotic, antiparasitic and insecticidal activities [9]. Moreover, the volatile constituents of plants are highly popular in the food, cosmetic and pharmaceutical industries because of their broad acceptance by consumers, relative safety, and their potential multipurpose effect [10,11].

The Apiaceae family is well-known for its rich aromatic plants, which are categorized under approximately 112 genera and nearly 316 species. Anise, chervil, celery, coriander, cumin, caraway, dill, fennel, ferula and galbanum are significant members of this family and they are characterized by their notable odor owing to the presence of considerable amounts of essential oils or the oleoresin predominant in their different organs [3]. These plants are widely used for culinary purposes either for their aroma or as nutrients [12].

*Ferula* constitutes the third largest genus in the Apiaceae family with nearly 180 species. The members of this genus are very popular for their essential oils, which are recognized as having many biological activities including antibacterial, antifungal, antiviral, antispasmodic, anticonvulsant, and antioxidant activity as well as having high nutritive value [13,14].

This study aimed to investigate the contents of the essential oil from six *Ferula* species growing in Uzbekistan, namely, *F. caratavica* (Fc), *F. kuchistanica* (Fk), *F. pseudoreoselinum* (Fp), *F. samarcandica* (Fs), *F. tenuisecta* (Ft) and *F. varia* (Fv) using GC analyses. Discrimination of these species was carried by coupling the data obtained from GC-analyses with chemometrics employing unsupervised pattern recognition techniques represented by principal component analysis (PCA). Furthermore, the antioxidant potential of the different essential oil samples using different assays, namely, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC), ferric reducing power (FRAP), and the phosphomolybdenum (PM) assay were evaluated in vitro. In addition, an evaluation of the possible enzymatic inhibitory activities of essential oils against tyrosinase and α-amylase was done using standard in vitro bioassays.

### 2. Results and Discussion

#### 2.1. GC-MS and GC-FID Quantitative and Qualitative Determination

The differences in the composition of the essential oils obtained from the aerial parts of *Fc*, *Fk*, *Fp*, *Fs*, *Ft* and *Fv* were detected both qualitatively and quantitatively using GC-MS and GC-FID analyses, respectively. All of the essential oils are yellow in color and possess a characteristic odor. Characterization of the essential oils using GC analyses revealed the presence of 106 metabolites (Table 1, Figures 1 and 2) that account for 92.10, 96.43, 87.43, 95.95, 92.90 and 89.48% of *Fc*, *Fk*, *Fp*, *Fs*, *Ft* and *Fv* whole essential oils, respectively. Twenty-nine compounds were detected in *Fc* with α-pinene (21.17%), 10,13-docosadienoic acid methyl ester (15.20%), β-caryophyllene oxide (13.23%) and caryophyllene (10.88%) representing the predominant compounds. Meanwhile, thirty-nine compounds were identified in *Fk* essential oil with α-pinene (36.79%) and verbenol (8.49%) being the major compounds. In *Fp*, forty-five compounds were characterized with 4-terpineol (16.28%), α-pinene (10.99%), β-myrcene (6.04%), β-caryophyllene oxide (5.69%), p-cymen-8-ol (5.36%) and spathulenol (5.34%) as the main metabolites in the oil. Furthermore, 15 compounds were determined in *Fs* oil with
the main compounds, palmitic acid, β-myrecene, heptacosane, octacosane, hexacosane and pentacosane accounting for 39.09, 10.75, 10.27, 9.60, 8.99 and 6.29%, respectively. For Ft, 62 compounds were detected of which α-pinene (42.0%), camphene (8.34%) and α-cadinol (8.14%) exist in high percentages in the oil. Finally, 25 compounds were identified in the Fv oil with 10,13-docosadienoic acid methyl ester (69.61%) constituting the major component (Figure 3). From the data shown in Table 1, it was concluded that monoterpenes are the predominate class of essential oil metabolites in Fc, Fk and Ft, where they represent 24.90, 42.91 and 61.95%, respectively, while oxygenated monoterpenes are the dominant class of metabolites in Fp (35.60%), and they also exist in a high percentage in Fk (34.82%). On the contrary, fatty acids are highly predominate in Fs and Fv and account for 82.55 and 79.84%, respectively.

Table 1. Composition of volatile oil in the aerial parts of F. caratavica (Fc), F. kuchistanica (Fk), F. pseudoreoselinum (Fp), F. samarcardica (Fs), F. tenuisecta (Ft) and F. varia (Fv).

| Compound | RI Content, (%) | Identification Methods |
|----------|----------------|------------------------|
| 1. n-Nonane | 889 900 0.37 | tr 0.16 | - | - | - | MS, RL |
| 2. Tricyclene | 913 913 - | 0.37 | - | - | - | MS, RL |
| 3. 3-Thujiene | 919 919 - | 0.50 | 0.36 | - | - | MS, RL, AU |
| 4. α-Pinene | 952 925 21.17 | 36.79 | 10.99 | 1.5 | 42.0 | MS, RI, AU |
| 5. Camphene | 941 941 2.91 | 0.47 | - | - | 8.34 | MS, RL |
| 6. Sabine | 970 970 - | 0.94 | - | 0.35 | - | MS, RL |
| 7. β-Pinene | 973 973 0.82 | 1.88 | 3.11 | - | 3.59 | MS, RI, AU |
| 8. 6-Methyl-5-heptene-2-one | 986 986 - | 0.35 | - | - | - | MS, RI, AU |
| 9. β-Mycene | 989 989 - | 0.19 | 6.04 | 10.75 | 1.90 | MS, RL |
| 10. n-Decane | 998 1000 1.00 | 0.16 | 0.47 | 2.44 | 0.06 | 0.62 | MS, RI, AU |
| 11. α-Phellandrene | 1003 1003 - | 1.81 | tr | - | - | - | MS, RI, AU |
| 12. (3E)-3-Hexenyl acetate | 1007 1006 - | - | - | - | 1.40 | - | MS, RL |
| 13. 3-Carene | 1009 1009 - | tr 0.97 | - | 0.07 | - | MS, RL |
| 14. 2-Carene | 1016 1018 - | - | tr | - | - | MS, RL |
| 15. β-Cymene | 1024 1025 - | tr 2.59 | - | 0.39 | - | MS, RL |
| 16. Limonene | 1028 1028 - | 1.77 | 0.83 | 11.5 | 4.80 | - | MS, RI, AU |
| 17. α-Terpinene | 1059 1059 - | 0.30 | - | - | - | MS, RI, AU |
| 18. Linalool oxide | 1074 1074 | 0.25 | - | - | - | - | MS, RL |
| 19. Terpinolene | 1089 1089 - | - | - | tr | - | MS, RL |
| 20. Dehydro-p-cymene | 1090 1090 - | - | - | 0.15 | - | - | MS, RL |
| 21. n-Undecane | 1098 1100 tr 1.60 | 1.56 | - | 0.43 | - | MS, RL |
| 22. β-Linalool | 1100 1100 tr | tr 2.03 | - | 0.54 | - | MS, RI, AU |
| 23. cis-β-Menth-2,6-dienol | 1108 1104 - | 0.80 | 1.78 | - | 0.40 | - | MS, RL |
| 24. Fenchol | 1116 1117 - | - | - | - | 0.04 | - | MS, RL |
| 25. 6-Camphenol | 1128 1131 - | 2.75 | - | 0.05 | - | MS, RL |
| 26. Limonene oxide | 1135 1133 - | - | - | - | tr | - | MS, RL |
| 27. 4-Isopropenyl-1-methyl-2-cyclohexen-1-ol | 1137 1142 - | 0.32 | 0.49 | - | 0.12 | - | MS, RL |
| 28. 1-pinocarveol | 1141 1141 - | 3.90 | 0.74 | - | 0.33 | - | MS, RL |
| 29. Verbenol | 1146 1148 | 8.49 | - | - | 1.25 | - | MS, RL |
| 30. trans-2-Nonenal | 1160 1161 - | - | - | - | 0.03 | 0.73 | MS, RL |
| 31. 3-Finone | 1163 1160 - | tr | - | - | - | MS, RL |
| 32. Verbenone | 1165 1173 | 1.43 | - | - | - | - | MS, RL |
| 33. Borneol | 1169 1169 - | - | - | - | 0.11 | - | MS, RL |
| 34. 4-Terpineol | 1179 1179 - | 0.39 | 16.28 | - | 0.36 | - | MS, RL |
| 35. p-Cymen-8-ol | 1186 1186 tr 2.85 | 5.36 | - | 0.80 | 0.49 | MS, RL |
| 36. α-Terpinol | 1193 1193 - | 0.52 | 5.00 | - | 0.41 | - | MS, RL |
| 37. Myrtenol | 1199 1199 | 2.30 | 0.65 | - | 0.21 | - | MS, RL |
| 38. cis-Geraniol | 1210 1210 - | - | 0.35 | - | tr | - | MS, RL |
| 39. Verbezone | 1214 1214 - | 3.95 | 1.11 | - | 0.18 | - | MS, RL |
| 40. Fenchyl acetate | 1222 1223 - | 4.51 | - | - | - | - | MS, RL |
| 41. cis-Carveol | 1225 1220 - | tr | - | 0.46 | - | MS, RL |
| 42. β-Citronellol | 1230 1230 - | - | - | - | 0.05 | - | MS, RL |
| 43. trans-Carveol | 1234 1229 - | - | - | - | 0.04 | - | MS, RL |
| 44. Thymol methyl ether | 1237 1237 - | 0.19 | 0.23 | - | 0.03 | - | MS, RL |
| 45. D-Carvone | 1249 1249 - | 1.68 | - | tr | - | - | MS, RL |
Table 1. Cont.

| Compound | RI Content, (%) | Identification Methods |
|----------|----------------|------------------------|
|          | Cal. Rep. Fc Fk Fp Fs Ft Fv |                      |
| 46. Nerol | 1252 1251 - tr - - tr - | MS, RL                  |
| 47. Bornyl acetate | 1290 1290 - 0.49 0.39 - 0.31 - | MS, RL                  |
| 48. (-)-trans-Pinocarvyl acetate | 1305 297 - 1.19 - - - | MS, RL                  |
| 49. Carvacrol | 1306 1306 - tr - - - | MS, RL                  |
| 50. α-Cubebene | 1353 1353 - - - - 0.16 | MS, RL                  |
| 51. D-longsolene | 1370 1370 - tr - - - | MS, RL                  |
| 52. α-Copaene | 1380 1380 - 0.38 0.41 - 0.18 0.39 | MS, RL                  |
| 53. β-Curcumene | 1386 1388 - 4.81 - - - | MS, RL                  |
| 54. β-Bourbonene | 1390 1390 - - - tr - | MS, RL                  |
| 55. β-Elemene | 1395 1395 - - - - 0.24 tr | MS, RL                  |
| 56. Jasnone | 1403 1399 - tr - - - | MS, RL                  |
| 57. β-Caryophyllene | 1425 1425 10.88 - 0.91 - 0.08 tr | MS, RL, AU               |
| 58. τ-Elemene | 1438 1438 - 0.12 - - | MS, RL                  |
| 59. Patchoulen | 1440 1440 tr - 0.38 - | MS, RL                  |
| 60. Alloaromadendrene | 1447 1442 - - - - 0.63 | MS, RL                  |
| 61. Geranyl acetone | 1456 1455 - 4.48 0.58 - | MS, RL                  |
| 62. α-Humulen | 1461 1461 2.98 tr - 0.04 | MS, RL                  |
| 63. τ-Murolene | 1467 1467 0.27 - - 0.21 0.87 | MS, RL                  |
| 64. α-Curcumene | 1487 1486 - tr - - | MS, RL                  |
| 65. Germacrene D | 1489 1489 - - - - 0.13 | MS, RL                  |
| 66. β-βudesmol | 1495 1495 - - - - 0.20 | MS, RL                  |
| 67. β-Guaiene | 1503 1500 0.65 - tr - 0.38 tr | MS, RL                  |
| 68. α-Murolene | 1508 1508 - 0.35 - 0.74 | MS, RL                  |
| 69. Cypene | 1514 1513 3.09 0.23 - | MS, RL                  |
| 70. α-Seinene | 1514 1517 tr - - 0.07 0.34 | MS, RL                  |
| 71. τ-Cadinene | 1523 1521 - - 0.75 0.50 | MS, RL                  |
| 72. β-Cadinene | 1524 1529 tr - - - | MS, RL                  |
| 73. δ-Cadinene | 1531 1531 1.37 tr - 3.07 | MS, RL                  |
| 74. Elemol | 1557 1577 - - - - tr | MS, RL                  |
| 75. Nerolidol | 1566 1564 1.70 - - 1.74 | MS, RL                  |
| 76. Germacrene B | 1572 1569 - - - - 1.07 | MS, RL                  |
| 77. Germacrene D-4-ol | 1585 1583 - - - - 0.75 | MS, RL                  |
| 78. Spathulenol | 1587 1587 - 5.34 - 0.38 0.65 | MS, RL                  |
| 79. Globulol | 1590 1590 1.06 3.25 - 0.18 | MS, RL                  |
| 80. Caryophyllene oxide | 1594 1594 13.23 tr 5.69 - 2.14 | MS, RL, AU               |
| 81. Guaiol | 1602 1602 - - - 0.53 | MS, RL                  |
| 82. Cubenol | 1606 1605 - tr - - 1.13 | MS, RL                  |
| 83. β-βudesmol | 1612 1613 - - - - 0.20 | MS, RL                  |
| 84. τ-βudesmol | 1631 1631 - - 0.66 | MS, RL                  |
| 85. δ-Cadinol | 1661 1656 2.82 - - 3.49 1.02 | MS, RL                  |
| 86. τ-Murolol | 1665 1664 4.64 - - - | MS, RL                  |
| 87. α-βudesmol | 1666 1662 - 2.54 tr - | MS, RL                  |
| 88. δ-Cadinol | 1669 1669 - - - - 4.14 | MS, RL                  |
| 89. Cadinol | 1682 1688 2.17 - - - | MS, RL                  |
| 90. Cadinol | 1692 1692 - - - - 0.56 | MS, RL                  |
| 91. Farnesol | 1726 1725 0.82 - - - - 1.13 | MS, RL                  |
| 92. Hexadecanol | 1817 1819 - - - - 1.16 | MS, RL                  |
| 93. Hexadecanol | 1817 1819 - - - - 1.16 | MS, RL                  |
| 94. Hexahydrofarnesyl acetone | 1845 1845 - tr - - | MS, RL                  |
| 95. Palmitic acid | 1977 1975 - - - 39.03 - | MS, RL, AU               |
| 96. trans-9-Octadec-1-olio | 2068 2068 1.31 - - - | MS, RL                  |
| 97. Heptadecanoic acid ethyl ester | 2080 2082 1.06 - - - | MS, RL                  |
| 98. trans-Phytol | 2120 2122 2.69 - 0.42 - - | MS, RL                  |
| 99. Docosane | 2200 2200 - - 0.60 1.47 - | MS, RL                  |
| 100. Tricosane | 2301 2300 - 0.34 tr - 2.66 | MS, RL                  |
| 101. Tetracosane | 2395 2400 - 0.51 4.49 - | MS, RL                  |
| 102. 10,13-13-ol Docosadienoic acid methyl ester | 2449 2449 15.2 - - - - 69.61 | MS, RL                  |
| 103. Pentacosane | 2498 2500 0.66 - 0.95 6.26 - | MS, RL                  |
| 104. Heptacosane | 2548 2600 0.63 - 1.10 8.99 3.23 | MS, RL                  |
| 105. Heptacosane | 2697 2700 1.26 - 1.35 10.27 - | MS, RL                  |
| 106. Octacosane | 2790 2800 0.77 - 1.13 9.60 - tr | MS, RL                  |

**Monoterpene hydrocarbons**

|                      | 24.9 42.91 26.27 13.40 61.95 - |
|----------------------|---------------------------------|
| Oxygenated monoterpene | tr 34.82 35.60 - 5.69 0.49 |
| Sesquiterpene hydrocarbons | 18.97 5.69 2.05 tr 6.37 3.80 |
| Oxygenated sesquiterpene | 24.59 10.9 15.52 tr 18.49 5.35 |
| Others               | 23.64 2.11 7.87 82.55 0.40 79.84 |
| Total                | 92.10 96.43 87.31 95.95 92.90 89.48 |

Compounds were identified based on a comparison of the compounds' mass spectral data and retention indices with those of the NIST Mass Spectral Library (December 2011), the Wiley Registry of Mass Spectral Data, 8th edition and by comparison with the authentic standard (AU). The content (%) was calculated using the normalization method based on the GC-FID data generated from the average of three independent chromatographic runs.
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Figure 1. GC-MS chromatograms of F. caratavica (A), F. kuchistanica (B) and F. pseudoreoselinum (C).

Figure 2. GC-MS chromatograms of F. samarcandica (A), F. tenuisecta (B) and F. varia (C).

| Compound | identifier | RI | Content (%) | Identification Methods |
|----------|------------|----|-------------|------------------------|
| Docosane 2200 | 0.60 | 1.47 | | |
| Tricosane 2301 | 0.34 | 2.66 | | |
| Tetracosane 2395 | 0.51 | 4.49 | | |
| Docosadienoic acid methyl ester | 2449 | 15.2 | | |
| Pentacosane 2498 | 0.66 | 0.95 | 6.26 | MS, RI, |
| Hexacosane 2598 | 0.63 | 1.10 | 0.89 | MS, RI, |
| Heptacosane 2697 | 1.26 | 1.35 | 10.27 | MS, RI, |
| Octacosane 2790 | 0.77 | 1.13 | 9.60 | MS, RI, |
| Monoterpene hydrocarbons | 24.90 | 42.91 | 26.27 | MS, RI, |
| Oxygenated monoterpene | 34.82 | 35.60 | 0.00 | MS, RI, |
| Sesquiterpene hydrocarbons | 18.97 | 5.69 | 2.05 | MS, RI, |
| Oxygenated sesquiterpene | 24.59 | 10.90 | 15.52 | MS, RI, |
| Others | 23.64 | 2.11 | 7.87 | MS, RI, |
| Total | 92.10 | 0.00 | 96.43 | MS, RI, |
2.2. Chemometric Analysis

It is extremely difficult to identify the qualitative and quantitative differences between the *Ferula* species under evaluation with the naked eye. So, the data obtained from GC analyses were subjected to unsupervised pattern recognition chemometric analysis utilizing PCA to improve the visualization of these differences. The results of the PCA, as represented by the obtained score plot shown in Figure 4A effectively discriminated the six *Ferula* species into five clusters along the first component (PC1) and the second component (PC2) that account for 57% and 30%, respectively, or 87% of the total variance. From the obtained results, it is obvious that both *Fk* and *Ft* are very closely related to each other as they are gathered together in one cluster in the lower left quadrant. However, PC1 successfully discriminated between *Fk* and *Ft* with negative values of PC1 as they are located in the lower left quadrant and *Fc* and *Fv*, which show positive values of PC1 are located in the lower right quadrant. Meanwhile, PC2 significantly discriminated between *Fk* and *Ft*, which show negative values of PC2 as they are located in the lower left quadrant and between *Fs* and *Fp*, which show positive values of PC2 and are located in the upper left quadrant. Furthermore, both PC1 and PC2 significantly discriminated between *Fc* and *Fv*, which show positive values for PC1 and negative values for PC2 as they are located in the lower right quadrant and between *Fs* and *Fp*, displaying negative values for PC1 and positive values for PC2 as they are located in the upper left quadrant. The major discriminatory signals are α-pinene, 10,13-docosadienoic acid methyl ester and palmitic acid as revealed in the loading plot shown in Figure 4B.

The Pearson correlation coefficient (r) between the essential oil contents of different studied samples indicated that *Fc* had a highly significant positive correlation with *Ft* (r = 0.71), *Fk* (r = 0.58), *Fv* (r = 0.47) and *Fp* (r = 0.35), while a non-significant negative correlation was observed between *Fc* and *Fs* (the highest correlations were observed between *Ft* and *Fk* (r = 0.89, p < 0.001), between *Fc* and *Ft* (r = 0.71, p < 0.001), and between *Fc* and *Fk* (r = 0.58, p < 0.001) as seen in Table 2. These data indicate that three samples, *Ft*, *Fk*, and *Fc* have highly similar essential oil content.
2.2. Chemometric Analysis

It is extremely difficult to identify the qualitative and quantitative differences between the essential oil contents of different samples. In the qualitative and quantitative analyses using principal component analysis (PCA), the major discriminatory signals are $\alpha$-pinene (4), palmitic acid (95) and 10,13-docosadienoic acid methyl ester (102).

**Table 2.** The Pearson correlation matrix of the essential oils content of different samples.

|     | $F_c$  | $F_k$  | $F_p$  | $F_s$  | $F_t$  | $F_v$  |
|-----|--------|--------|--------|--------|--------|--------|
| $F_c$ | -      | 0.58***| 0.35***| -0.02  | 0.71***| 0.47***|
| $F_k$ | 0.58***| -      | 0.43***| -0.02  | 0.89***| -0.03  |
| $F_p$ | 0.35***| 0.43***| -      | 0.05   | 0.45***| -0.03  |
| $F_s$ | -0.02  | -0.02  | 0.05   | -      | -0.002 | -0.02  |
| $F_t$ | 0.71***| 0.89***| 0.45***| -0.002 | -      | -0.03  |
| $F_v$ | 0.47***| -0.03  | -0.03  | -0.02  | -0.03  | -      |

The data is represented as the $r$ value of the correlation coefficient and *** is the level of significance, $p < 0.001$.

2.3. Biological Evaluation

2.3.1. Antioxidant Potential of Different Ferula Species

The antioxidant potential of the different essential oil samples was performed in vitro using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the cupric ion reducing antioxidant capacity (CUPRAC), The ferric reducing antioxidant power (FRAP) and the phosphomolybdenum...
method (PM) assays. The results displayed in Table 3 reveal that most of the samples showed considerable antioxidant potential in the performed assays. Fc (41.36 mgTE/g oil) exhibited the most antioxidant activity in ABTS assays, followed by Fk (29.12 mgTE/g oil) and Ft (28.03 mgTE/g oil). However, in CUPRAC assay, Fp (289.45 mgTE/g oil) showed the most superior antioxidant potential followed by Ft (278.87 mgTE/g oil) and Fk (120.43 mgTE/g oil). Furthermore, Ft exhibited the most significant antioxidant power in both FRAP and PM assays with antioxidant activity equivalent to 136.81 mgTE/g oil and 76.66 mmolTE/g oil, respectively, followed by Fp, which showed antioxidant potential of 121.64 mgTE/g oil and 50.86 mmolTE/g oil in FRAP and PM assays, respectively. Thus, it can be concluded that the essential oil from both Ft and Fp exhibited the most notable antioxidant properties as evidenced by their pronounced activities in most of the performed antioxidant assays, followed by Fc. α-Pinene, the predominant compound in Ft and Fp has previously been shown to possess notable antioxidant activity [15]. Additionally, the significant antioxidant activity found in this study, which can be interpreted as a result of the synergistic action between the different components that exist in the oils, was in accordance with that previously reported for many other Ferula species such as F. microcolea, F. orantalis and F. communis. Various mechanisms can be used to interpret antioxidant potential including the prohibition of chain initiation, peroxide decomposition, obstruction of continual hydrogen removal as well as the scavenging of free radical and uniting transition metal ion catalysts [3,16,17]. Additionally, α-pinene, the main constituent in both Ft and Fp, has previously been shown to be a potent antioxidant in both DPPH and FRAP assays, displaying EC50 values equal to 310 and 238 μg/mL, respectively [18].

Table 3. Antioxidant activities of the essential oil samples of Ferula species using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the ferric ion reducing antioxidant capacity (CUPRAC), The ferric reducing antioxidant power (FRAP) and the phosphomolybdenum method (PM) assays.

| Samples          | ABTS (mgTE/g Oil) | CUPRAC (mgTE/g Oil) | FRAP (mgTE/g Oil) | PM (mmolTE/g Oil) |
|------------------|-------------------|---------------------|-------------------|-------------------|
| *F. caratavica*  (Fc)  | 41.36 ± 1.27 a     | 83.54 ± 3.13 c      | 47.34 ± 0.65 e    | 5.59 ± 0.01 f     |
| *F. kuchistania* (Fk)  | 29.12 ± 0.85 b    | 120.43 ± 9.36 b     | 80.74 ± 0.25 c    | 36.42 ± 0.07 c    |
| *F. pseudoreoselinum* (Fp) | 22.66 ± 1.03 c   | 289.45 ± 7.30 a     | 121.64 ± 0.01 b   | 50.86 ± 0.07 b    |
| *F. samarcandica* (Fs)  | 11.84 ± 1.37 d    | 74.39 ± 4.73 c,d    | 43.21 ± 0.48 f    | 14.37 ± 0.04 e    |
| *F. tenuisecta* (Ft)  | 28.03 ± 3.89 b    | 278.87 ± 8.53 a     | 136.81 ± 1.98 a   | 78.66 ± 0.15 a    |
| *F. cura* (Fv)        | 7.04 ± 0.47 e     | 65.90 ± 1.66 d      | 55.00 ± 0.18 d    | 15.33 ± 0.07 d    |

Values are reported as mean ± S.D of three parallel measurements. TE: Trolox equivalents. Different superscripts (a–f) indicate significant differences in the tested Ferula species (p < 0.05).

2.3.2. Tyrosinase and α-Amylase Inhibitory Potential

Tyrosinase enzyme is an oxidase enzyme containing copper that assists in the completion of the first two steps of mammalian melanogenesis, which leads to undesirable hyperpigmentation. Thus, the search for effective tyrosinase inhibitors has recently become vital so that they can be incorporated in cosmetics for effective skin whitening and to counteract hyperpigmentation [19]. Fk showed the most effective tyrosinase inhibitory potential, which was estimated as 119.67 mgKAE/g oil followed by Fv, which showed an inhibitory potential equivalent to 118.42 mgKAE/g oil, where KAE is a Kojic acid equivalent, a potent tyrosinase inhibitory drug. Fv oil is rich in 10,13 docosadienoic acid methyl ester, a polyunsaturated fatty acid, which greatly accounts for its promise as a tyrosinase inhibitor [20]. The underlying tyrosinase inhibitory mechanism mainly relies on the essential oils being rich in components that possess a hydrophobic portion that competitively inhibits the active sites of tyrosinase enzyme with subsequent interference of melanin synthesis. This inhibition may be achieved via interaction with Cu+2 that exists in the active sites of tyrosinase in addition to the prohibition of tautomerization to dopachrome triggered by the oil, which behaves as a reducing agent and blocks of the oxidation reaction during the formation of melanin intermediates during the conversion of tyrosinase/DOPA into melanin, thus reducing skin pigmentation [21].
The α-amylase enzyme is critical in assisting in the catalysis of the first steps in the conversion of starch into maltose, and subsequently to glucose [22,23]. Nowadays, α-amylase inhibitors are used in therapeutic approaches to counteract hyperglycemia. Fp and Fv exhibited the most potent α-amylase inhibitory potential as evidenced by their pronounced inhibitory activity, which was equivalent to 2.61 and 1.40 mmol ACAE/g oil, respectively, in which ACAE is the acarbose equivalent, a potent α-amylase inhibitor (Figure 5). 4-Terpinol as well as α-pinene, which predominate the essential oil of Fp, were previously reported to possess considerable α-amylase inhibitory activity [24]. Similarly, the potent α-amylase inhibitory potential is mainly due to the synergistic action between the different components, which is in accordance to different previously reported studies that confirmed the α-amylase inhibitory effect of different terpenes and different Ferula species such as F. gummosa essential oil [24,25].

![Figure 5. In vitro tyrosinase inhibition (A) and α-amylase inhibition (B) of the essential oil of different Ferula species, F. caratavica (Fc), F. kuchistanica (Fk), F. pseudoreoselinum (Fp), F. samarcandica (Fs), F. tenuisecta (Ft) and F. varia (Fv). Different letters (a–f) indicate significant differences in the tested Ferula species (p < 0.05).](image_url)

3. Materials and Methods

3.1. Plant Material

Aerial parts (flowers, leaves and stems) of F. caratavica Regel & Schmalh. (N2004), F. pseudoreoselinum (Regel & Schmalh.) Koso-Pol., p.p. (N1489), F. tenuisecta Korovin (N1488) were collected from the Tashkent region of Uzbekistan. F. varia (Schrenk ex Fisch., C.A.Mey. & Avé-Lall.) Trautv. (N1407) was collected from the Bukhara region (Uzbekistan), while F. kuchistanica Korovin (N1425) and F. samarcandica Korovin (N1919) were collected from the Samarkand region of Uzbekistan. The plants were collected during the flowering stage in June–July 2018. Their taxonomic authentication was accomplished by Dr. A. Nigmatullaev at the Institute of the Chemistry of Plant Substances (Tashkent, Uzbekistan).

3.2. Preparation of Essential Oil Samples

All the plant materials were air-dried in the shade for 7 days at room temperature and powdered using a mortar and pestle to get particles of a uniform, reduced size. Preparation of the essential oil samples was achieved by hydrodistillation of the air-dried aerial parts of the different Ferula species, F. caratavica (Fc), F. kuchistanica (Fk), F. pseudoreoselinum (Fp), F. samarcandica (Fs), F. tenuisecta (Ft) and F. varia (Fv) for 2 h by Clevenger-type apparatus. Anhydrous Na₂SO₄ was used to dehydrate the prepared essential oils, yielding 0.4, 0.7, 0.3, 0.3, 0.8 and 0.5 % v/w of dry weight for Fc, Fk, Fp, Fs, Ft and Fv, respectively. Then the various oil samples were maintained at −30 °C in dark-colored stoppered glasses until their analyses [26,27].
3.3. GC-FID and GC-MS Analyses

A Shimadzu GC-17A gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an FID detector and DB-5 fused-bonded cap column (Phenomenex; 29 m × 0.25 mm i.d., film thickness 0.25 μm; Torrance, California, USA) was utilized for the semi-quantitative determination of the different components of the essential oils using the normalization method to get the relative percentage of each component and applying GC-FID data that is highly sensitive using GC solution® software ver. 2.4 (Shimadzu Corporation, Kyoto, Japan). The areas under the peaks (AUP) were determined using three independent runs where the total area is considered as 100%. Meanwhile, the Shimadzu GC-2010 plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) supplied with Rtx-5MS (Restek, Bellefonte, PA, USA) in addition to a quadrupole mass spectrometer was used for the identification of the essential oil different metabolites. Instrument settings were adjusted according to what was previously reported [28,29]. The Wiley Registry of Mass Spectral Data 8th edition, NIST MassSpectral Library (December 2011), and previously reported data were employed to confirm the identity of the compounds and the retention indexes were calculated to corroborate the identification of the volatile compounds [30,31].

3.4. Chemometric and ANOVA Analysis

To examine the differences between the essential oils’ components prepared from different Ferula species, the data collected from the different GC-MS spectra were subjected to chemometric analysis of unsupervised pattern recognition represented by PCA, which was processed by employing Unscrambler 9.7 (CAMO SA, Oslo, Norway) [28,32]. Meanwhile, other statistical analyses used for biological assessment were performed using ANOVA assay (with Tukey’s test, significant value: p < 0.05) and Xlstat 2017 software.

3.5. Biological Evaluation

3.5.1. Determination of the Antioxidant Potential

The antioxidant activity of the different essential oil samples from different Ferula species was evaluated using ABTS, CUPRAC, FRAP and PM assays. These assays were performed following the methods described by Mamadalieva et al. [33]. The antioxidant activities were reported as Trolox equivalents and the samples were analyzed in triplicate.

3.5.2. Determination of Enzyme Inhibitory Effects

The possible inhibitory potential of the essential oil samples was investigated against tyrosinase and α-amylase enzymes using standard in vitro bioassays as previously reported by Mamadalieva et al. [33] in which all the samples were analyzed in triplicate. Results are expressed in mgKAE/g oil for tyrosinase inhibitory activity and in mmol ACAE/g oil for α-amylase inhibition.

4. Conclusions

The essential oils obtained from different Ferula species, F. caratavica, F. kuchistanica, F. pseudoreoselinum, F. samarcardica, F. tenuisecta and F. varia showed significant variation as revealed by GC analyses. Furthermore, this variation became more clearly observable when coupled with a chemometric approach as represented by PCA used as an unsupervised pattern recognition technique. Additionally, the obtained essential oils showed notable antioxidant as well as tyrosinase and α-amylase inhibitory activities with variable degrees, which is mainly related to the differences in the secondary metabolites that predominate in the oils. Thus, it was concluded that the different Ferula species could serve as a promising natural antioxidant drug that could be included in different products and used as spices to alleviate hyperglycemia and as a natural ingredient in pharmaceutical cosmetics to counteract hyperpigmentation. Chemometric study based on gathering the different biological activities of many
additional *Ferula* species will be considered. It is recommended that further in vivo studies such as animal and bioavailability studies be carried out to confirm the obtained results.

**Author Contributions:** F.S.Y., identification of the essential oil compounds, chemometric analysis, writing the whole manuscript; M.A.M., N.Z.M., S.F.A., collection of the plants, isolation of the essential oil samples and revising the manuscript; G.Z., performing the biological studies; E.A. and M.L.A., supervising the study and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

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**References**

1. Rehman, R.; Hanif, M.A.; Mushtaq, Z.; Al-Sadi, A.M. Biosynthesis of essential oils in aromatic plants: A review. *Food Rev. Int.* 2016, 32, 117–160. [CrossRef]
2. Sharifi-Rad, J.; Sureda, A.; Tenore, G.C.; Daglia, M.; Sharifi-Rad, M.; Valussi, M.; Tundis, R.; Sharifi-Rad, M.; Loizzo, M.R.; Ademiluyi, A.O. Biological activities of essential oils: From plant chemoecology to traditional healing systems. *Molecules* 2017, 22, 70. [CrossRef] [PubMed]
3. Sahebkar, A. Biological activities of essential oils from the genus *Ferula* (Apiaceae). *Asian Biomed. Res. Rev.* News. 2010, 4, 835–847. [CrossRef]
4. Burits, M.; Bucar, F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res.* 2000, 14, 323–328. [CrossRef]
5. Koleva, I.I.; Van Beek, T.A.; Linssen, J.P.; Groot, A.d.; Evstatieva, L.N. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem. Anal.* 2002, 13, 8–17. [CrossRef]
6. Lee, S.E.; Hwang, H.J.; Ha, J.-S.; Jeong, H.-S.; Kim, J.H. Screening of medicinal plant extracts for antioxidant activity. *Life Sci.* 2003, 73, 167–179. [CrossRef]
7. Moniruzzaman; Shahinuzzaman; Haque, A.; Khatun, R.; Yaakob, Z. Gas chromatography mass spectrometry analysis and in vitro antibacterial activity of essential oil from *Trigonella foenum-graecum*. *Asian Pac. J. Trop. Biomed.* 2015, 5, 1033–1036. [CrossRef]
8. Rashid, S.; Rather, M.A.; Shah, W.A.; Bhat, B.A. Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica* Willd. *Food Chem.* 2013, 138, 693–700. [CrossRef]
9. Adorjan, B.; Buchbauer, G. Biological properties of essential oils: An updated review. *Flav. Frag. J.* 2010, 25, 407–426. [CrossRef]
10. Sun, J.; Wang, X.; Wang, P.; Li, L.; Qu, W.; Liang, J. Antimicrobial, antioxidant and cytotoxic properties of essential oil from *Dictamnus angustifolius*. *J. Ethnopharmacol.* 2015, 159, 296–300. [CrossRef]
11. Martins, C.D.M.; Nascimento, E.A.D.; de Morais, S.A.; de Oliveira, A.; Chang, R.; Cunha, L.; Martins, M.M.; Martins, C.H.G.; Moraes, T.D.S.; Rodrigues, P.V. Chemical constituents and evaluation of antimicrobial and cytotoxic activities of *Kielmeyera coriacea* Mart. & Zucc. essential oils. *Evid.-Based Complement. Altern. Med.* 2015, 2015, 842047.
12. Safari, O.; Sarkheil, M.; Paolucci, M. Dietary administration of *Ferula* (*Ferula asafoetida*) powder as a feed additive in diet of koi carp, *Cyprinus carpio* koi: Effects on hematological-immunological parameters, mucosal antibacterial activity, digestive enzymes, and growth performance. *Fish. Physiol. Biochem.* 2019, 45, 1277–1288. [CrossRef] [PubMed]
13. Mohammadhosseini, M.; Venditti, A.; Sarker, S.D.; Nahar, L.; Akbarzadeh, A. The genus *Ferula*: Ethnobotany, phytochemistry and bioactivities—A review. *Ind. Crops Prod.* 2019, 129, 350–394. [CrossRef]
14. Iranshahy, M.; Iranshahi, M. Traditional uses, phytochemistry and pharmacology of *Asafoetida* (*Ferula asafoetida* oleo-gum-resin)—A review. *J. Ethnopharmacol.* 2011, 134, 1–10. [CrossRef] [PubMed]
15. Him, A.; Ozbek, H.; Turel, I.; Oner, A.C. Antinociceptive activity of alpha-pinene and fenchone. *Pharmacologyonline* 2008, 3, 363–369.
16. Amiri, H. Chemical composition and antioxidant activity of essential oil and methanolic extracts of *Ferula microcolea* (Boiss.) Boiss (Apiaceae). *Int. J. Food Prop.* 2014, 17, 722–730. [CrossRef]
17. Nguir, A.; Mabrouk, H.; Douki, W.; Ismail, M.B.; Jannet, H.B.; Flamini, G. Chemical composition and bioactivities of the essential oil from different organs of *Ferula communis* L. growing in Tunisia. *Med. Chem. Res.* 2016, 25, 515–525. [CrossRef]

18. Bouzenna, H.; Hfaiedh, N.; Giroux-Metges, M.-A.; Elfeki, A.; Talarmin, H. Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells. *Biomed. Pharmacother.* 2017, 93, 961–968. [CrossRef]

19. Chang, T.-S. An updated review of tyrosinase inhibitors. *Int. J. Mol. Sci.* 2009, 10, 2440–2475. [CrossRef]

20. Guo, Y.-J.; Pan, Z.-Z.; Chen, C.-Q.; Hu, Y.-H.; Liu, Y.-H.; Shi, Y.; Yan, J.-H.; Chen, Q.-X. Inhibitory effects of fatty acids on the activity of mushroom tyrosinase. *Appl. Biochem Biotechnol.* 2010, 162, 1564–1573. [CrossRef]

21. Taherkhani, M. Chemical constituents, total phenolic content, antimicrobial, antioxidant and radical scavenging properties, chelating ability, tyrosinase inhibition and in vitro cytotoxic effects of *Artemisia aucheri* herbs. *Pharm. Chem. J.* 2017, 50, 736–745. [CrossRef]

22. Youssef, F.S.; Ashour, M.L.; Ebada, S.S.; Sobeh, M.; El-Beshbishy, H.A.; Singab, A.N.; Wink, M. Antihyperglycaemic activity of the methanol extract from leaves of *Eremophila maculata* (Scrophulariaceae) in streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol.* 2017, 69, 733–742. [CrossRef] [PubMed]

23. Thabet, A.A.; Youssef, F.S.; El-Shazly, M.; El-Beshbishy, H.A.; Singab, A.N.B. Validation of the antihyperglycaemic and hepatoprotective activity of the flavonoid rich fraction of *Brachychiton rupestris* using in vivo experimental models and molecular modelling. *Food Chem. Toxicol.* 2018, 114, 302–310. [CrossRef] [PubMed]

24. Jelenkovic, L.; Jovanovic, V.S.; Palic, I.; Mitic, V.; Radulovic, M. In vitro screening of α-amylase inhibition by selected terpenes from essential oils. *Trop. J. Pharm. Res.* 2014, 13, 1421–1428. [CrossRef]

25. Heydari-Majd, M.; Rezaeinia, H.; Shadan, M.R.; Ghorani, B.; Tucker, N. Enrichment of zein nanofibre with chemometrics analysis. *Authentication and discrimination of green tea samples using UV–vis, FTIR and HPLC techniques coupled with chemometrics analysis.* J. Pharm. Biomed. Anal. 2019, 164, 653–658. [CrossRef] [PubMed]

26. Mamadalieva, N.Z.; Youssef, F.S.; Ashour, M.L.; Akramov, D.K.; Sasmakov, S.A.; Ramazonov, N.S.; Azimova, S.S. A comparative study on chemical composition and antimicrobial activity of essential oils from three *Phlomis* species from Uzbekistan. *Nat. Prod. Res.* 2019, 1–6. [CrossRef]

27. Youssef, F.S.; Hamoud, R.; Ashour, M.L.; Singab, A.N.; Wink, M. Volatile oils from the aerial parts of *Eremophila maculata* and their antimicrobial activity. *Chem. Biodivers.* 2014, 11, 831–841. [CrossRef]

28. Thabet, A.A.; Youssef, F.S.; El-Shazly, M.; Singab, A.N.B. GC-MS and GC-FID analyses of the volatile oil and validation of its anti-inflammatory activity using molecular modelling and bleomycin-induced inflammation in *albino* mice. *Molecules* 2017, 22, 1384. [CrossRef]

29. Mamadalieva, N.Z.; Youssef, F.S.; Ashour, M.L.; Sasmakov, S.A.; Tiezzi, A.; Azimova, S.S. Chemical composition, antimicrobial and antioxidant activities of the essential oils of three Uzbek Lamiaceae species. *Nat. Prod. Res.* 2019, 33, 2394–2397. [CrossRef]

30. Ayoub, I.M.; Youssef, F.S.; El-Shazly, M.; Ashour, M.L.; Singab, A.N.B.; Wink, M. Volatile constituents of *Dietes bicolor* (Iridaceae) and their antimicrobial activity. *Z. Naturforsch. C* 2015, 70, 217–225. [CrossRef] [PubMed]

31. Aboulwafa, M.M.; Youssef, F.S.; Gad, H.A.; Sarker, S.D.; Nahar, L.; Al-Azizi, M.M.; Ashour, M.L. Authentication and discrimination of green tea samples using UV–vis, FTIR and HPLC techniques coupled with chemometrics analysis. *J. Pharm. Biomed. Anal.* 2019, 164, 653–658. [CrossRef] [PubMed]

32. Mamadalieva, N.Z.; Böhmdorfer, S.; Zengin, G.; Bacher, M.; Potthast, A.; Akramov, D.K.; Janibekov, A.; Rosenau, T. Phytochemical and biological activities of *Silene viridiflora* extractives. Development and validation of a HPTLC method for quantification of 20-hydroxyecdysone. *Ind. Crop. Prod.* 2019, 129, 542–548. [CrossRef]

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