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Fatty acid profiles in the rhizome essential oil of *Iris germanica* cultivated in Kuna, Idaho

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Abstract
The rhizome essential oil of *Iris germanica*, known as oil of orris or orris butter, is an important commodity in the perfume industry. In this work, nine samples of *Iris germanica* rhizomes were obtained by hydrodistillation and analyzed by gas chromatography. The essential oils were dominated by fatty acids, particularly caprylic acid (4.1-29.3%), capric acid (13.3-25.4%), lauric acid (19.3-64.5%), and myristic acid (8.4-54.2%). However, the important perfume components, the irones, were negligible (0.0-0.1%).

Keywords: Orris butter, caprylic acid, capric acid, lauric acid, myristic acid

1. Introduction
There are around 330 species of *Iris* Tourn. ex L. distributed throughout the world [1]. *Iris* × *germanica* L., the "bearded iris" is a natural hybrid between *Iris pallida* Lam. and *Iris variegata* L. [2]. *Iris germanica* is thought to have originated in the Mediterranean region, but has become naturalized across Europe, North Africa, North and South America, Central Asia, and Australia [1]. It is a popular garden plant and there are estimated to be more than 60,000 cultivars [3]. The rhizomes of *I. germanica* and *I. pallida*, known as orris root, are popular as an herbal medicine, a food additive, and for use in perfumery and cosmetics [4,5]. Orris root is the source of oil of orris (orris butter), the essential oil from the dried (at least three years) rhizomes, which is an ingredient in perfumery [5-7]. In this work, we have obtained the fresh rhizome essential oils from nine samples of *I. germanica* cultivated in Kuna, Idaho.

2. Materials and Methods
2.1 Plant Materials
The fresh rhizomes from nine different *I. germanica* plants (Figure 1) were obtained on 29 September 2024 (post flowering) from a garden located in Kuna, Idaho (43°30′49″ N, 116°25′53″ W, 818 m elevation). The rhizomes were washed with water to remove soil and other debris, allowed to air dry at room temperature for three days, chopped, and stored frozen (-20 °C) until processed.

Fig 1: Photographs (taken on 16 May 2025) of *Iris germanica* plants analyzed in this study.

2.2 Hydrodistillation

Each rhizome sample was hydrodistilled for 6 h using a Likens-Nickerson apparatus with continuous extraction of the distillate with dichloromethane. Evaporation of the dichloromethane with a stream of warm air gave the essential oils as pale-yellow liquids that solidified upon cooling. The essential oils were stored under refrigeration (-20 °C) until analyzed. The hydrodistillation details are summarized in Table 1.

Table 1: Details of hydrodistillation of *Iris germanica* rhizomes.

Iris germanica sample	Mass rhizome (g)	Mass essential oil (g)	% Yield
#1	285.62	2.2666	0.794
#2	231.41	1.9452	0.841
#3	252.66	2.2646	0.896
#4	227.31	1.8367	0.808
#5	243.28	2.2086	0.908
#6	217.99	1.6745	0.768
#7	255.84	2.2789	0.891
#8	170.21	2.2044	1.295
#9	235.29	1.9924	0.847

2.3 Gas Chromatographic Analysis

The *I. germanica* rhizome essential oils were analyzed by GC-MS as previously described [8] using a Shimadzu GC-MS-QP2010 Ultra (Shimadzu Scientific Instruments), fitted with a ZB-5ms GC column (60 m × 0.25 mm × 0.25 µm film thickness, Phenomenex). Retention index (RI) values were calculated using the method of van den Dool and Kratz [9]. The components in the essential oils were identified by comparison of both RI values and MS fragmentation patterns with those from the Adams [10], FFNSC 3 [11], NIST20 [12], and Satyal [13] databases.

2.4 Multivariate Analyses

Agglomerative hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The percentages of the most abundant components (α-pinene, caprylic acid, capric acid, lauric acid, myristoleic acid, myristic acid, and palmitic acid) were used for the HCA and PCA. Dissimilarity using Euclidean distance and Ward's method for agglomeration were used for the HCA. The PCA (type Pearson Correlation) was carried out to corroborate the HCA analysis.

3. Results and Discussion

Hydrodistillation of *I. germanica* rhizomes gave pale-yellow essential oils in yields ranging from 0.768% to 1.295%. The rhizome essential oils were analyzed by gas chromatography - mass spectrometry. A total of 70 compounds were identified, which accounted for 99.5-100.0% of the total compositions (Table 2).

Table 2: Chemical compositions of *Iris germanica* rhizome essential oils.

RI_{calc}	RI_{db}	Compounds	#1	#2	#3	#4	#5	#6	#7	#8	#9
787	780	3-Methyl-2-butenal	tr	tr	tr	-	tr	tr	0.2	0.1	0.1
798	801	Hexanal	tr	tr	tr	tr	tr	tr	tr	tr	-
831	831	Furfural	tr	tr	tr	-	tr	tr	0.1	tr	0.1
933	933	α-Pinene	0.5	0.7	0.4	0.3	0.1	0.2	0.3	0.1	0.2
949	950	Camphene	tr	tr	tr	tr	-	tr	-	-	-
969	971	Hexanoic acid (= Caproic acid)	tr	tr	0.1	-	0.1	0.1	tr	-	-
977	978	β-Pinene	tr	tr	tr	tr	-	tr	tr	tr	tr
982	982	6-Methyl-5-hepten-2-one	tr	tr	tr	-	tr	tr	tr	tr	tr
990	990	Dehydro-1,8-cineole	-	tr	tr	tr	-	-	-	-	-
1018	1018	α-Terpinene	-	-	tr	tr	-	tr	-	-	-
1025	1025	*p*-Cymene	tr	tr	tr	tr	tr	tr	tr	tr	tr
1029	1030	Limonene	tr	tr	tr	tr	-	tr	-	tr	-
1032	1031	1,8-Cineole	0.4	0.5	tr	tr	-	tr	tr	tr	-
1043	1045	Phenylacetaldehyde	tr	tr	tr	-	0.1	tr	-	-	-
1052	1052	Bergamal	-	-	-	-	-	-	tr	tr	tr
1058	1058	γ-Terpinene	tr	tr	tr	tr	-	tr	-	-	-
1086	1086	trans-Linalool oxide (furanoid)	-	-	-	tr	tr	-	-	-	-
1090	1091	*p*-Cymenene	tr	tr	tr	-	-	tr	-	-	-
1096	1099	6-Camphenone	tr	tr	0.1	0.1	tr	tr	tr	tr	tr
1101	1101	Linalool	-	-	-	tr	0.1	-	-	-	-
1104	1104	Nonanal	-	-	-	-	tr	tr	tr	-	-
1123	1123	Methyl caprylate	-	-	tr	tr	-	tr	tr	-	-
1147	1145	Camphor	tr	-	-	tr	-	tr	-	-	-
1173	**1171**	**Octanoic acid (= Caprylic acid)**	**1.3**	**4.3**	**17.8**	**14.9**	**12.7**	**29.3**	**8.5**	**4.5**	**4.1**
1180	1180	Terpinen-4-ol	tr	tr	-	-	-	-	tr	tr	tr
1188	1186	*p*-Cymen-8-ol	0.1	0.1	tr	-	tr	-	-	tr	-
1195	1196	Myrtenal	-	-	-	-	-	-	tr	-	tr
1197	1195	α-Terpineol	0.2	0.1	tr	tr	tr	tr	tr	tr	-

1198	1198	Safranal	-	-	-	-	tr	-	tr	tr	tr
1217	1217	Coumaran	-	-	tr	-	0.1	tr	0.1	0.1	0.2
1228	1229	Thymyl methyl ether	0.1	0.1	tr	tr	tr	tr	tr	tr	tr
1283	1282	Bornyl acetate	tr	-	tr	tr	-	-	-	-	-
1290	1289	Thymol	-	-	-	-	-	-	-	-	tr
1298	1300	Carvacrol	tr	tr	-	-	-	-	-	-	tr
1307	1309	4-Vinylguaicol	tr	tr	0.1	tr	0.1	0.1	0.2	0.1	0.2
1322	1322	Methyl decanoate (= Methyl caprate)	tr	tr	tr	-	-	tr	tr	tr	-
1324	1325	Silphiperfol-5-ene	tr	tr	tr	tr	tr	tr	tr	tr	tr
1346	1349	7-*epi*-Silphiperfol-5-ene	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1
1356	1356	Eugenol	-	-	-	0.3	-	-	-	-	-
1373	**1371**	**Decanoic acid (= Capric acid)**	**20.4**	**25.4**	**16.5**	**16.3**	**15.1**	**17.1**	**17.7**	**15.0**	**13.3**
1392	1392	Vanillin	-	-	-	-	-	-	-	tr	tr
1408	1409	Dodecanal	-	-	tr	tr	-	tr	tr	tr	-
1410	1411	Thymohydroquinone dimethyl ether	tr	tr	-	-	-	tr	-	tr	-
1460	1458	Undecanoic acid	0.1	tr	-	-	-	-	-	tr	-
1479	1480	*ar*-Curcumene	-	-	tr	tr	-	-	-	-	-
1516	1518	δ-Cadinene	tr	tr	-	-	-	tr	-	-	-
1521	1527	Methyl dodecanoate	tr	tr	-	-	-	-	-	-	-
1529	1526	α-Irone	0.1	0.1	tr	tr	-	tr	0.1	0.1	0.1
1564	**1560**	**Dodecanoic acid (= Lauric acid)**	**64.5**	**59.9**	**19.3**	**19.8**	**39.6**	**22.1**	**32.1**	**32.4**	**26.2**
1580	1587	Caryophyllene oxide	-	-	-	-	-	tr	0.1	0.1	0.2
1592	1592	Methoxyeugenol	-	-	-	-	-	-	tr	tr	tr
1595	1590	Gleenol	-	-	-	tr	-	tr	tr	-	-
1609	1611	Humulene epoxide II	-	-	-	tr	-	-	tr	tr	tr
1627	1628	1-*epi*-Cubenol	tr	tr	-	-	-	-	-	-	-
1636	1633	γ-Eudesmol	-	tr	-	-	tr	-	tr	-	-
1656	1659	Tridecanoic acid	-	-	-	-	-	-	-	0.1	tr
1659	1655	α-Cadinol	-	tr	-	-	-	tr	-	-	-
1659	1655	α-Eudesmol	-	-	-	-	0.1	-	-	-	-
1686	1688	α-Bisabolol	-	-	tr	-	-	0.1	-	-	-
1720	1723	Methyl myristate	-	-	tr	tr	-	tr	tr	tr	tr
1743	1746	(9*Z*)-Tetradecenoic acid (= Myristoleic acid)	0.2	0.1	0.4	0.7	0.5	0.8	0.4	0.8	0.7
1758	**1758**	**Tetradecanoic acid (= Myristic acid)**	**11.8**	**8.4**	**44.8**	**47.8**	**31.4**	**29.8**	**39.7**	**45.2**	**54.2**
1900	1900	Nonadecane	-	-	-	-	-	-	-	tr	tr
1940	1941	(9*Z*)-Hexadecenoic acid (= Palmitoleic acid)	0.1	tr	0.1	-	tr	tr	0.1	0.1	0.1
1960	1958	Hexadecanoic acid (= Palmitic acid)	0.2	0.2	0.4	-	0.1	0.2	0.3	0.6	0.4
2117	2118	Linoleic acid	-	-	-	-	-	-	-	0.1	tr
2143	2143	Serratol	-	tr	-	-	tr	-	0.1	-	tr
2300	2300	Tricosane	tr	tr	tr	-	tr	tr	tr	tr	tr
2500	2500	Pentacosane	0.1	tr	0.1	-	tr	0.1	tr	0.1	tr
2700	2700	Heptacosane	tr	tr	tr	-	tr	tr	tr	0.1	-
		Monoterpene hydrocarbons	0.5	0.7	0.4	0.3	0.1	0.2	0.3	0.1	0.2
		Oxygenated monoterpenoids	0.8	0.8	0.1	0.1	0.1	tr	tr	tr	tr
		Sesquiterpene hydrocarbons	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1
		Oxygenated sesquiterpenoids	tr	tr	tr	tr	0.1	0.1	0.1	0.1	0.2
		Diterpenoids	0.0	tr	0.0	0.0	tr	tr	0.1	tr	tr
		Benzenoid aromatics	tr	tr	0.1	0.3	0.2	0.1	0.3	0.3	0.3
		Fatty acids and esters	98.4	98.3	99.4	99.4	99.5	99.4	98.8	98.6	98.9
		Alkanes	0.1	tr	0.1	tr	tr	0.1	tr	0.2	tr
		Others	0.1	0.1	tr	tr	tr	tr	0.3	0.2	0.3
		Total identified	99.8	99.9	100.0	100.0	100.0	99.9	100.0	99.5	99.9

RI_{calc} = Retention index determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column. RI_{db} = Retention index from the databases [10-13]. tr = trace (< 0.05%). The major fatty acid components are highlighted in blue bold font.

The *I. germanica* rhizome essential oils were dominated by fatty acids, particularly caprylic acid (4.1-29.3%), capric acid (13.3-25.4%), lauric acid (19.3-64.5%), and myristic acid (8.4-54.2%). There is wide variation in the relative concentrations of these four fatty acid components. In order to visualize the fatty acid distributions in *I. germanica*, an agglomerative hierarchical cluster analysis (HCA) was carried out (Figure 2).

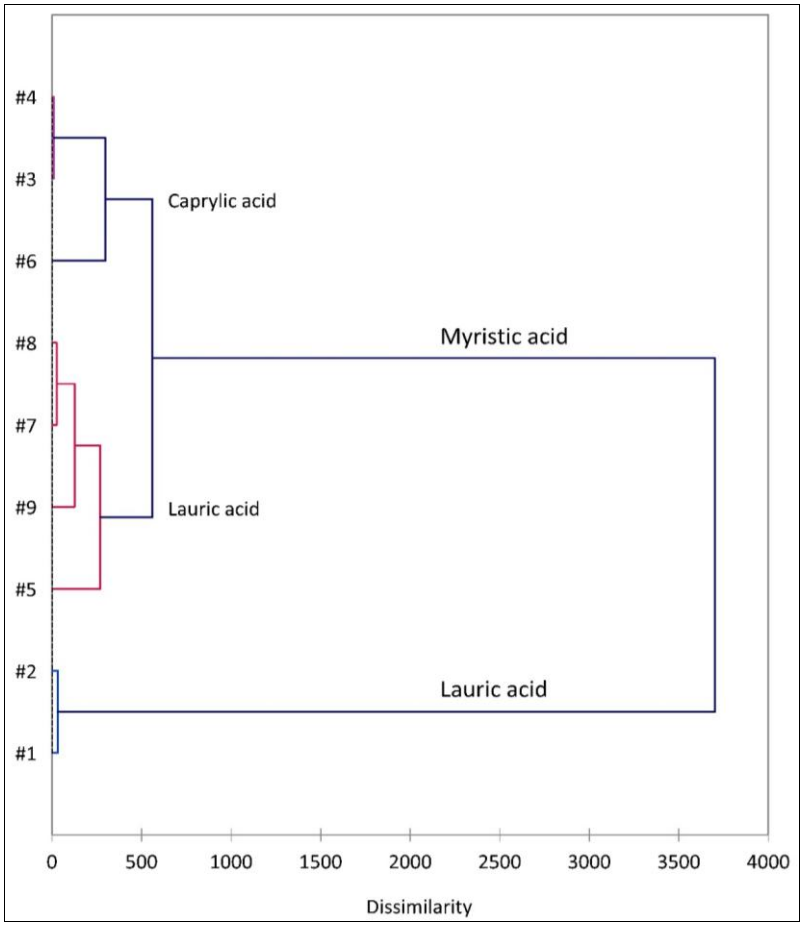

Fig 2: Dendrogram based on agglomerative hierarchical cluster analysis of *Iris germanica* rhizome essential oil compositions.

There are three well-defined clusters from the HCA. One cluster (#1 and #2) is dominated by lauric acid (62.2 ± 3.2%), but with low levels of myristic acid (10.1 ± 2.4%). The other two clusters are dominated by myristic acid (41.8 ± 8.8%). The two clusters are separated based on the relative concentrations of caprylic acid (20.7 ± 7.6% and 7.5 ± 4.0%) and lauric acid (20.4 ± 1.5% and 32.5 ± 5.5%). A principal component analysis corroborates the relationships between the samples and their fatty acid profiles (Figure 3).

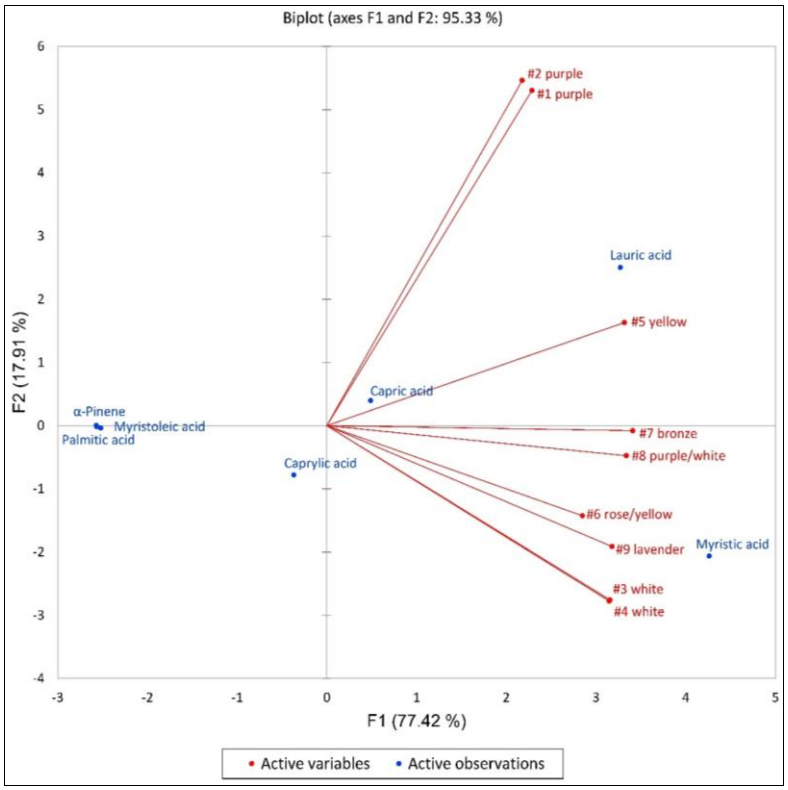

Fig 3: Biplot based on principal component analysis of *Iris germanica* rhizome essential oil compositions.

It is tempting to try to correlate fatty acid profiles with floral colors. Both purple-colored samples (#1 and #2) are tightly correlated and the two white samples (#3 and #4) are correlated. The two yellowish samples (#5 and #7) have similar fatty acid profiles. Samples #8 and #9 have lavender-like flowers, while the flowers of #6 is rose with yellow. Thus, correlating floral colors with rhizome fatty acid profiles is tenuous at best.

Ghasemi and co-workers [5] analyzed the dried (30 days) rhizomes from 33 cultivars of *I. germanica* cultivated in Mahallat, Iran. In contrast to the compositions observed in the samples from Idaho, the samples from Iran were rich in the monoterpenes α-pinene (3.6-40.1%) and γ-terpinene (1.2-23.1%), with caprylic acid not observed in any samples, capric acid and lauric acid in relatively low concentrations (0.0-3.4% and 0.0-13.9%, respectively), but myristic acid in high concentrations (2.4-49.8%). Interestingly, one of the samples from the Iran study showed high levels of α-irone (25.5%) and β-irone (40.0%), which are important constituents with respect to the perfume industry [4,5]. The samples from Idaho showed only low levels of α-irone (0.0-0.1%).

Gürbüzer and Kara carried out a seasonal variation study of *I. germanica* rhizomes from Kuyucak, Türkiye [14]. These authors observed no α-pinene, γ-terpinene, caprylic acid, capric acid, or lauric acid, and only low concentrations of myristic acid (1.1-9.9%) in the fresh rhizome essential oils. However, both α-irone (16.1-27.7%) and γ-irone (23.4-50.8%) were abundant. An *I. germanica* rhizome essential oil sample from Damascus, Syria, showed a fatty acid profile of caprylic acid (0.0%), capric acid (0.1%), lauric acid (5.7%), myristic acid (61.4%), and palmitic acid (4.9%) [15]. Neither α-pinene nor γ-terpinene were observed, but a small amount of α-irone (0.3%) was detected.

4. Conclusion

The essential oils obtained from fresh rhizomes of *I. germanica* were obtained and analyzed by GC-MS revealing high concentrations of fatty acids, but with different relative concentrations. There seems to be little correlation between floral color and fatty acid profiles of the rhizome essential oils. Concentrations of irones were negligible, indicating the importance of prolonged drying of the rhizomes before distillation. Nevertheless, the particular individuals in this study do not appear to be important for future consideration as sources of perfume materials; the irone concentrations in fresh rhizome essential oils from Iran and Türkiye were much higher.

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Conflicts of Interest

The authors declare no conflicts of interest.

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