

Article

The Impact of Geographical Location on the Chemical Compositions of *Pimpinella lutea* Desf. Growing in Tunisia

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Citation: AL-Hmadi, H.; El Mokni, R.; Joshi, R.K.; Ashour, M.L.; Hammami, S. The Impact of Geographical Location on the Chemical Compositions of *Pimpinella lutea* Desf. Growing in Tunisia. *Appl. Sci.* **2021**, *11*, 7739. <https://doi.org/10.3390/app11167739>

Academic Editor: Claudio Medana

Received: 29 July 2021

Accepted: 19 August 2021

Published: 23 August 2021

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Abstract: Essential oils are generally produced to confer the protection of medicinal plants against several natural enemies. Variations of chemical and physical environmental factors exert significant influences on plant development. They hence may affect the quality and quantity of volatile organic metabolites of interest and, therefore, the economic applications of essential oils. This research focused on the effects of the harvest region on the production and analytes present in Tunisian *Pimpinella lutea* Desf. Apiaceae that were collected in three different growing environments (North and South Bizerta and Tabarka). Essential oils extracted from a variety of genotypes were analyzed, for the first time, using gas chromatography and mass spectrometry (GC/FID and GC/MS). The determination of the percentage of essential oil components allowed the recognition of three chemotypes: α -trans-Bergamotene quantified at a percentage of 18.1% in North Bizerta (NBEO), muurola-4,10(14)-dien-1- β -ol identified in South Bizerta (10.1%, SBEO) and acora-3,7(14)-dien present in a high level of 29.1% in Tabarka population (TEO). The richness of different populations in sesquiterpenes (60.2–78.1%) suggests that *Pimpinella lutea* Desf. may be used in different industrial segments.

Keywords: *Pimpinella lutea* (Desf.); essential oils; different locations; chemical composition; gas chromatography-mass spectrometry

1. Introduction

Up to date, approximately 90% of Cancer patients are unresponsive to therapy because of multidrug resistance [1]. Consequently, several researchers have renewed their interest in studying the biological activities of medicinal plant extracts and discovering plant-based remedies, including volatile oils [2]. Essential oils are naturally occurring metabolites of high desirability known for their positive effects and beneficial characteristics. They have a long history in inhalation therapies and dermal application by different cultures globally. Their powerful valuable chemicals possess a profound physiological effect, restoring balance and vitality [3]. The use of essential oils obtained from various aromatic plants generally spread worldwide is considered an essential part of the traditional pharmacopeia [4,5]. Presently, essential oils are increasingly crucial in the food and perfume industry, pesticides, and pharmaceutical industries due to their pleasant aroma, diverse

biological activities, and ease of availability [6–9]. The use of many essential oils as an alternative to synthetic products in destroying bacteria, viruses, and fungi that cause infections, may help avoid toxic side effects in the environment and human health [10,11]. Many factors can affect the qualities of essential oils, including the chemotype, chemical composition, biological properties, and yields. Seasonal changes in geographic, environmental, and climatic conditions are among the most prominent factors [12–14].

The oil production also varies depending on different plant species and the extraction method [15,16]. The method used to produce an essential oil depends on the type of plant material. True essential oils are extracted through various distillation methods (water and steam distillation). However, some volatile constituents are fragile and susceptible to heat, reactive to moisture during extraction, and difficult to liberate from the surrounding plant material. Thus, other new suitable methods of extraction, such as enfleurage and supercritical carbon dioxide, are utilized to save solvent and energy and extract thermolabile phytochemicals [17]. The crop yields, the chemical compositions, and the physicochemical characteristics determine the usefulness of oil in various applications aside from edible uses, such as in foods, cosmetics, and pharmaceuticals [18]. Many studies have proven that the disparity of locations may affect volatile oils composition and efficiency [19]. Many studied medicinal plants were widely recognized in local herbal medicine as foods and safe medical products [20] due to their wide range of pharmacological uses [21]. A great deal of research has been conducted on the chemical composition and the biological activities of numerous species belonging to the Apiaceae family, including common vegetables (e.g., parsnips, carrots, and celery/celeriac) and condiments (e.g., anise, coriander, chervil, caraway, cumin, dill, and parsley). *Pimpinella lutea* Desf. Syn. *Reutera lutea* (Desf.) Maire Figure 1 is known as one of the most excellent flowering Apiaceae plants [22]. It is a perennial plant with fat vertical root; strong principal stem, glabrous, branched at the top, reaching 1.5 m in height, leaves are pubescent, the lower ones numerous, soft, very large, up to 40 cm long, pinnate with ovoid or rounded segments, toothed or incised, the others reduced to the sheath or almost, inflorescences in umbel with 3–6 capillary rays. Involucre and involucre almost absent. Petals are yellow rounded at the base, fruits smooth, oval-elongated with prominent ribs [23].

Previous studies conducted on the investigation of the chemical composition of *Reutera lutea* (Desf.) Maire growing in Algeria led to the isolation of the flavonoid C-glucosides: Isoorientin, isoorientin 6''-O-acetate, and the coumarin: 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin [24]. It has also been demonstrated that the methylene dichloride and butanol extracts are effective corrosion inhibitors for carbon steel in HCl solution [25]. However, the volatile chemical constituents of *Reutera lutea* have not yet been identified. Therefore, this is the first study on the hydrodistilled essential oils from the fragrant *Pimpinella lutea* aerial parts collected from three regions in Tunisia. The main objective is to study its basic properties, the variability in yields and phytochemicals constituting the plant material, and to create awareness and interest in its possible utilization as a source of natural bioactive compounds (Table 1).

Table 1. Characteristics of sampling sites of *Pimpinella lutea* harvested during the flowering-fruiting period.

Locality	Coordinates	Altitude	Substrate (Soil)	Voucher Specimens
Bizerta North (Northeast of Tunisia)	Lat. 37°17'13'' N Long. 09°47'44'' E	143	Calcareous marley	(API/Reu.lu; 0027/2017)
Bizerta south (Northeast of Tunisia)	Lat. 36°55'14'' N Long. 09°23'40'' E	417	Clay-loamy	(API/Reu.lu; 0141/2017)
Tabarka (Northwest of Tunisia)	Lat. 36°56'55'' N Long. 08°46'39'' E	04	Sandy	(API/Reu.lu; 0334/2017)



Figure 1. Tunisian *Pimpinella lutea* Desf. (Tabarka, NW of Tunisia). (A) The habit of the plant in one of its natural habitats; (B) Long, glabrous stems with leaves reduced to sheaths; (C) Basal leaves long, typically pubescent, soft, pinnate with ovoid or rounded segments. (Photographs by Ridha El Mokni).

2. Materials and Methods

2.1. The Plant Material

The harvest of *Pimpinella lutea* aerial parts was done during the flowering-fruiting period from three different geographical areas of Tunisia (North and South Bizerta and Tabarka) (Table 1). The botanical identification of the plant material was carried out and authenticated by Dr. Ridha EL MOKNI affiliated to the Laboratory of Botany, Cryptogamy and Plant Biology, Department of Pharmaceutical Sciences “A”, Faculty of Pharmacy, Monastir, Tunisia.

2.2. Extraction Method

Air-dried aerial parts of the *Pimpinella lutea* aromatic plant (100 g of each sample) were extracted via a hydrodistillation process for three hours using a Clevenger-type extractor [26]. The collected essential oils were dehydrated over anhydrous sodium sulphate and then stored in airtight and watertight glass containers at a low temperature ($-4\text{ }^{\circ}\text{C}$) until analysis.

2.3. GC–FID and GC–MS Analyses of Essential Oils Extracted by Hydrodistillation

2.3.1. Gas Chromatography–Flame Ionization Detector (GC–FID)

Under the recorded experimental conditions [27,28], GC analysis of essential oils was performed on a Varian 450 gas chromatograph fitted with FID, using a ZB-5 stationary phase ($30\text{ m} \times 0.25\text{ mm i.d.}$, 0.25 m film thickness) column. The temperature program was (60 to 220) $^{\circ}\text{C}$ at $3\text{ }^{\circ}\text{C}/\text{min}$; for the injector and detector, temperatures were 230 and $240\text{ }^{\circ}\text{C}$, respectively. The injection volume was $1.0\text{ }\mu\text{L}$ of 1% solution diluted in *n*-hexane, the split ratio was 1:50.

2.3.2. Gas Chromatography–Mass Spectrometry (GC–MS)

The critical oils were GC–MS analyzed using a Thermo Scientific Trace Ultra GC coupled to a Thermo Scientific ITQ 1100 Mass Spectrometer with a ZB-5 ($30\text{ m} \times 0.25\text{ mm i.d.}$; 0.25 m film thicknesses) column. The oven temperature was programmed from 60 – $220\text{ }^{\circ}\text{C}$ at $3\text{ }^{\circ}\text{C min}^{-1}$ using helium as a carrier gas at 1 mL min^{-1} . The injector temperature was $230\text{ }^{\circ}\text{C}$; the injection volume was $0.1\text{ }\mu\text{L}$ of 1% solution prepared in *n*-hexane, split ratio 50:1. The mass spectrometry (MS) spectrum was taken at 70 eV with a mass scan range of 40 – 450 amu . All of the experimental parameters were applied based on those reported earlier [27,29,30].

2.3.3. Identification of the Constituents

The retention index values were used to assess the already listed constituents (RI, determined concerning homologous series of *n*-alkanes C 8 –C 25 , analyzed under identical experimental conditions), MS library quest (NIST 08 Mass Spectra Library (Version 2.0 f), and WILEY’S Library of Mass Spectra 9th Edition), MS literature data comparison [31], and co-injection of commercial samples from Sigma-Aldrich, India (≥ 98 percent purity). The relative amount of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

3. Results and Discussion

The hydrodistillation of samples of dried *Pimpinella lutea* aerial parts collected in separate bioclimatic zones (North Bizerta, South Bizerta, and Tabarka) afforded three yellowish essential oils, with yields of 0.49% , 0.46% , and 0.41% , respectively). Table 2 displays the findings of the initial chemical analysis of essential oils extracted from different Tunisian locations. The volatile oil’s chemical composition was determined using GC–FID and GC–MS techniques. To increase the reliability of identification, the mass spectra and the calculated LRI were compared with those of the same substances computerized in the data bank. According to the resulting analysis, a total of 73 organic volatiles were identified: 39 from NBEO (North Bizerta location), 44 from SBEO (South Bizerta location), and 51 from

TEO (Tabarka location), representing 76.2%, 94.6%, and 97.6% of the whole essential oil constituents, respectively. Despite some variations in chemotype structures depending on the environmental sources, common patterns in qualitative composition were established.

Pimpinella lutea essential oils consisted of monoterpenes and sesquiterpene hydrocarbons, oxygenated sesquiterpenes and phenyl derivatives, as shown in Figure 2.

A specific feature of *Pimpinella lutea* essential oil is the predominance of sesquiterpenes constituting the main groups in NBEO (72.2%), SBEO (78.1%), and TEO (60.2%). Even though this fraction dominated in all volatile oils, some qualitative and quantitative chemogeographical variability was clear in all samples. In particular, the acorane sesquiterpenoid acora-3,7(14)-dien Figure 3 was quantified at a reasonably high concentration (29.1%) in TEO but in a significantly lower amount in NBEO (3.4%) and SBEO (4.7%), respectively. Moreover, β -pinene ranked second in abundance for *Pimpinella lutea* collected in the sandy soil of Tabarka. At the same time, it occurred at a shallow mean content in SBEO (1.1%) and was completely absent in the sample harvested in the calcareous marly soil (NBEO). It is worth mentioning that several studies have demonstrated the anti-cancer properties and the high activity of β -pinene against both Gram-positive and Gram-negative bacteria and *Candida albicans* yeast [32]. α -trans-Bergamotene identified as the primary sesquiterpene metabolite in NBEO (18.1%) was less observed in SBEO (6.4%) and as a trace element in Tabarka's sample (1.5%) (Table 2). In addition, It is interestingly noticeable that NBEO and SBEO were distinguished by almost equal amounts of muurola-4,10(14)-dien-1- β -ol ranging from 8.9% to 10.1% in both samples, respectively. In contrast, this compound was not detected in TEO. Fokienol, the second interesting odorant in SBEO (9.1%) against a deficient level in NBEO, was utterly absent in TEO. As evidenced by previously published studies, this variation in terpene metabolites might be attributed to different climatic and edaphic conditions.

On reviewing the literature, several studies were performed on the chemical composition of essential oils extracted from *Pimpinella anisum* L. seeds, widely used as a culinary ingredient. The results revealed that in contrast to *Pimpinella lutea* (Desf.), Tunisian aniseeds contained noticeable amounts of phenylpropanoids (more than 95%) and were primarily composed of trans anethole (94.3%). However, *P. anisum* essential oil was very poor on sesquiterpenes hydrocarbons [33].

Table 2. The chemical profiles of essential oils extracted from *Pimpinella lutea* (Desf.) subspecies from various locations of Tunisia.

No	Compound	RI	AI	Content (%)		
				NBEO	SBEO	TEO
1	α -Thujene	943	930	-	0.1	0.3
2	α -pinene	947	939	Tr	0.5	0.1
3	<i>trans</i> -Pinene	968	975	0.5	-	-
4	Sabinene	977	976	0.7	-	-
5	β -pinene	981	980	-	1.1	18.2
6	Myrcene	993	979	-	-	0.1
7	dehydro-1,8-Cineol	995	991	-	Tr	tr
8	α -Phellandrene	1007	1002	-	-	tr
9	α -Terpinene	1018	1017	-	0.7	0.5
10	<i>p</i> -Cymene	1026	1024	0.2	Tr	1.7
11	Limonene	1030	1029	0.1	0.1	0.2
12	1,8-Cineol	1031	1031	Tr	-	-
13	γ -Terpinene	1061	1059	-	0.3	0.4
14	<i>Cis</i> -Sabinene hydrate	1070	1070	-	0.2	0.9
15	<i>m</i> -Cresol	1080	1076	0.8	-	0.5
16	Terpinolene	1093	1088	-	0.1	0.3
17	Linalool	1104	1096	0.1	0.5	0.8

Table 2. Cont.

No	Compound	RI	AI	Content (%)		
				NBEO	SBEO	TEO
18	<i>cis-p</i> -Menth-2-en-1-ol	1130	1121	-	0.9	0.5
19	<i>trans-p</i> -Menth-2-en-1-ol	1151	1140	-	0.5	1.1
20	Geijerene	1153	1143	0.9	-	tr
21	(<i>Z</i>)- <i>Tagetone</i>	1163	1152	-	-	0.3
22	Sabina ketone	1173	1159	-	0.9	0.6
23	Menthofuran	1176	1164	0.1	-	0.1
24	Terpin-4-ol	1197	1177	0.1	1.9	5.9
25	<i>p</i> -Cymen-8-ol	1205	1182	-	0.6	1.2
26	α -Terpineol	1212	1188	-	Tr	0.2
27	Myrtenal	1215	1195	-	0.3	-
28	<i>cis</i> -Piperitol	1217	1196	-	-	0.3
29	<i>trans</i> -Piperitol	1232	1208	-	0.3	0.7
30	<i>cis</i> -Sabinene hydrate acetate	1244	1221	0.5	-	0.1
31	Coahuilensol, methyl ether	1269	1221	-	-	0.5
32	<i>trans</i> -Sabinene hydrate acetate	1285	1256	-	-	0.1
33	(<i>E</i>)-Anethole	1326	1284	-	0.5	0.4
34	Pregeijerene	1330	1287	-	-	0.5
35	<i>p</i> -Cymene-7-ol	1332	1290	-	0.9	0.8
36	Carvacrol	1346	1299	0.1	6.1	0.1
37	δ -Elemene	1388	1338	0.2	0.7	-
38	Piperitenone	1391	1343	Tr	-	-
39	α -Cubebene	1402	1351	Tr	0.5	0.6
40	α -Copaene	1434	1376	0.5	0.8	0.2
41	Daucene	1437	1381	-	0.3	-
42	β -Cubebene	1451	1388	-	-	tr
43	Acora-3,7(14)-dien	1478	1408	3.4	4.7	29.1
44	β -Cedrene	1490	1420	8.9	0.2	0.6
45	α - <i>trans</i> -Bergamotene	1502	1433	18.1	6.4	1.5
46	α -Humulene	1525	1452	-	-	0.7
47	(<i>E</i>)- β -Farnesene	1529	1456	0.3	0.9	0.5
48	<i>cis</i> -Muurola-4(14),5-diene	1544	1466	3.1	1.3	0.2
49	Germacrene D	1555	1480	-	-	5.9
50	ar-Curcumene	1556	1483	0.7	-	-
51	β -Selinene	1564	1490	-	-	6.5
52	α -Selinene	1573	1492	-	-	4.1
53	α -Alaskene	1593	1512	1.0	4.7	1.6
54	<i>cis</i> -Calamene	1601	1522	6.1	4.5	-
55	δ -Cadinene	1604	1523	-	-	0.8
56	<i>trans</i> -Calamene	1615	1529	3.1	3.2	0.6
57	α -Calacorene	1626	1545	1.5	-	-
58	<i>cis</i> -Cadinene ether	1638	1553	-	-	0.5
59	Elemicin	1639	1557	Tr	-	-
60	<i>trans</i> -Cadinene ether	1647	1558	-	2.5	0.9
61	Spathulenol	1664	1578	1.2	5.9	-
62	Fokienol	1687	1596	0.5	9.1	-
63	β -Atlantol	1696	1608	1.3	5.6	-
64	2- <i>epi</i> - α -Cedren-3-one	1710	1627	-	5.5	-
65	Muurola-4,10(14)-dien-1- β -ol	1719	1631	8.9	10.1	-
66	Cedr-8(15)-en-9- α -ol	1743	1651	2.5	1.0	-
67	<i>epi</i> -Zizanone	1768	1670	6.5	7.5	-
68	α -Bisabolol	1778	1685	1.4	-	-
69	<i>epi</i> -Nootkatol	1784	1699	0.8	1.5	-
70	10- <i>nor</i> -Calamenene-10-one	1806	1702	0.3	-	-
71	(<i>Z</i>)- α -Atlantone	1810	1718	1.0	1.2	-
72	Zerumbone	1819	1733	0.8	-	5.6
73	Cyclocolorenone	1844	1760	-	-	0.3

Table 2. Cont.

No	Compound	RI	AI	Content (%)		
				NBEO	SBEO	TEO
	Oxygenated Monoterpenes			0.7	6.1	12.7
	Sesquiterpene Hydrocarbons			46.9	28.2	52.9
	Oxygenated Sesquiterpenes			25.2	49.9	7.3
	monoterpene Hydrocarbons			1.5	2.9	21.8
	Phenyl derivatives			1.9	7.5	2.9
	Total identified			76.2	94.6	97.6

RI: experimentally determined retention indices by co-injection of a homologous series of *n*-alkanes C₈–C₂₅ on a ZB5-MS fused silica column, AI: Adams retention indices, tr: trace and (-) not detected. OM: Oxygenated monoterpene, SH: Sesquiterpene hydrocarbon, MH: Monoterpene hydrocarbon, OS: Oxygenated sesquiterpene, PD: Phenyl derivative.

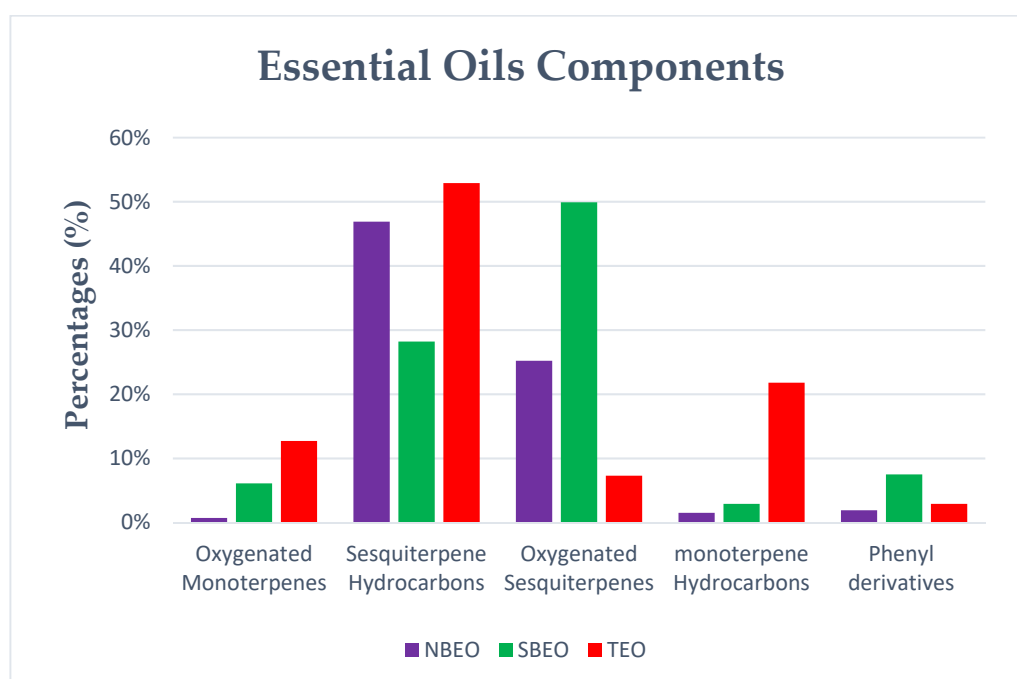
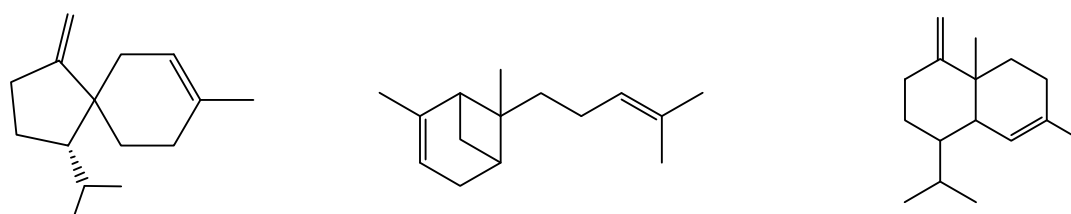


Figure 2. The semi-quantitative distribution of the main classes of organic volatiles in *Pimpinella lutea* growing in three different environmental conditions (North and South Bizerta and Tabarka) of Tunisia.



Acora-3,7(14)-dien (29.1% in TEO) α -trans-Bergamotene (18.1% in NBEO) Muurolo-4,10(14)-dien-1- β -ol (10.1% in SBEO)

Figure 3. The major organic volatiles of essential oils from *Pimpinella lutea* (Desf.), growing in three different environmental conditions (North and South Bizerta and Tabarka) of Tunisia.

4. Conclusions

The present work constitutes a first comparison of the chemical characteristics of essential oils produced by *Pimpinella lutea* growing wild in three Tunisian sites (North and South Bizerta and Tabarka). Regardless of the subspecies' type oil similarity, some qualitative and quantitative variations have been noticed due to several factors, including climatic, edaphic, or altitude. The overall results demonstrated the predominance of

sesquiterpenes in all studied samples. The use of sesquiterpenes can help patients with squamous cell carcinoma of the esophagus have a better prognosis. Furthermore, it has the potential to reduce the adverse effects of chemoradiotherapy [34]. Thus, this analysis suggests that *Pimpinella lutea* essential oils have the potential to be used for pharmaceutical and commercial purposes and may supply market demands.

Author Contributions: Conceptualization, H.A.-H.; R.E.M.; R.K.J.; M.L.A. and S.H. methodology, R.K.J.; software, R.K.J. and S.H.; validation, R.K.J., M.L.A. and S.H.; formal analysis, R.K.J.; M.L.A. and S.H.; investigation, H.A.-H. and S.H.; resources, R.K.J. and S.H.; data curation, H.A.-H.; R.E.M.; R.K.J.; M.L.A. and S.H. writing-original draft preparation, R.K.J.; M.L.A. and S.H.; writing—review and editing, R.K.J. and S.H.; supervision, S.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors are grateful to the Director of the ICMR-National Institute of Traditional medicine for instrumental facilities to analyze the chemical constituents.

Conflicts of Interest: The authors declare no conflict of interest.

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