J Appl Biotechnol Rep. 2025 March;12(1):1528-1536

Journal of
Applied Biotechnology Reports

doi 10.30491/jabr.2024.451602.1716

Original Article

Effect of Phenological Stages on the Composition and Antimicrobial Activity of *Phagnalon saxatile* L. Essential Oils

Rachida Kerzabi [1], Ilyas Chikhi [2,3], Samir Cherigui [2,3], Hanane Chaker [2,3], Hocine Allali [4], Mohammed El Amine DIB [5*], Alain Muselli [6]

[1] Research Center in Agropastoralism (CRAPast), Djelfa, Algeria
[2] Catalysis and Organic Synthesis Laboratory, BP 119, University of Tlemcen, Algeria
[3] Belhadj Bouchaib University, Route Sidi Bel Abbes, PB 284, University of Aïn Témouchent, Aïn Témouchent 46000, Algeria
[4] Department of Chemistry, Faculty of Sciences, University of Tlemcen, Tlemcen, Algeria
[5] Laboratory of Natural and Bioactive Substances (LASNABIO), Faculty of Sciences, University of Tlemcen, Tlemcen, Algeria
[6] Laboratory of Natural Product Chemistry, Pascal Paoli University, Corte, France

Corresponding Author: Mohammed El Amine DIB, PhD, Professor, Laboratory of Natural and Bioactive Substances (LASNABIO), Faculty of Sciences, University of Tlemcen, Tlemcen, Algeria. Tel: +213554378192, E-mail: a_dibdz@yahoo.fr

Received April 8, 2024; **Accepted** August 22, 2024; **Online Published** March 20, 2025

Abstract

Introduction: The primary aim was to analyze *Phagnalon saxatile* essential oils at the three vegetative stages, exploring their chemical composition. Additionally, antimicrobial activity was assessed during these developmental phases.

Materials and Methods: The essential oils extracted from the three phenological stages were examined using gas chromatography coupled with mass spectrometry. Their antimicrobial properties were evaluated on nine reference strains using the minimum inhibitory concentration (MIC) method. This method determines the lowest concentration of the antimicrobial substance that prevents visible growth of the microorganisms in a series of dilutions.

Results: The oil yield rate varied with the stage of development, with the highest concentration recorded at 0.08% (w/w) during the growth phase. Chemical analysis of the essential oils using GC and GC-MS revealed the presence of 112 different compounds. Significant variations were noted in the main classes of compounds. Notably, hydrocarbon monoterpenes showed a significant increase from 7.4% at the beginning of the vegetative cycle to 66.6% during the flowering period. In contrast, non-oxygenated compounds decreased significantly from 49.6% to 15.0% and non-oxygenated hydrocarbons decreased from 7.5% to 2.6%. During the early vegetative cycle, the essential oil of *P. saxatile* showed a more marked antimicrobial activity against the nine bacterial strains. In contrast, at the beginning of flowering and full flowering, the essential oils exhibited less significant antimicrobial activity.

Conclusions: Analyses of *P. saxatile* essential oils throughout its development cycle have shown promising bactericidal properties. These results could be utilized for the development of new drugs in the pharmaceutical field.

Keywords: Antimicrobial Activity, Phenological Phases, Hydrocarbon Monoterpenes, Essential Oils

Citation: Kerzabi R, Chikhi I, Cherigui S, Chaker H, Allali H, DIB MEA, et al. Effect of Phenological Stages on the Composition and Antimicrobial Activity of *Phagnalon saxatile* L. Essential Oils. J Appl Biotechnol Rep. 2025;12(1):1528-1536. doi:10.30491/jabr.2024.451602.1716

Introduction

Medicinal plants have been used for millennia in various cultures around the world to treat a wide variety of diseases. The growing interest in the use of medicinal plants as an alternative or complement to synthetic drugs, particularly in the field of fighting microbial agents, is of interest to many researchers. Modern scientific research confirms what our ancestors already knew: many medicinal plants are extremely effective against microbial infections.[1-3] Herbal medicine, an ancient practice widespread in different cultures, uses plant extracts. These extracts contain various beneficial compounds such as essential oils, which can have a positive impact on health.[4] Essential oils have been shown to be effective against various types of microorganisms, including bacteria, fungi and viruses. However, their effectiveness may vary depending on the specific type of essential oil and the target microorganism.[5]

The genus *Phagnalons* includes twenty species native to the Mediterranean region or Central Asia. In Algeria, there are mainly four types of *Phagnalons*: *P. sordidum* (L.) DC., *P. garamantum* M., *P. saxatile* (L.) Cass., and *P. rupestre* (L.) DC. Several hybrid species have also been reported.[6-8] In folk medicine, the *Phagnalons* plant is widely used to treat conditions such as asthma, headaches, and as an anesthetic to relieve dental pain.[8] Only one study has dealt with the composition of the essential oil of *P. saxatile* (L.) Cass., of Italian origin. The essential oil was mainly characterized by predominance of sesquiterpenes (23.9%), fatty acids (21.8%), waxes (19.3%), and monoterpenes

(14.6%).[9] So far, no research has explored the chemical composition of *P. saxatile* essential oils through its three stages of development. This study aims to analyze the chemical composition of essential oils extracted from *P. saxatile* at different stages of its development, while evaluating their antimicrobial properties throughout the three vegetative stages. The objective was to determine the potential benefits of these essential oils in terms of antimicrobial activity.

Materials and Methods

Vegetable Materials and Extraction of Essential Oils

The aerial parts of *P. saxatile* were harvested between January and February 2022 in the Tlemcen region of western Algeria, where they grow in abundance. Fresh aerial parts (250 g) were separated and then introduced into the balloon (6 liters) of a Clevenger-type apparatus. After 5 hours of hydrodistillation, extraction yields were determined. The plant material was identified in the Department of Biology and deposited in the laboratory herbarium.

Identification of Components

The analysis of the volatile part was performed using two chromatographic techniques: gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). For GC, the analyses were conducted on a Perkin Elmer Clarus 600 chromatograph equipped with two flame ionization detectors (FID), a split injector, and two columns: a polar column (Rtx-Wax) and an apolar column (Rtx-1, polydimethylsiloxane). Helium was used as the carrier gas with a column head pressure of 25 psi. The injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. The temperature programming involved an increase from 60 to 230 °C at a rate of 2 °C/min, followed by a 45-minute hold at 230 °C. Injection was performed in split mode with a division ratio of 1/50. The amount of essential oil injected varied from 0.1 to 0.2 µl depending on its purity or dilution in a solvent. For each compound, polar and apolar retention indices (Ir) were calculated from the retention times of a range of alkane standards from C_5 to C_{30} in the temperature programming.

For the GC/MS coupling, the analyses were performed on a Perkin Elmer Autosystem XL chromatograph, equipped with an automatic injector and two columns: a polar column (Rtx-Wax) and an apolar column (Rtx-1). These two columns were coupled to a Perkin Elmer TurboMass quadrupole mass detector, operating under the chromatographic conditions described above. This comparison was made with reference to genuine compounds or data from the literature.[10,11] Then, computer matching was performed using commercial mass spectral libraries[12,13], and the spectra were compared to those stored in our internal laboratory library.

Evaluation of Antimicrobial Activity: Dilution Technique (CMI)

The plate micro-dilution method, used in accordance with the 2006 CLSI guidelines, involves several steps. Initially, the bacteria were collected from a culture in a solid medium and then suspended in a nutrient broth until a desired cell concentration is obtained. This suspension was further diluted to obtain a final inoculum of 10^6 cells/ml. Then a microplate was used, with 12 wells reserved for each bacterial strain to be tested. The wells were filled with the test medium, followed by successive dilutions of the antimicrobial to be studied. After stirring and incubation, the INT colored indicator was added to each well, and the microplate was incubated again to allow the reaction. The presence or absence of color indicates bacterial growth, thus providing an indication of antimicrobial activity.[14]

Statistical Analysis

Statistical analysis of variance (ANOVA) was performed using the SAS software, and means were compared using the least significant difference (LSD) test with a significance threshold of $p \leq 0.05$. Each test was repeated three times to ensure reliable results. The Principal Component Analysis (PCA) was carried out using the XLSTAT 2014 software.

Results

Table 1 shows the change in the essential oil yield of the aerial parts of *P. saxatile* through three growth stages. Significant variation in essential oil yield was observed throughout the different growth stages. At the beginning of the vegetative cycle, the essential oil yield was 0.02%. This yield increased to 0.03% at the early flowering stage. In full bloom, the essential oil yield increased significantly to 0.08% (Table 1).

Table 1: Plant Material, Dates, Vegetative Cycle and Oil Yields of *P. saxatile*

Harvest dates	Vegetative cycle	Essential oil yield (%)
15/01/2022	Early vegetative cycle	0.02
28/02/2022		0.03
26/03/2022	Early flowering	0.04
30/04/2022	Flowering period	0.08
25/05/2022		
30/06/2022		

Coll-EO Analysis of P. saxatile

The collected essential oil (Coll-EO) from the aerial parts of *P. saxatile* was analyzed using CPG/IR and CPG/SM-IE. The analyses identified 112 compounds representing 92.9%

Table 2: The Chemical Compositions of *P. saxatile* Essential Oils Vary According to Stage of the Vegetative Cycle

N°[a]	Compounds	RIL[b]	RIa[c]	RIp[d]	Coll-EO	January	February	March	April	May	Jun	Identification[e]
1	Hexanal	780	780	1074	0.3	0.3	0.1	0.3	0.2	0.6	0.6	RI. MS
2	Z-3-Hexene-1-ol	851	845	1341	0.1	5.1	0.4	0.1	0.1	0.2	-	RI. MS
3	1-Hexanol	855	850	1340	0.1	0.4	0.1	0.1	0.1	0.1	0.2	RI. MS
4	Heptanal	882	875	1170	0.1	tr	0.1	0.1	0.1	0.2	0.1	RI. MS
5	Nonane	900	898	900	0.1	0.1	0.1	0.2	0.1	0.2	0.2	RI. MS
6	α-Thujene	932	927	1026	0.1	0.1	0.2	0.2	0.1	0.3	0.1	RI. MS
7	α-Pinene	936	929	1022	3.1	0.2	1.5	2.5	3.1	5.5	5.8	RI. MS
8	Sabinene	973	967	1124	1.4	0.1	0.5	0.9	1.5	2.3	2.5	RI. MS
9	β-Pinene	978	971	1115	31.3	2.0	19.1	28.3	34.4	40	46.7	RI. MS
10	6-methyl-5-hepten-2-one	978	976	1340	0.6	0.9	1.2	0.9	0.4	0.8	0.6	RI. MS
11	Myrcene	987	980	1157	0.7	0.1	1.2	0.8	0.5	1.1	0.9	RI. MS
12	Octanal	980	984	1282	0.4	0.1	0.7	0.5	0.5	0.3	0.5	RI. MS
13	(Z)-Hex-3-enyl acetate	987	986	1309	0.1	0.1	0.2	0.3	0.2	0.1	0.1	RI. MS
14	α-Phellandrene	1002	996	1165	0.2	0.1	0.2	0.2	0.4	0.3	0.8	RI. MS
15	p-Cymene	1015	1011	1266	2.9	0.7	9.0	2.5	2.6	1.8	2.7	RI. MS
16	Limonene	1025	1020	1201	1.6	0.3	1.9	1.9	2.5	3.2	4.9	RI. MS
17	β-Phellandrene	1023	1021	1210	1.2	0.2	0.6	1.2	1.1	1.1	1.3	RI. MS
18	(Z)-β-Ocimene	1029	1030	1234	0.3	0.2	0.3	tr	0.4	0.3	0.1	RI. MS
19	(E)-2-Octenal	1034	1033	1420	0.1	0.2	0.2	0.3	tr	0.1	tr	RI. MS
20	(E)-β-Ocimene	1041	1036	1242	0.1	0.1	0.2	0.1	-	0.1	tr	RI. MS
21	γ-Terpinene	1051	1047	1242	0.3	0.2	0.4	0.5	0.5	0.3	0.5	RI. MS
22	Isolyratol	1051	1051	1550	0.2	0.8	0.4	0.2	0.2	0.2	0.1	RI. MS
23	p-Cymenene	1075	1075	1429	0.1	tr	0.1	0.1	tr	0.1	tr	RI. MS
24	Terpinolene	1082	1077	1280	0.2	0.1	0.2	0.3	0.3	0.2	0.3	RI. MS
25	Nonanal	1086	1082	1392	6.0	9.1	8.4	6.2	6.5	4.7	5.0	RI. MS
26	Undecane	1110	1110	1100	0.1	0.1	0.1	0.1	0.1	0.1	0.1	RI. MS
27	(Z)-P-2-menth-2-ene-1-ol	1108	1106	1564	0.1	0.2	0.1	0.9	tr	0.1	0.2	RI. MS
28	(E)-Pinocarveol	1126	1122	1652	0.9	0.3	0.4	0.3	1.3	0.5	0.2	RI. MS
29	(E)-2-Nonenal	1139	1136	1525	0.5	0.6	0.7	0.6	0.5	0.4	0.3	RI. MS
30	Pinocarvone	1137	1137	1558	0.4	-	0.1	0.5	-	0.3	0.1	RI. MS
31	Cryptone	1160	1154	1666	0.4	tr	-	0.3	0.1	0.4	0.2	RI. MS
32	Terpinene-4-ol	1164	1158	1599	0.4	0.8	0.2	0.6	0.5	0.8	0.1	RI. MS
33	P-Cymene-8-ol	1169	1162	1830	0.7	0.8	-	tr	0.6	0.2	-	RI. MS
34	Myrtenal	1172	1171	1626	0.6	0.9	0.8	0.5	0.3	0.7	0.8	RI. MS
35	Myrtenol	1178	1173	1782	0.1	0.1	0.5	0.4	0.1	0.1	0.1	RI. MS
36	α-Terpineol	1176	1178	1690	0.6	0.1	0.2	0.8	0.6	0.3	0.1	RI. MS
37	Decanal	1180	1184	1497	4.3	7.8	7.6	5.3	3.8	3.5	2.3	RI. MS
38	(E-E)-2.4-Nonacadienal	1188	1191	1677	tr	0.2	0.2	0.2	tr	0.1	-	RI. MS
39	β-Cyclocitral	1195	1196	1601	0.2	0.1	0.3	0.2	0.1	0.1	0.1	RI. MS
40	Dodecane	1200	1198	1200	0.3	1.2	0.5	0.2	tr	0.2	0.2	RI. MS
41	nerol	1210	1211	1795	0.1	0.2	0.4	0.2	0.1	0.1	-	RI. MS
42	Methyl thymyl oxide	1215	1209	1585	0.1	0.1	0.1	0.1	0.1	0.1	tr	RI. MS

43	Cuminaldehyde	1215	1212	1763	tr	0.1	0.1	0.1	0.1	0.1	-	RI. MS
44	Geraniol	1235	1236	1838	0.6	0.9	0.6	0.8	0.8	0.6	0.3	RI. MS
46	E-2-decenal	1240	1246	1640	0.2	0.2	0.2	0.1	tr	-	tr	RI. MS
47	Thymol	1267	1269	2172	tr	0.3	0.2	0.1	0.4	0.2	tr	RI. MS
48	Bornyl acetate	1270	1271	1553	0.1	0.8	-	0.6	0.3	0.8	0.4	RI. MS
49	2-Undecanone	1273	1274	1593	1.2	0.2	1.9	1.0	1.4	0.3	0.1	RI. MS
50	Undecanal	1290	1285	1604	1.4	3.1	3.7	1.4	1.2	0.8	0.8	RI. MS
51	(E. E)-2.4-Decadienale	1291	1288	1820	1.0	1.2	0.9	1.5	1.4	0.8	0.4	RI. MS
52	(Z)-3-Hexenyl tiglate	1291	1296	1653	0.2	0.3	0.1	0.1	-	0.1	-	RI. MS
53	Tridecane	1300	1302	1300	0.1	0.1	0.2	0.1	tr	tr	0.1	RI. MS
54	E-2-Undecanal	1345	1338	1741	0.6	0.2	0.1	0.9	0.8	0.4	tr	RI. MS
55	Neryle acetate	1342	1341	1740	0.1	0.8	0.5	0.1	0.2	0.1	0.2	RI. MS
56	Acide decanonque	1347	1342	2079	0.1	0.1	0.1	0.2	0.2	0.1	0.1	RI. MS
57	Geranyle acetate	1362	1359	1744	0.4	0.5	0.4	0.7	0.4	0.8	0.3	RI. MS
58	α-Copaene	1379	1373	1489	0.5	0.2	0.3	0.2	0.2	0.3	0.2	RI. MS
59	β-Bourbonene	1386	1380	1510	0.2	0.1	0.3	0.6	0.5	0.5	0.3	RI. MS
60	β-elemene	1389	1383	1598	0.1	tr	tr	0.1	0.3	0.1	-	RI. MS
61	Dodecanal	1385	1387	1703	1.1	3.0	1.4	1.0	1.1	0.6	0.6	RI. MS
62	Tetradecane	1400	1400	1400	0.1	0.5	1.3	0.8	0.2	0.1	0.1	RI. MS
63	β-Ionol	1400	1405	1911	0.1	0.1	0.1	tr	0.2	0.2	0.1	RI. MS
64	α-Gurjunene	1413	1409	1515	0.2	0.2	0.2	0.3	0.1	0.1	0.1	RI. MS
65	(E)-β-caryophyllene	1421	1418	1596	0.9	1.2	1.6	1.0	0.8	0.7	0.5	RI. MS
66	β-copaene	1430	1323	1591	0.3	0.4	0.4	0.3	0.2	0.2	tr	RI. MS
67	Geranyle-acetone	1430	1427	1842	0.5	0.3	0.3	0.4	0.4	0.2	0.1	RI. MS
68	α-Humulene	1455	1450	1662	0.1	0.1	0.1	0.1	0.1	tr	tr	RI. MS
69	Alloaromadendrene	1462	1458	1632	1.6	1.7	1.0	2.1	1.8	1.4	0.8	RI. MS
70	β-Ionone	1464	1466	1927	0.1	0.5	0.2	0.1	0.1	0.1	-	RI. MS
71	γ-Muurolene	1474	1471	1671	1.2	2.8	2.3	0.7	0.8	0.8	0.8	RI. MS
72	Tridecan-2-one	1477	1479	1809	tr	0.1	0.1	0.1	0.1	0.1	tr	RI. MS
73	Germacrene D	1479	1473	1700	2.0	0.1	1.7	3.4	2.4	2.7	1.9	RI. MS
74	β-Selinene	1486	1482	1707	0.1	0.1	0.1	0.2	0.1	0.1	0.1	RI. MS
75	4-Epi-Cubebol	1490	1492	1881	0.1	0.2	-	0.2	0.1	0.1	0.1	RI. MS
76	Tridecanal	1493	1490	1808	4.1	10.9	5.4	3.8	4.2	2.6	2.4	RI. MS
77	Pentadecane	1500	1499	1500	0.1	0.3	0.1	0.1	0.1	0.1	tr	RI. MS
78	γ-Cadinene	1507	1505	1749	0.4	0.2	0.3	0.5	0.3	0.3	0.3	RI. MS
79	τ--Cadinene	1520	1516	1752	0.8	0.6	0.5	1.0	0.9	0.8	0.5	RI. MS
80	Cadina -1 4-diene	1523	1528	1763	0.1	0.1	0.1	0.1	0.1	tr	-	RI. MS
81	1.5-Epoxy salvial-4(14)-ene	1554	1559	1905	0.1	0.4	tr	0.1	-	0.1	0.3	RI. MS
82	Palustrol	1569	1567	1912	0.2	0.2	0.2	0.1	0.3	0.1	0.1	RI. MS
83	Spathulenol	1572	1568	2107	0.7	0.6	0.1	0.6	0.7	0.4	tr	RI. MS
84	Dodecanoic acid	1573	1569	2468	tr	0.2	-	0.3	0.1	0.1	0.1	RI. MS
85	Germacrene-D-4-ol	1571	1570	2037	0.1	-	0.1	tr	0.1	tr	tr	RI. MS

86	Caryophyllene oxide	1578	1578	1980	0.1	1.0	0.1	0.1	0.1	0.1	0.1	RI. MS
87	Tetradecanal	1596	1591	1851	1.1	2.3	1.2	1.2	1.3	0.8	0.5	RI. MS
88	Ledol	1600	1596	2029	0.3	0.1	0.3	0.1	0.1	tr	tr	RI. MS
89	Hexadecane	1600	1597	1600	0.1	0.7	-	0.2	0.2	0.1	tr	RI. MS
90	Epi-Cubenol	1623	1619	2052	0.1	0.2	0.1	0.3	0.3	0.2	0.2	RI. MS
91	τ-Cadinol	1633	1627	2154	0.4	0.5	0.2	0.4	0.3	0.3	0.2	RI. MS
92	T-Muurolol	1633	1629	2149	0.2	0.1	-	0.1	tr	0.1	0.1	RI. MS
93	α-Cadinol	1643	1639	2221	0.6	0.8	0.2	0.6	0.4	0.4	0.4	RI. MS
94	Eudesma-4 (15) 7-diene 1β-ol	1671	1668	2341	0.4	0.4	0.2	0.2	0.2	0.1	0.1	RI. MS
95	Pentadecanal	1696	1693	1990	1.3	2.4	1.8	1.5	1.1	1.1	0.8	RI. MS
96	Heptadecane	1700	1698	1700	0.1	0.2	0.1	0.1	0.1	0.1	tr	RI. MS
97	α-Oxa-Bisabolene	1719	1718	2267	0.4	0.4	0.2	0.2	0.1	0.2	0.1	RI. MS
98	Tetradecanoic acid	1761	1755	2641	0.3	0.1	0.1	-	0.1	-	-	RI. MS
99	Hexadecanal	1795	1795	2113	0.7	0.8	0.4	0.2	0.2	0.1	0.1	RI. MS
100	Octadecane	1800	1798	1800	0.1	0.1	-	-	0.2	0.1	-	RI. MS
101	Phytone	1823	1828	2121	1.4	5.3	2.6	2.5	2.1	1.9	1.2	RI. MS
102	Pentadecanoic acid	1857	1865	2671	0.3	0.2	0.1	0.2	tr	0.1	0.1	RI. MS
103	(E.E)-Farnesyl acetone	1895	1894	2347	tr	0.1	0.1	0.1	0.1	0.1	tr	RI. MS
104	Nonadecane	1900	1900	1900	0.9	1.4	0.6	0.3	0.3	0.3	0.3	RI. MS
105	Isophytol	1949	1948	2290	0.5	0.2	0.2	0.2	0.4	0.1	0.2	RI. MS
106	Eicosane	2000	1999	2000	0.2	0.1	0.6	0.1	0.1	0.1	0.1	RI. MS
107	Heneicosane	2100	2100	2100	0.1	0.3	0.1	0.1	0.1	0.1	tr	RI. MS
108	(E)-phytol	2114	2107	2606	tr	2.2	0.3	0.1	0.1	0.1	0.2	RI. MS
109	Docasane	2200	2199	2000	0.1	0.2	0.1	0.1	0.1	tr	tr	RI. MS
110	n-Tricosane	2300	2299	2300	tr	0.2	0.2	0.3	0.3	0.5	0.3	RI. MS
111	n-Tetracosane	2400	2399	2400	tr	0.6	0.1	0.1	0.1	0.1	0.1	RI. MS
112	n-Pentacosane	2500	2501	2500	tr	1.2	1.0	0.5	0.7	1.0	0.8	RI. MS
	% Identification				92.9	91.0	97.6	95.6	96.6	98.1	96.4	
Hydrocarbon monoterpenes					43.5	7.4	35.4	39.5	47.4	56.6	66.6	
Oxygenated monoterpenes					7.0	9.0	6.0	8.1	6.9	7.2	3.4	
Hydrocarbon Sesquiterpenes					8.4	7.8	8.9	10.5	8.6	7.9	5.5	
Oxygenated sesquiterpenes					3.7	1.9	1.7	3.0	2.7	2.1	1.7	
Diterpenes					1.9	7.8	3.2	2.9	2.7	2.2	1.6	
Oxygenated non terpene compounds					25.8	49.6	37.2	28.1	25.4	18.7	15.0	
Non terpene hydrocarbons compounds					2.6	7.5	5.2	3.5	2.9	3.4	2.6	

[a]Order of elution is given on apolar column (Rtx-1); [b]RILit: apolar column retention indices; [c]RIa: apolar column retention indices Rtx-1; [d]RIa: polar column retention indices Rtx-1; [e]RI: retention indices; MS; mass spectrometry in electronic impact mode; Coll-EO: Collective essentiel oil.

of the chemical composition of the collective essential oil obtained (Table 2). The identifications were established based on the "Arômes" libraries specific to the Laboratory of the University of Corsica, as well as commercial libraries. GC-IR and GC/MS analyses revealed that the essential oil of the aerial parts of *P. saxatile* was characterized by a predominance of hydrocarbon monoterpenes (43.5%), with β-pinene (31.3%) as the majority compound. This was followed by non-terpene oxygenated compounds (25.8%), with the main compounds in this class being nonanal (6.0%), decanal (4.3%), tridecanal (4.1%), and pentadecanal (1.3%). Additionally, oxygenated monoterpenes, hydrocarbon sesquiterpenes,

oxygenated sesquiterpenes and diterpenes were present at percentages of 7.0%, 8.4%, 3.7%, and 1.9%, respectively (Table 2).

Chemical Composition of the Essential Oil according to the Vegetative Cycle

Principal component analysis (PCA) was employed to assess the variation in the chemical composition of the essential oil throughout the vegetative cycle of *P. saxatile*. This method involved monitoring the chemical composition from the winter rest period through the stages of early, full, and post-flowering (Figure 1). According to Figure 1, the chemical composition of the essential oil of *P. saxatile* varies significantly depending on the period of plant harvest. There are notable variations in the main classes of compounds, including monoterpenes, sesquiterpenes and monoterpene compounds. As the vegetative cycle progresses from January to June, there is a noticeable increase in the proportion of monoterpene hydrocarbons, rising from 7.4% to 66.6%. In contrast, significant decreases were observed for non-terpene oxygenated compounds (from 49.6% to 15.0%), diterpenes (from 7.8% to 1.6%), and non-terpene hydrocarbons (from 7.5% to 2.6%) (Figure 1, Table 2).

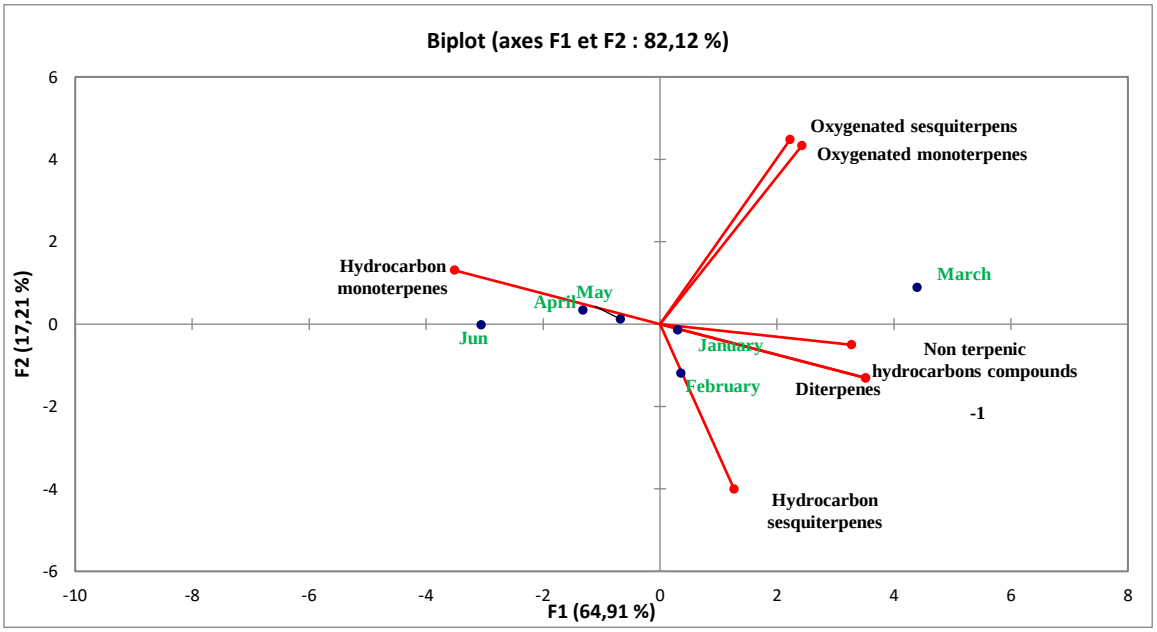

Figure 1. Principal Component Analysis (PCA) of *P. saxatile* by stage of life cycle.

Table 3. Antibacterial Activities of Essential Oils during the Vegetative Cycle (January-Jun)

Strains	Early vegetative cycle	Early flowering	Flowering period	Gentamycin
	CMI (g/L)			
E. coli ATCC 25922	1.2 ± 0.5	1.5 ± 0.1	11.5 ± 0.5	0.4 ± 0.01
C. freundii ATCC 8090	0.9 ± 0.1	4.8 ± 0.6	5.9 ± 0.1	0.2 ± 0.02
P. aeruginosa ATCC 27853	1.6 ± 0.1	3.6 ± 0.2	2.6 ± 0.1	0.8 ± 0.04
P. mirabilis ATCC 35659	1.2 ± 0.2	2.2 ± 0.1	5.2 ± 0.2	0.2 ± 0.01
A. baumanni ATCC 19606	1.8 ± 0.2	2.8 ± 0.1	2.8 ± 0.2	0.8 ± 0.02
B. cerius ATCC 10876	1.6 ± 0.4	2.6 ± 0.2	2.8 ± 0.4	0.2 ± 0.01
S. typhimurium ATCC 13311	1.1 ± 0.6	2.1 ± 0.1	5.1 ± 0.6	0.8 ± 0.02
S. aureus ATCC 25923	1.2 ± 0.4	2.2 ± 0.1	5.2 ± 0.4	0.2 ± 0.01
E. faecalis ATCC 49452	5.7 ± 0.8	8.7 ± 0.6	18.7 ± 0.8	3.1 ± 0.02

Evaluation of Antimicrobial Activity at the Beginning and End of the Vegetative Cycle

The antibacterial activities of the essential oils of *P. saxatile* were studied during different months corresponding to specific stages of its growth cycle. These included April, May, and June (flowering period), January and February (early vegetative cycle), and March (early flowering). These studies evaluated their effectiveness against nine reference bacterial strains:

E. faecalis ATCC 49452, *S. aureus* ATCC 25923, *S. typhimurium* ATCC 13311, *E. coli* ATCC 25922, *C. freundii* ATCC 8090, *P. aeruginosa* ATCC 27853, *P. mirabilis* ATCC 35659, *A. baumannii* ATCC 19606, and *B. cerius* ATCC 10876. The selection of these bacterial strains was based on their pathogenicity. The results of the MIC of essential oils, evaluated using the direct contact liquid method, are detailed in Table 3. Gentamicin was used as a control antibiotic. As shown in Table 3, the Minimum

Inhibitory Concentrations (MIC) of the essential oils studied ranged from 0.9 to 18.7 g/L over the three periods of the plant cycle. During the early vegetative stage (January and February), the essential oil presented an interesting antibacterial activity against all bacterial strains, with MICs ranging from 0.9 to 5.7 g/L. The highest activity was observed against *C. freundii*, *P. aeruginosa*, *S. typhimurium* and *E. faecalis*, with MIC of 0.9, 1.6, 1.1, and 5.7 g/L, respectively, similar to gentamicin. However, at the beginning of flowering (March) and during the flowering period (April, May and June), the essential oils showed significantly lower activity compared to the reference antibiotic (gentamicin).

Discussion

Increasing essential oil yield in aromatic plants is a major topic of interest for researchers and producers, due to its therapeutic and economic implications. The increase in essential oil yield in the aerial parts of *P. saxatile* throughout the growth stages results from various biological and environmental factors. As plants mature, they invest more in the production of essential oils, which is enhanced by optimal conditions of light, temperature, and humidity during flowering, as well as adequate nutrient availability. Studies on similar plants, such as lavender, rosemary, peppermint, and basil,[15-18] show a comparable increase in essential oil yield at the flowering stage, similar to that observed in *P. saxatile*. These findings are promising for improving agricultural practices and maximizing yields in the essential oil industry. On the other hand, there are few studies on the specific chemical composition of *P. saxatile*.[9] It is noteworthy that the chemical profile of this species different from that of the essential oils found in other *Phagnalon* species documented in the literature.[19-22] The work of Orhan et al.[19] *P. graecum* essential oil contains mainly germacrene-D (21.3%), hexahydrofarnesyl acetone (9.6%), β-caryophyllene (9.4%), hexadecanoic acid (6.1%), caryophyllene oxide (6.0%), and δ-cadine (3.2%). while the essential oil of *P. sordidum* from Corsica was mainly composed of (E)-β-caryophyllene (14.4%), β-pinene (11.0%), thymol (9.0%) and hexadecanoic (5.3%).[20] The predominant constituents identified in Algerian *P. sordidum* essential oil were β-pinene (26.0%), (E)-β-caryophyllene (10.0%), limonene (8.5%), myrcene (4.7%), decanal (4.5%), thymol (3.9%), germacrene-D (3.8%), and p-cymene (3.4%).[21] GC-MS analysis of *P. sinaicum* essential oil was found to be rich in terpenoid compounds, the main compound being artemisinin ketone (22.3%), followed by α-thujone (17.7%) and santoline alcohol (14.8%).[22] On the other hand, the essential oil of aerial parts of *P. saxatile* was composed of hexadecanoic acid (17.4%) and low quantity of terpenes β-pinene (5.4%), ρ-cymene (2.1%) caryophyllene (4.6%), γ-cadinene (3.0%), aromadendrene (2.2%), and δ-cadinene (1.8%).[9] The chemical composition of the essential oil of *P. saxatile* varies significantly throughout its vegetative cycle, particularly showing notable changes in the predominant classes of compounds. Towards the end of the flowering period, there is a marked increase in the quantity of hydrocarbon monoterpenes. Specifically, essential oil samples collected in April, May and June exhibited high levels of hydrocarbon monoterpenes. During the January and February harvests, the essential oils of *P. saxatile* were predominantly composed of non-terpene oxygenated compounds and diterpenes. In contrast, during March, there was a notable increase in the quantities of sesquiterpenes and oxygenated monoterpenes compared to other stages of vegetative growth. The vegetative cycle of plants plays a crucial role in plant ecology and their interactions with the environment. Variations in the chemical composition of essential oils produced at each phase can significantly impact their antimicrobial properties, thereby determining their effectiveness against different pathogens.[23] With regard to the antimicrobial activity of the essential oils of *P. saxatile*, it appears that the period of the beginning of the vegetative cycle is that which favors a high antibacterial activity, while the beginning and flowering period show a reduction in this activity, suggesting a variation in the chemical composition of essential oils throughout the plant growth cycle. The analysis of chemical compositions reveals that essential oils harvested during the early periods (January and February) are rich in compounds such as pinene, nonanal, decanal, and tridecanal. It has been shown that α-pinene and β-pinene each demonstrate varying efficacy against a range of pathogenic microorganisms.[23] Studies have shown that pinene exerts inhibitory effects on Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria like *Escherichia coli*, as well as pathogenic fungi, including *Candida albicans*. The mechanism of action of pinene includes the disruption of microbial cell membranes, leading to leakage of intracellular components and denaturation of proteins, which compromises the viability of microbial cells. [24] On the other hand, the lipophilic and hydrophilic character of the functional groups are of crucial importance in the antimicrobial activity of the essential oil components. Phenols have been shown to have greater antimicrobial activity than aldehydes, ketones, alcohols and esters. However, aldehydes are known for their strong antimicrobial activity. It is suggested that an aldehyde group combined with a carbon-carbon double bond forms a highly electronegative arrangement, which could explain their activity.[25] Nonanal, decanal and tridecanal are long-chain aldehydes extensively studied for their antimicrobial properties. Nonanal, a compound found in the essential oils of many plants, has demonstrated notable efficacy against various Gram-positive and Gram-negative bacteria, as well as pathogenic fungi. Similarly, decanal and tridecanal have

shown comparable antimicrobial activities, inhibiting the growth of pathogenic microorganisms by disrupting cell membranes and inhibiting the synthesis of essential proteins.[26,27] The antimicrobial properties of these aldehydes make them promising for applications in the food, pharmaceutical, and cosmetic industries as natural preservatives. However, during the early and full bloom periods, a significant decrease in antimicrobial activity has been observed. This reduction may be attributed to the lower levels of oxygenated compounds such as aldehydes and the increased percentage of hydrocarbon compounds.[28-32] Studies have indicated that aliphatic alcohols exhibit strong to moderate antimicrobial activity against various bacteria.[33-35] Moreover, research suggests that even minor components in essential oils play a crucial role in their antibacterial activity. These components often act synergistically with others to enhance effectiveness through different mechanisms of action.[33-38] For instance, certain compounds can disrupt bacterial cell membranes, leading to cell death, while others may interfere with internal metabolic processes or inhibit microbial reproduction.[39]

Conclusion

The objective of this study was to investigate the chemical composition of the essential oil of *P. saxatile* and assess its antimicrobial efficacy across the plant's life cycle, aiming to discover new molecules or compound families with antimicrobial potential. The GC and GC-MS analyses showed that *P. saxatile* oils are rich in hydrocarbon monoterpenes. These compounds showed a marked variation with the stage of plant growth, and the maximum amounts were detected at the full-flowering stage. However, oxygenated non-terpene compounds were the main components at the early vegetative stage. Essential oils have demonstrated significant antimicrobial activity against bacterial strains at the beginning of the vegetative cycle, likely due to the high concentrations of pinene, nonanal, decanal, and tridecanal. However, the decrease in this activity during the flowering period could be related to the reduction in concentrations of aldehydes and oxygenated compounds. It is crucial to emphasize the importance of further research before considering the practical application of these essential oils. Additional studies are needed to thoroughly investigate their antimicrobial properties, safety profiles, and potential applications in various fields such as pharmaceuticals or natural antimicrobial agents.

Authors' Contributions

IC and DMA design of activity tests and experiments; RK performed the experiments; SC and AH analyzed and interpreted the data; AM and DMA wrote and edited the article.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

References

1. Bohlin L, Bruhn JG, editors. Bioassay methods in natural product research and drug development. Springer Science & Business Media; 1999.
2. Canel C, Lopes-Cardoso MI, Whitmer S, van der Fits L, Pasquali G, van der Heijden R, et al. Effects of over-expression of strictosidine synthase and tryptophan decarboxylase on alkaloid production by cell cultures of *Catharanthus roseus*. Planta. 1998;205:414-9. doi:10.1007/s004250050338
3. Halla N, Boucherit K, Zeragui B, Hellal D, Abenia T, Belouafi L, et al. Essential Oil of *Artemisia judaica* L.(ssp. Sahariensis) from Algerian Sahara: Antimicrobial Effects and Mechanisms of Action. Phytothérapie. 2021;19(1): 25-32. doi:10.3166/phyto-2019-0187
4. Benyoucef F, Dib ME, Arrar Z, Costa J, Muselli A. Synergistic antioxidant activity and chemical composition of essential oils from *Thymus fontanesii*, *Artemisia herba-alba* and *Rosmarinus officinalis*. J Appl Biotechnol Rep. 2018;5(4):151-6. doi:10.29252/JABR.05.04.03
5. Ainseba N, Soulimane A, DIB ME, Djabou N, Muselli A. Comparative Study of the Antioxidant, Antimicrobial and Anti-Inflammatory Activity between Essential Oil and Hydrosol Extract of the Aerial Parts of *Inula viscosa* L. J Appl Biotechnol Rep. 2023;10(4):1169-75. doi:10.30491/jabr.2023.409230.1654
6. Quйzel P, Santa S. Nouvelle flore de l'Algйrie et des rйgions dйsertiques mйridionales. Paris: Editions du Centre National de la Recherche Scientifique; 1963.
7. Ali-Shtayeh MS, Yaghmour RM, Faidi YR, Salem K, Al-Nuri MA. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J Ethnopharmacol. 1998;60(3):265-71. doi:10.1016/S0378-8741(97)00153-0
8. Góngora L, Giner RM, Máñez S, del Carmen Recio M, Rнos JL. Phagnalon rupestre as a source of compounds active on contact hypersensitivity. Planta med. 2002; 68(06):561-4. doi:10.1055/s-2002-32566
9. Senatore F, Formisano C, Grassia A, Rigano D, Bellone G, Bruno M. Chemical composition of the essential oil of *Phagnalon saxatile* (L.) Cass.(Asteraceae) growing wild in Southern Italy. J Essent Oil-Bear Plants. 2005;8(3):258-63. doi:10.1080/0972060X.2005.10662136
10. Babushok VI, Linstrom PJ, Reed JJ, Zenkevich IG, Brown RL, Mallard WG, et al. Development of a database of gas chromatographic retention properties of organic compounds. J Chromatogr A. 2007;1157(1-2):414-21. doi:10.1016/j.chroma.2007.05.044
11. Knorr A, Monge A, Stueber M, Stratmann A, Arndt D, Martin E, et al. Computer-Assisted Structure Identification (CASI)- An Automated Platform for High-Throughput Identification of Small Molecules by Two-Dimensional Gas Chromatography Coupled to Mass Spectrometry. Anal Chem. 2013;85(23):11216-24. doi:10.1021/ac4011952
12. McLafferty FW, Stauffer DB, Stenhagen E, Heller SR. The Wiley/NBS registry of mass spectral data. J Chem Educ. 1989;66(10):A256.
13. Wallace WE, Moorthy AS. NIST Mass Spectrometry Data Center standard reference libraries and software tools: Application to seized drug analysis. J Forensic Sci. 2023;68(5):1484-93. doi:10.1111/1556-4029.15284
14. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev. 2020;33(3):10-128. doi:10.1128/cmr.00069-19
15. Erdogan Orhan I, Senol FS, Demirci B, Ozturk N, Baser KH, Sener B. Phytochemical characterization of *Phagnalon graecum* Boiss. by HPLC and GC-MS with its

enzyme inhibitory and antioxidant activity profiling by spectrophotometric methods. Food Anal Methods. 2013;6:1-9. doi:10.1007/s12161-012-9386-6
16. Brunel M, Vitrac C, Costa J, Mzali F, Vitrac X, Muselli A. Essential Oil Composition of *Phagnalon sordidum* (L.) from C orsica, Chemical Variability and Antimicrobial Activity. Chem Biodivers. 2016;13(3):299-308. doi:10.1002/cbdv.201500078
17. Chikhi I, Allali H, Costa J. Chemical composition and biological activities of essential oils of *Phagnalon sordidum* (L.) Rchb.(Asteraceae) from Algeria. Agric Conspec Sci. 2019;84(3):271-81.
18. Badalamenti N, Di Napoli M, Castagliuolo G, Varcamonti M, Bruno M, Zanfardino A. The Biological Properties of the Essential Oil from the Jordan Accession of *Phagnalon sinaicum* Bornm. & Kneuck. Plants. 2023;12(23):4007. doi:10.3390/plants12234007
19. Kalemba DA, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem. 2003;10(10):813-29. doi:10.2174/0929867033457719
20. Lahlou M. Methods to study the phytochemistry and bioactivity of essential oils. Phytother Res. 2004;18(6): 435-48. doi:10.1002/ptr.1465
21. Michavila A, De la Torre MC, Rodrıguez B. 20-nor-abietane et ont réarrangé les diterpénorıdes abiétains de la racine de *Salvia argentea*. Phytochem. 1986;25(8): 1935-37.
22. Kusumoto N, Ashitani T, Murayama T, Ogiyama K, Takahashi K. Antifungal abietane-type diterpenes from the cones of Taxodium distichum Rich. J Chem Ecol. 2010;36:1381-6. doi:10.1007/s10886-010-9875-2
23. Kofujita H, Fujino Y, Ota M, Takahashi K. Antifungal diterpenes from the bark of *Cryptomeria japonica* D. Don Holzforschung. 2006;60(1):20-3.
24. Güllüce M, Sökmen M, Daferera DI, Ağar G, Özkan H, Kartal NU, et al. *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. J Agric Food Chem. 2003;51(14):3958-65. doi:10.1021/jf0340308
25. Moleyar V, Narasimham P. Antifungal activity of some essential oil components. Food Microbiol. 1986;3(4): 331-6. doi:10.1016/0740-0020(86)90017-1
26. Kurita N, Miyaji M, Kurane R, Takahara Y, Ichimura K. Antifungal activity and molecular orbital energies of aldehyde compounds from oils of higher plants. Agri Biol Chem. 1979;43(11):2365-71. doi:10.1080/00021369.1979.10863805
27. Kurita N, Miyaji M, Kurane R, Takahara Y. Antifungal activity of components of essential oils. Agri Biol Chem. 1981;45(4):945-52. doi:10.1271/bbb1961.45.945
28. Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int J Food Microbiol. 2001;67(3):187-95. doi:10.1016/S0168-1605(01)00447-0
29. Lattaoui N, Tantaoui-Elaraki A. Individual and combined oils. Riv. Ital EPPOS. 1994;13:13-9.
30. Paster N, Menasherov M, Ravid UZ, Juven B. Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J Food Prot. 1995;58(1):81-90. doi:10.4315/0362-028X-58.1.81
31. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6(12):1451-74. doi:10.3390/ph6121451