SUPPELEMENTARY MATERIAL

Terpenoid composition of Essential Oil from a new chemotype of *Selinum wallichianum* Raizada & Saxena

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Abstract

Essential oil samples obtained by steam distillation of the whole aerial parts and roots of ***Selinum wallichianum*** **Raizada & Saxena** (syn *S. tenuifolium*) growing wild in theHimalayan region of Uttarakhand, India were analyzed by capillary gas chromatography(GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of twenty-fourand forty-three constituents representing 97.0% and 95.4% of the oil composition, respectively were identified. Both the oil samples were constituted mainly of monoterpenehydrocarbons 68.1% and 77.4%, with sabinene 31.0% and 11.5%, β-phellandrene 18.2% and34.5%, α-bisabolol 16.0% and 1.8% and α-phellandrene 3.5% and 11.2%, respectively as amajor constituents. Presence of sabinene, β-phellandrene, α-phellandrene and completeabsence of previously reported 3,5-nonadiyne (53.8 to 90.0%) justifies it to be a new chemotype of *S. wallichianum.*

Keywords: *Selinum wallichianum*, Essential Oil, Chemotype, Sabinene, Phellandrenes, α-Bisabolol and 3,5-Nonadiyne.

3. Experimental

Plant material

The root and aerial parts of ***Selinum wallichianum*** were collected at full flowering stage of the plant in the month of September from Kilbari in the Nainital district and from Khuliyatop, Munsiyari in Pithoragarh district (Uttarakhand, India), at an elevation of about 2,200 m and 3,200 m, respectively. Both samples were identified at the Botany Department, Kumaun University, Nainital, and its botanical identity was further confirmed from Botanical Survey of India, Dehradun.

Herbarium voucher No.-

Selinum wallichianum from Nainital- BSI/NRC Tech./115909/2015-16/631
Selinum wallichianum from Munsiyari- BSI/NRC Tech./115912/2015-16/631

Isolation of the essential oil

The oil was obtained by the steam distillation of fresh plant material (6 kg; two collections of 3 kg for each population) using a copper still fitted with Spiral glass condensers. Each time 3 kg material was loaded and distilled for 3 hrs. The aqueous distillate was saturated with NaCl and the oil was extracted with hexane. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed using rotavap under reduced pressure and at 30°C. The oil was kept at -4^0C until analyzed by Gas chromatography/ Flame ionization detector (GC/FID) and Gas chromatography/Mass spectrometry (GC/ MS) systems.

Gas chromatographic (GC–FID) analysis

A gas chromatographic analysis of essential oil samples were carried out on a Shimadzu GC-2010 Plus gas chromatograph with a flame ionization detector (FID) equipped with a Rtx-5MS (30 m × 0.25 mm, 0.25 μm film thickness) fused silica capillary column, which was perfused with He adjusted to 1.21 mL/min flow rate at 84.2 kPa pressure and operated with a split ratio of 10:1. Essential oil injection (mode:split) volume was 2.0 μL with dilution (5% solution of oil in hexane) whereas the injection temperature was set at 260°C. The column oven temperature was programmed isothermally at 50°C for 2 minutes, after which the temperature was increased to 210°C at a rate of 3°C/minute. The sequence was terminated after 18 minutes of isothermal operation at 280°C. The detector temperature was 270°C.

Gas chromatography-mass spectrometry (GC–MS) analysis

The GC/MS analysis of the oil samples were carried out using a GC-2010 Plus gas chromatograph interfaced with a Shimadzu QP 2010 Plus mass-spectrometer fitted with an Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) programmed at 50°C for the first 2 minutes followed by 3°C/minute to 210°C and held isothermal at 280°C for the next 11 minutes. The mass spectral data were acquired under electronic impact (EI) at 70 eV at 2.41 scan/second and a mass range of 41–350 m/z. Carrier gas: He, linear velocity: 39.9 cm/sec; flow rate: 1.21 mL/minute; Inlet pressure: 69.0 kPa; Injection (split, 10:1). A 5% solution of oil in hexane (Injection volume: 1.0 μL) was injected and injection temperature was set at 260°C.

The constituents of essential oil samples were identified by comparison of their retention indices (RI) on Rtx-5MS capillary column, determined with reference to the homologous C_7–C_{33} (Supplier-Restek's ISO 9001:2008) n-alkane series injected in the GC and the GC-MS under the same temperature-programmed and experimental conditions. Identification of individual compounds were made by comparison of their mass spectra with those of the internal reference mass spectra library (WILEY 8 and NIST 11), the literature report and Retention Indices (Adams 2007). For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Isolation of major component - 1.3 ml of the root oil (Nainital collection) was loaded onto a column packed with 30 g silica gel (230-400 mesh, Merck). The column was first eluted with hexane (5 ml) and there after with 5 ml portions of mixture of diethyl ether and hexane (5%-30%). A total of 39 fractions of about 2 ml each were collected and screened by TLC. The major constituent was isolated in hexane fraction. The ^{1}H and ^{13}C NMR data were acquired with varia Mercury 300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C using $CDCl_3$ as a solvent and TMS as internal reference.

Table-S1: Terpenoid composition of the essential oil from a new chemotype of *Selinum wallichianum*

Compounds[A]	**RI**[O]	**Percentage**							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
butanoic acid	811	-	-	-	-	-	-	-	0.5
heptanal	904	-	0.4	-	-	-	-	-	-
α- thujene	930	0.4	0.3	-	-	-	-	tr	-
α- pinene	939	3.0	**8.0**	0.4	1.0	1.7	0.3	1.2	-
camphene	956	0.3	1.0	-	0.1	0.6	0.1	-	-
sabinene	978	**31.0**	**11.5**	-	-	-	-	2.0	-
β-pinene	979	-	1.0	0.1	0.1	0.4	-	0.2	-
myrcene	992	4.5	2.5	0.3	0.2	-	-	0.2	-
3*E*-hexenyl acetate	1002	-	-	-	-	-	0.9	-	-
***α*-phellandrene**	1005	**3.5**	**11.2**	-	-	-	-	-	-
δ-3-carene	1011	-	0.1	0.2	0.1	-	-	0.3	-
α-terpinene	1017	-	0.4	-	-	0.9	-	-	-
p-cymene	1030	-	-	-	-	1.7	0.1	-	0.2
limonene	1031	-	-	4.7	0.2	-	-	1.2	-
***β*-phellandrene**	1033	**18.2**	**34.5**	-	-	0.2	0.2	0.2	-
Z-*β*-ocimene	1041	1.0	0.3	0.3	-	-	-	0.2	-
E-*β*-ocimene	1052	**5.0**	**5.6**	**5.6**	-	-	-	tr	-
γ-terpinene	1059	0.6	1.0	0.6	0.2	-	-	-	-
cis sabinene hydrate	1068	-	0.3	-	-	-	-	0.5	-
n-octanol	1070	-	0.1	-	-	-	-	-	-
terpinolene	1089	0.6	-	-	-	-	-	-	-
2-nonanone	1091	-	2.0	-	-	-	-	-	-
nona-3,5-diyne	1094	-	-	**53.8**	**90.5**	**65.4**	**89.7**	-	**85.6**
amyl isovalerate	1093	-	-	-	-	-	-	0.2	-
trans-sabinene hydrate	1097	-	-	-	-	-	-	0.1	-
3-caren-10-al	-	-	-	-	-	3.1	-	-	-
isopentylisovalerate	1103	-	0.3	-	-	-	-	-	-
isopentyl-2-methyl butanoate	1106	1.0	-	-	-	-	-	-	-
2-methyl butyl isovalerate	1110	-	0.8	-	-	-	-	-	-
3-octanol acetate	1115	-	-	-	0.1	-	-	-	-
cis-*p*-menth-2-en-1-ol	1128	-	0.4	-	-	-	-	-	-
citronellal	1156	1.6	-	-	-	-	-	-	-
isoborneol	1160	-	-	-	-	0.3	-	-	-
2*E*- nonen-1-al	1166	-	0.4	-	-	-	-	-	-
iso-pinocampheol	1176	-	-	-	-	-	-	0.2	-
terpinen-4-ol	1180	0.7	3.5	-	-	-	-	-	-
dill-ether	1184	-	-	-	-	-	-	0.2	-
cryptone	1188	-	0.2	-	-	-	-	-	-
α-terpineol	1188	-	0.4	-	-	-	0.4	-	-
2*E*-octanol acetate	1211	-	-	-	0.6	-	-	-	-
nona-3,5-diyn-2-one	1211	-	-	-	-	-	-	-	3.0
nona-4,6-diyn-3-one	1222	-	-	-	-	-	-	-	2.5
endo-fenchyl acetate	1223	1.3	-	-	-	-	-	-	-

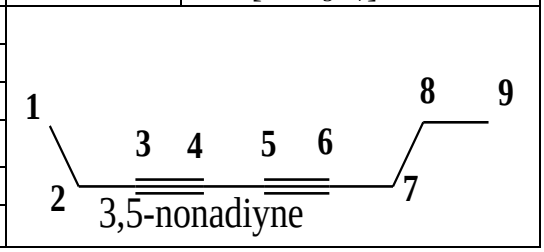
citronellol	1226	-	0.8	-	-	-	-	-	-
perilla ketone	1248	-	-	-	-	1.3	-	-	-
Z-citral	1253	-	-	-	-	-	-	0.4	-
nona-3,5-diyn-2-ol	1261	-	-	-	-	-	-	-	2.2
nona-4,6-diyn-3-ol	1263	-	-	-	-	-	-	-	3.1
2*E*-decenal	1268	-	0.4	-	-	-	-	-	-
isobornyl acetate	1288	0.4	-	-	-	-	-	-	-
bornyl acetate	1288	-	0.3	-	0.1	0.1	0.4	-	0.3
E-anethole	1291	-	0.4	-	-	-	-	-	-
p-vinyl-guaiacol	1294	-	0.7	-	-	-	-	-	-
myrtenyl acetate	1326	-	0.7	-	-	-	-	-	0.3
α-copaene	1379	-	-	0.3	0.1	-	0.2	0.1	-
3,4-dihydrocoumarin	1380	-	-	-	-	0.1	-	-	-
β-bourbonene	1388	0.4	-	0.3	-	-	-	1.2	-
β-elemene	1390	-	-	-	-	0.7	0.4	-	-
ledon oxide	-	-	-	-	-	1.0	-	-	-
methyl eugenol	1403	-	-	-	-	-	0.3	-	-
2*β*-*epi*-funebrene	1417	-	-	-	0.1	-	-	-	-
trans-caryophyllene	1423	0.6	-	6.0	0.1	-	0.4	1.2	-
aromadendrene	1439	-	-	-	-	-	-	0.2	-
α-guaiene	1439	-	-	-	-	-	0.2	-	-
citronellylpropanoate	1444	-	-	-	-	-	-	2.4	-
β-barbatene	1446	-	-	-	0.2	-	-	-	-
α-himachalene	1449	-	-	-	-	-	-	0.2	-
geranyl acetone	1458	-	0.4	-	-	-	-	-	-
E-*β*-farnesene	1460	1.4	0.2	3.0	0.2	-	-	1.5	-
geranylpropanoate	1469	-	-	0.4	-	-	-	-	-
γ-decalactone	1476	-	0.1	-	-	-	-	-	-
β-chamigrene	1477	-	-	-	-	0.7	-	-	-
γ-muurolene	1482	-	-	0.5	-	-	-	-	-
γ-himachalene	1482	-	-	-	0.1	-	0.3	-	-
α-amorphene	1483	-	-	-	-	-	-	0.1	-
germacrene D	1485	2.5	-	**18.0**	-	-	-	2.4	-
β-selinene	1491	-	-	0.3	-	1.2	-	-	-
phenyl ethyl 3-methyl butanoate	1496	-	0.6	-	-	-	-	-	-
bicyclogermacrene	1497	0.5	-	1.0	-	-	-	-	-
β-bisabolene	1505	-	-	-	0.1	-	-	tr	-
α-bisabolene	1506	-	-	-	-	-	-	0.3	-
E-methyl isoeugenol	1510	-	1.3	-	-	-	-	-	-
germacrene A	1512	-	-	0.8	-	-	-	-	-
δ-cadinene	1529	-	-	0.4	-	0.4	-	0.3	-
kessane	1529	-	-	-	-	-	-	0.9	-
γ-cuprenene	1537	-	-	-	tr	-	-	-	-
α-cadinene	1538	-	-	-	-	0.8	-	-	-
germacrene B	1562	-	-	0.8	-	-	-	-	-
longipinanol	1563	-	-	-	tr	-	-	-	-
E-nerolidol	1568	0.5	0.3	-	-	-	-	-	-
maaliol	1574	-	0.2	-	-	-	-	-	-
spathulenol	1578	-	-	-	-	2.1	-	0.9	-
germacrene D-4-ol	1581	-	-	0.4	-	-	-	-	-
caryophyllene oxide	1589	-	-	0.2	-	-	-	0.4	-
viridiflorol	1592	-	-	-	-	-	-	0.9	-
7-hexadecenal	1604	-	-	-	-	-	-	0.3	-

β-eudesmol	1650	-	-	-	-	7.2	0.9	-	-
α-bisabolol oxide B	1656	-	-	-	-	-	-	2.3	-
α-cadinol	1660	-	-	0.2	-	-	-	-	-
α-bisabolol	1694	**16.0**	**1.8**	**-**	**-**	**-**	**-**	**71.8**	**-**
2*Z*,6*Z*-farnesol	1698	**-**	-	-	-	-	-	3.6	-
7,14-*anhydro*-amorpha-4,9-diene	1752	**-**	0.2	-	-	-	-	-	-
drimenol	1767	**-**	-	-	-	1.1	0.1	-	-
bisabolen-12-ol	1761	-	-	-	-	0.4	-	-	-
n-pentadecanol	1783	**-**	0.1	-	-	-	-	-	-
neophytadiene	1838	-	-	-	-	-	-	0.7	-
14-hydroxy-*δ*-cadinene	1815	**-**	0.2	-	-	-	-	-	-
Z-falcarinol	2036	**-**	0.2	-	4.3	-	-	-	-
osthole	2145	2.0	0.3	-	**-**	**-**	**-**	**-**	-
Total compounds		**24**	**43**	**24**	**21**	**22**	**16**	**37**	**9**
Total identified		**97.0**	**95.4**	**98.6**	**98.4**	**91.4**	**94.9**	**98.8**	**97.7**
Monoterpene hydrocarbons		**68.1**	**77.4**	**12.2**	**1.9**	**5.5**	**0.7**	5.6	**0.2**
Sesquiterpene hydrocarbons		5.4	0.2	31.4	0.9	3.8	1.5	9.9	-
Oxygenated monoterpenes		3.3	11.2	-	0.7	4.8	1.3	1.5	-
Oxygenated sesquiterpenes		20.2	6.4	1.2	0.1	11.9	1.7	**81.8**	0.6
Others		-	0.2	**53.8**	94.8	65.4	89.7	**-**	96.9

(-) indicates absence, **tr**- Percentage <**0.1**, **A**-Munsiyari, Aerial Parts Oil, **B**- Munsiyari, Root Oil, **C**- Nainital, Aerial Parts Oil, **D**-Nainital, Root Oil, **E**-Mathela *et al*.(2003), Nainital, Aerial Parts Oil, **F**-Mathela *et al*. (2003), Nainital, Root Oil, **G**-Mohan M. *et al* (2013), Chamoli, Aerial Parts Oil, **H**-Chauhan R.S. *et al*.(2012), Rohtang, Root Oil

Notes: ^{A}Compounds listed in order of elution; RI^{O} (retention indices observed) measured relative to n-alkanes (C_7–C_{33}) on Rtx-5MS capillary column under conditions listed in the Experimental section and by comparison with Literature retention indices (Adams 2007).

Table-S2 NMR, IR and MS data of 3,5-Nonadiyne

Position		**Chemical shift(δ) ^{1}H- NMR(ppm)**	**Chemicalshift(δ) ^{13}C- NMR (ppm)**	**Infrared (cm^{-1})**	**Mass (m/z)**
1	CH_3	(t) 1.14(J=7.5)	13.50	2180 and 2280	120 $[M]^+$(100% abundance) 105 $[M-CH_3]^+$ 91 $[M-C_2H_5]^+$ 77 $[M-C_3H_7]^+$
2	CH_2	(q) 2.23(7.5)	12.20		
3	C	-	77.61		
4	C	-	64.50		
5	C	-	65.20		
6	C	-	76.5		
7	CH_2	(t) 2.25(J=7.2)	21.1		
8	CH_2	(sext.) 1.54(J=7.2)	21.9		
9	CH_3	(t) 0.97 (J=7.2)	13.3		

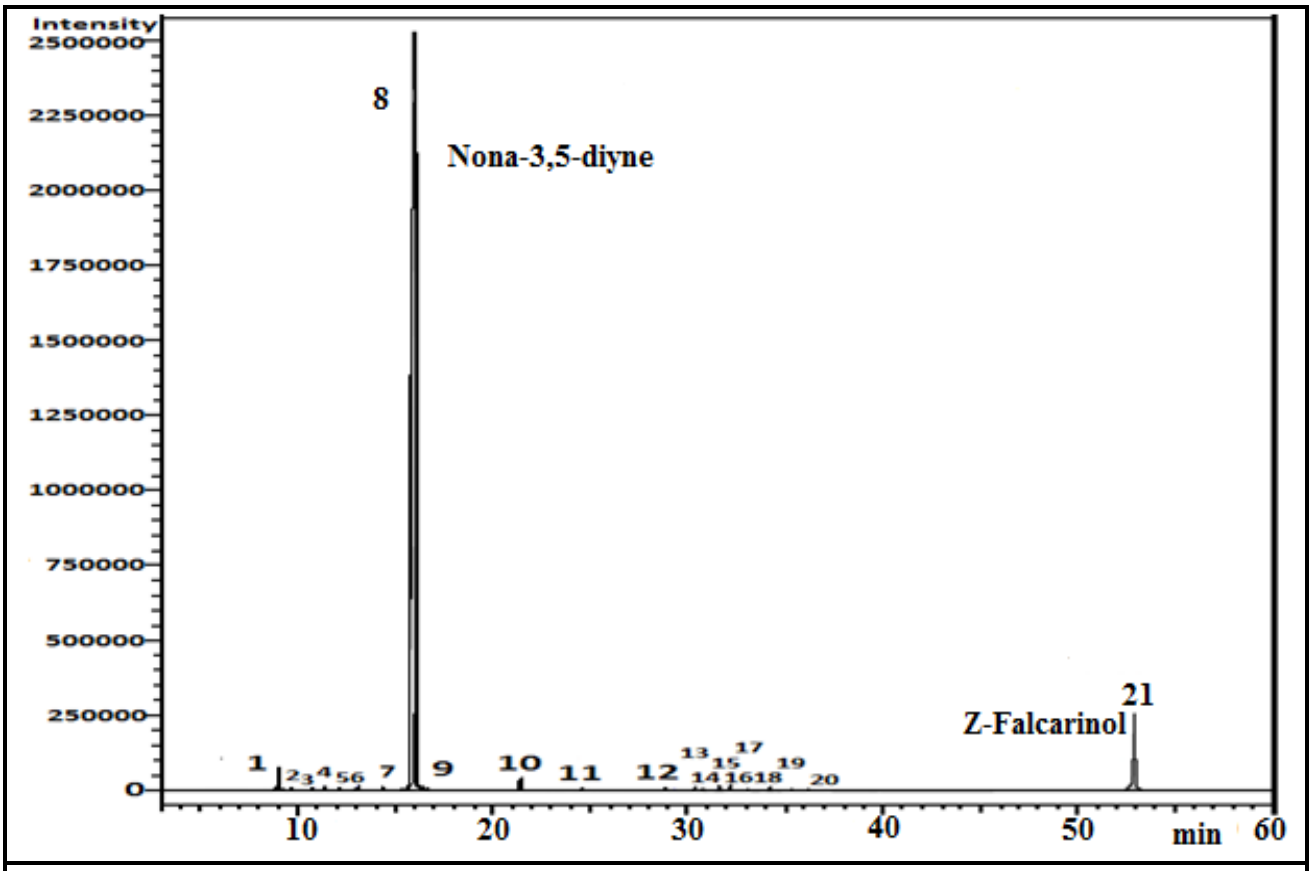


Fig.-S1. Gas Chromatogram of the essential oil from root parts of *Selinum wallichianum* (Nainital collection)

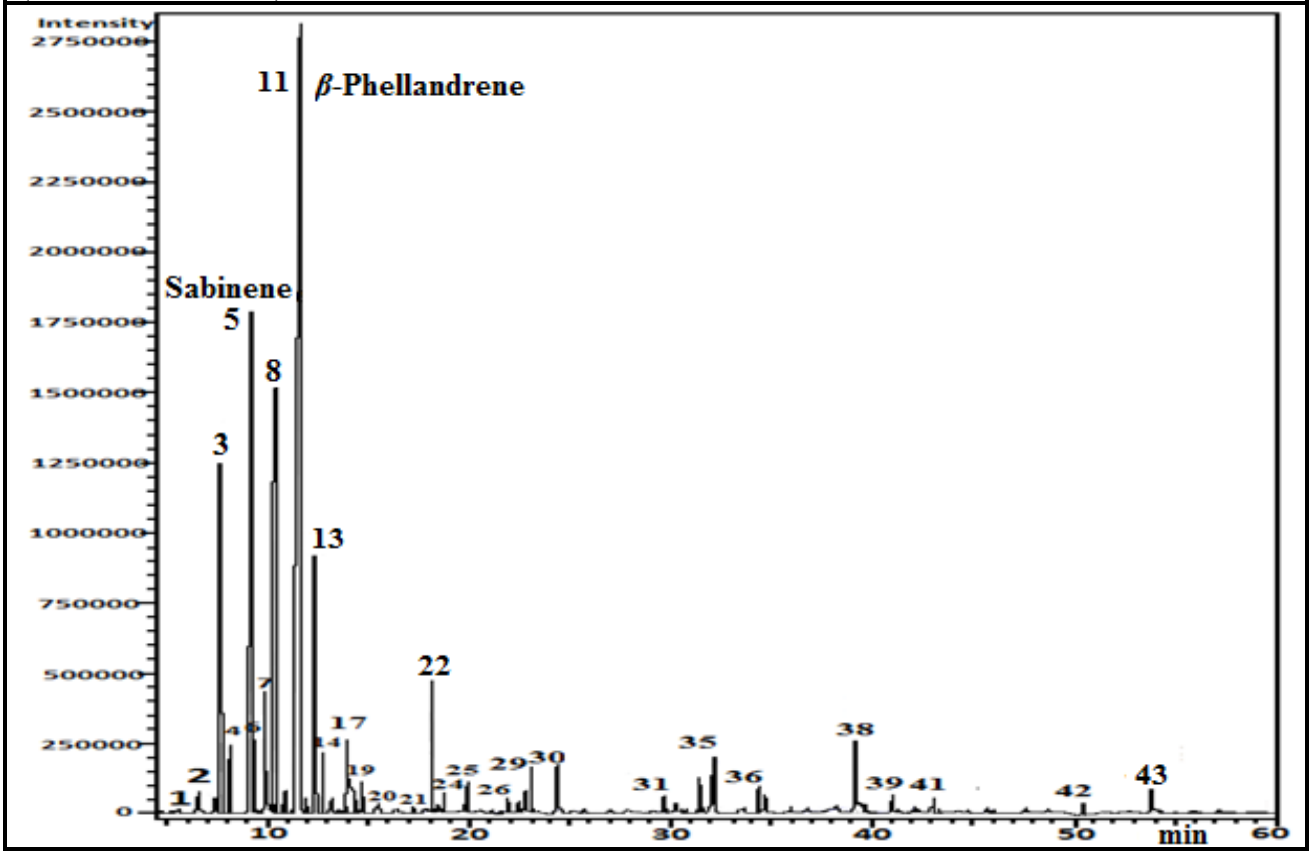


Fig.-S2. Gas Chromatogram of the essential oil from root parts of *Selinum wallichianum* (Munsiyari collection)

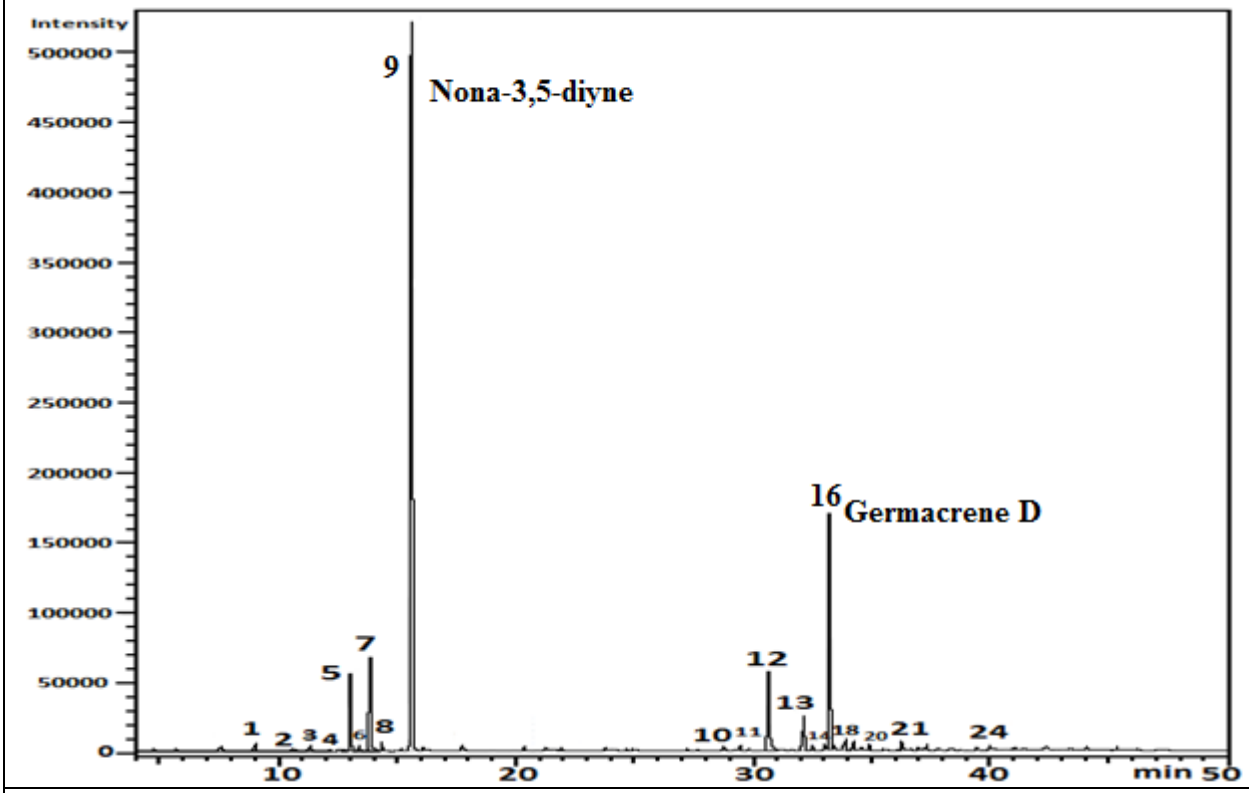


Fig.-S3. Gas Chromatogram of the essential oil from aerial parts of *Selinum wallichianum* (Nainital collection)

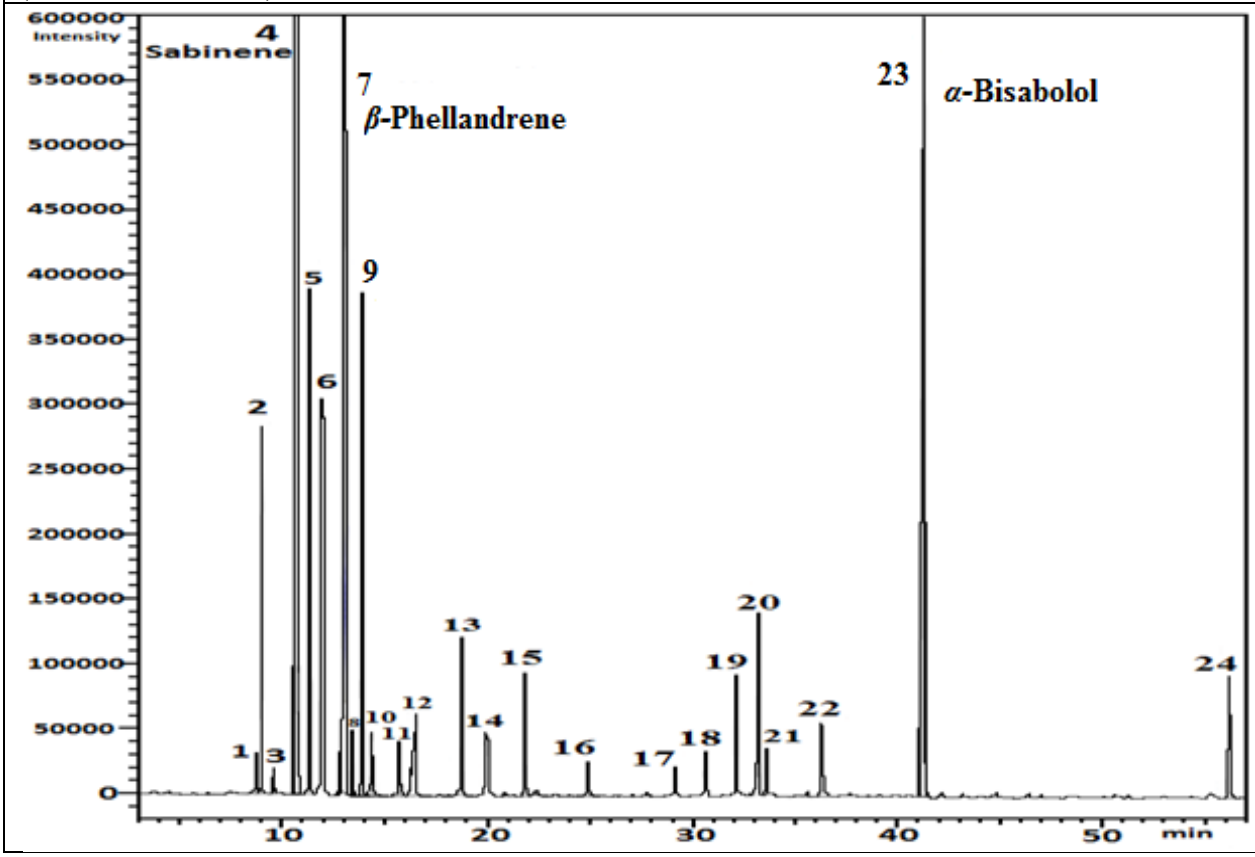


Fig.-S4. Gas Chromatogram of the essential oil from aerial parts of *Selinum wallichianum* (Munsiyari collection)