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Research article

Intraspecific variability of the essential oil of *Zanthoxylum alatum* from North-Western Himalaya

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ABSTRACT

Hydro-distilled essential oils of mature seeds of *Zanthoxylum alatum* DC. from nineteen populations of the North-Western Himalaya (Uttarakhand, India) were analysed using GC and GC/MS techniques to determine the intraspecific chemical variability. Altogether, 39 compounds were identified in the oils, and a relatively high variation in their contents was found. The oils were dominated by monoterpene hydrocarbons (17.44–68.53%) and oxygenated monoterpenes (13.53–69.12%). The main constituents of the essential oils were linalool (3.5–46.5%), limonene (0.82–41.5%), β-phellandrene (0.24–37.6%), terpinen-4-ol (0.02–21.9%), 2-undecanone (0.16–18.2%), sabinene (0.34–17%), E-methyl cinnamate (0.02–15.7%), 1,8-cineole (0.03–15.2%), myrcene (0.65–13.8%) and trans-caryophyllene (0.04–10.66%). For the determination of the chemotypes and the intraspecific chemical variability, the essential oil components were subjected to cluster analysis. The five different chemotypes characterized were Chemotype I (limonene), Chemotype II (linalool/ sabinene/ 2-undecanone), Chemotype III (linalool), Chemotype IV (limonene/ β-phellandrene/ linalool) and Chemotype V (β-phellandrene/ linalool/ sabinene).

INTRODUCTION

The genus *Zanthoxylum*, which includes trees and shrubs, belongs to the Rutaceae family. It is economically important because of its alimentary, industrial and medicinal applications (Chase et al., 1999; Seidemann, 2005). The genus comprises over 200 species, distributed worldwide mainly in tropical and temperate regions, in which 10 species are reported in India (Gaur, 1999). Among them, *Zanthoxylum alatum* DC. (*Z. alatum* Roxb.), a large evergreen shrub, is distributed mainly between 900 and 2100 elevation. It is locally known as Timur or Timru (Osmaston, 1926). It has high medicinal value and whole plant parts are used as medicine. Locally, it is used as a condiment and for water purification (Daudi et al., 2016). Young shoots are used to brush the teeth and are useful for curing gum diseases. The fruits, branches and thorns are generally used as carminative, stomachic, remedy for toothache, condiment and flavouring agent (Halliwell and Gutteridge, 1984; Barkatullah et al., 2013).

In India, different parts of the *Z. alatum* are used in Ayurvedic practices for the treatment of skin diseases, abdominal pain, anorexia, and ataxia (Chaudiere and Ferrari-Iliou, 1999). The fruit essential oil possesses antiseptic, disinfectant, deodorant, anthelmintic properties and antifungal activity, while the extract of fruits is useful in expelling roundworms (Chopra et al., 1956; Mehta et al., 1981).

A literature survey of *Z. alatum* revealed that the essential oil composition of the seeds (Ahmad et al., 1988; Jain et al., 2001; Tiwary et al., 2007), the pericarp of the wild fruit (Shah et al., 1991; Mohan et al., 2012; Kumar et al., 2014), leaves, aerial parts, terminal branches and bark (Nigam and Dhingra, 1960; Weyerstahl et al., 1999; Bisht and Chanotiya, 2011) has been analysed. The main constituents in the fruit pericarp oil were found as linalool (45.2%), limonene (19.6%), β-phellandrene (14.4%) and methyl cinnamate (9.3%), while leaf oil afforded 2-undecanone (43.1%) and linalool (33.4%), collected from North-Western Himalaya region of Uttarakhand (Kumar et al., 2014). Another study from this region reported linalool (71.2%) as the major compound in fruit pericarp oil, while oil from leaves contained 2-undecanone (30.9%) and linalool (39.4%) in high proportion (Mohan et al., 2012). Linalool (70.6%) was found to be main compound in the seed oil from northern India (Jain et al., 2011).

A literature survey revealed that no systematic work has been undertaken to explore the chemical variability of *Z. alatum* seed essential oil from Uttarakhand Himalaya. Hence, accessions from different locations were collected to determine the chemical variability in nineteen populations.

MATERIALS AND METHODS

Plant material

The mature seeds of *Zanthoxylum alatum* were collected in April 2014 from nineteen populations growing in different eco-climatic zones of the North-Western Himalaya region (Uttarakhand, India). The details of the sampling locations are as follows:

1. P1 (Adibadri, Altitude 1343m, Latitude 30°8'23.16"N, Longitude 79°14'33.06"E)
2. P2 (Agarchatti, 1363m, 30°0'38.28"N, 79°18'58.38"E)
3. P3 (Ratura, 712 m, 30°17'50.94"N, 79°3'32.04"E)
4. P4 (Munsiyari, 1677 m, 30°06'12.2"N, 80°14'58.3"E)
5. P5 (Sunow, 1140 m, 30°5'31.56"N, 79°27'4.8"E)
6. P6 (Kafrd, 1101 m, 29°42'6.6"N, 79°26'18.66"E)
7. P7 (Chamoli, 1051 m, 30°23'30.54"N, 79°19'7.38"E)
8. P8 (Bhimtal, 1635 m, 29°22'59.8"N, 79°31'0.2"E)
9. P9 (Mandal, 1618 m, 30°27'16.02"N, 79°16'13.68"E)
10. P10 (Tharali, 1193 m, 30°5'0.60"N, 79°29'20.16"E)
11. P11 (Almora, 1547 m, 29°35'21.33"N, 79°38'29.71"E)
12. P12 (Karnprayag, 1131 m, 30°15'33.6"N, 79°13'12.7"E)
13. P13 (Bagoli, 885 m, 30°11'43.68"N, 79°18'15.24"E)
14. P14 (Raragi, 807 m, 30°14'34.68"N, 79°14'50.46"E)
15. P15 (Dwarshow, 1690 m, 29°39'21.18"N, 79°32'33.66"E)
16. P16 (Dwarahat, 1398 m, 29°44'57.36"N, 79°25'52.14"E)
17. P17 (Lodhia, 1535 m, 29°33'53.75"N, 79°37'59.38"E)
18. P18 (Booj, 1342 m, 29°52'38.94"N, 79°35'36.78"E)
19. P19 (Niglat, 1503 m, 29°24'20.93"N, E79°30'53.78"E)

The samples were identified and voucher specimens (No. GBPIHED.XA: No. 1-19) from each population have been deposited with the Herbarium of the G.B. Pant National Institute of Himalayan Environment and Sustainable Development at Kosi-Katarmal, Almora.

Isolation of the essential oils

For extraction of essential oil, mature seeds were air-dried in shade (3 days) at room temperature. The seeds of each population (50 g) were subjected to hydro-distillation in a 1000 ml round-bottom flask for 4 h using a Clevenger-type apparatus, to isolate the essential oil. The essential oils were collected, dried (anhydrous Na_2SO_4), and then kept in a sealed vial at 4° until analysis.

GC and GC-MS analyses

GC analyses were performed using an Agilent (HP7890 GC) gas chromatograph equipped with a flame ionization detector (FID) and a fused silica capillary HP-5 column (30 m × 0.32 mm; film thickness 0.25 µm). The injector and detector temperatures were kept at 210°C and 230°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL/min; oven temperature program was 60° to 220° with an increase in rate of 3°/min and finally held isothermally for 5 min; the split ratio was 1:50. The injection volume was 0.2µL.

GC-MS analyses were carried out by using Agilent mass spectrometer (Model 5975C) coupled to an Agilent gas chromatograph DB5 column (60 m × 0.32 mm; film thickness 0.25 µm). Helium was used as carrier gas (flow rate 1 mL/min) with an ionization voltage of 70 eV. The mass spectrum was taken with a mass range of 40-600 amu. The oven temperature program was the same given above for the GC.

The constituents of the essential oil were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C_8-C_{32}). Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/ WILEY) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature (Adams, 2009). For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Statistical analysis

To classify and group the nineteen populations of *Z. alatum* based on their essential oil components and to identify the chemotype, the composition data matrix of the populations was analysed using cluster analysis with SPSS version 13.0.

RESULT AND DISCUSSION

The chemical constituents of nineteen populations of *Z. alatum* with their retention indices (RI) and percentage contents in the seed essential oils are listed in Table 1 and 2, according to their elution order on a DB-5 column. The essential oil in seed yields, calculated on the dry-weight basis, varied considerably from location to location (0.6–3.8%).

Altogether, 39 compounds were identified, accounting for 91.3–97.54% of the whole oil composition. In the oils, monoterpene hydrocarbons (17.44–68.53%) and oxygenated monoterpenes (13.53–69.12%) were present at appreciable amounts, *viz*., linalool (3.5–46.5%), limonene (0.82–41.5%), β-phellandrene (0.24–37.6%), terpinen-4-ol (0.02–21.9%), sabinene (0.34–17%), E-methyl cinnamate (0.02–15.7%), 1,8-cineole (0.03–15.2%)and myrcene (0.65–13.8%). Moreover, 2-undecanone (0.16–18.2%) and trans-caryophyllene (0.04–10.66%) were also found in major portions in some of the locations.

Table 1. Composition (%) of the essential oils of different populations of chemotype I and II of *Zanthoxylum alatum* from the North-Western Himalaya region

Compound	$RI_{Lit.}$	$RI_{Exp.}$	Composition (%)							
			Chemotype I					Chemotype II		
			P8	P15	P11	P9	P3	P1	P17	P7
α-thujene	924	927	0.75	0.10	1.18	0.51	0.39	0.98	0.41	–
α-pinene	932	930	2.35	1.89	2.83	2.56	4.37	6.29	6.94	11.33
benzaldehyde	952	951	0.67	0.45	–	0.56	2.11	2.23	0.51	0.89
sabinene	969	972	8.76	5.65	13.84	12.79	1.29	13.00	7.21	–
β-pinene	974	975	–	0.50	2.34	1.76	–	1.98	0.11	0.19
myrcene	988	987	7.83	10.32	8.54	7.47	8.77	8.07	2.53	2.22
α-terpinene	1014	1011	–	–	0.76	0.64	0.03	1.34	–	0.34
p-cymene	1020	1019	0.01	0.04	0.03	–	0.05	–	0.12	0.31
limonene	1024	1020	41.48	39.90	36.69	27.56	23.29	11.28	2.23	1.45
β-phellandrene	1025	1023	0.56	2.34	0.24	0.24	0.30	5.66	10.00	0.98
1,8-cineole	1026	1024	15.19	–	–	0.57	0.10	–	1.12	–
(E)-β-ocimene	1044	1044	0.22	0.09	–	0.32	0.04	0.97	0.44	–
γ-terpinene	1054	1053	–	–	2.08	1.20	0.10	0.23	0.04	0.40
cis-linalool oxide	1067	1065	–	0.47	0.04	0.12	0.06	0.47	0.21	0.03
trans-linalool oxide	1084	1081	–	–	0.03	0.07	–	0.05	0.56	0.33
α-terpinolene	1086	1084	–	0.08	–	–	0.11	0.42	0.21	0.22
linalool	1095	1098	6.42	8.61	3.50	11.81	20.55	34.24	32.36	40.12
nonanal	1100	1101	–	0.67	–	0.55	0.78	–	0.07	0.13
terpinen-4-ol	1174	1177	3.10	2.08	9.98	2.72	2.53	0.51	–	21.89
cryptone	1183	1185	–	1.14	0.10	–	3.33	–	0.98	0.08
α-terpineol	1186	1187	0.93	0.77	0.13	0.22	0.55	0.03	–	5.60
piperitone	1249	1243	0.11	–	0.09	0.38	0.23	–	0.68	0.31
geraniol	1249	1250	0.02	–	–	–	–	0.04	–	0.15
phellandral	1273	1280	0.33	0.33	0.51	–	1.38	1.11	2.95	–
2-undecanone	1293	1291	–	4.26	3.45	8.76	8.62	4.21	18.15	0.21
E-methyl cinnamate	1376	1375	2.12	1.27	0.02	3.39	3.77	1.01	0.23	0.69
trans-caryophyllene	1417	1415	0.20	2.03	0.13	0.82	4.15	0.53	3.13	2.47
Allo-aromadendrene	1458	1456	0.45	0.74	0.04	1.60	0.81	–	0.07	–
germacrene D	1484	1487	–	2.45	1.19	0.98	1.32	–	0.05	0.56
β-selinene	1489	1488	–	0.85	0.10	0.73	0.10	–	0.03	0.34
2-tridecanone	1495	1498	1.24	1.00	1.84	1.38	2.79	0.91	0.04	0.76
caryophyllene oxide	1582	1581	0.79	1.61	0.61	–	0.85	0.56	0.06	0.32
β-eudesmol	1649	1647	–	0.45	0.23	0.06	0.13	–	–	0.51
α-eudesmol	1652	1650	–	–	0.44	–	–	0.06	0.66	–
α-cadinol	1652	1653	–	0.29	–	0.06	–	0.03	–	0.31
tetradecanoic acid	-	1720	–	–	–	–	–	0.34	0.77	0.01
phytol	1942	1932	–	0.29	0.02	0.73	0.39	–	–	0.30
hexadecanoic acid	1959	1964	0.55	1.35	0.79	–	0.22	0.21	0.90	0.40
oleic acid	2141	2122	0.39	0.55	1.60	1.69	1.03	0.78	0.38	0.22
monoterpene hydrocarbons	-	-	61.96	60.91	68.53	55.05	38.74	50.22	30.24	17.44
oxygenated monoterpenes	-	-	28.22	13.53	14.3	19.28	29.17	37.46	38.11	69.12
sesquiterpene	-	-	0.65	6.07	1.46	4.13	6.38	0.53	3.28	3.37

hydrocarbons										
oxygenated sesquiterpenes	-	-	0.79	2.35	1.28	0.12	0.98	0.65	0.72	1.14
oxygenated diterpenes	-	-	–	0.29	0.02	0.73	0.39	–	–	0.30
others	-	-	2.85	9.42	7.78	12.94	18.88	8.68	21.8	2.7
Total identified (%)			**94.47**	**92.57**	**93.37**	**92.25**	**94.54**	**97.54**	**94.15**	**94.07**
Oil yield (%)			**2.4**	**1.8**	**3.0**	**1.9**	**2.9**	**2.3**	**0.6**	**1.5**

RI: Retention index on the DB-5 column; $RI_{Lit.}$: retention indices literature (Adams, 2009); $RI_{Exp.}$: retention indices (experiment); (–): absent

Table 2. Composition (%) of the essential oils of different populations of chemotype III, IV and V of *Zanthoxylum alatum* from the North-Western Himalaya region

Compound	**Composition (%)**										
	Chemotype III		**Chemotype IV**			**Chemotype V**					
	P4	**P13**	**P6**	**P12**	**P19**	**P2**	**P14**	**P16**	**P10**	**P5**	**P18**
α-thujene	0.41	–	1.16	0.87	0.15	0.85	0.67	0.60	0.83	0.63	–
α-pinene	0.71	7.91	1.24	2.37	2.10	3.13	2.33	2.20	8.27	1.36	4.17
benzaldehyde	–	0.29	0.35	–	1.34	0.78	0.60	0.69	0.43	1.32	0.70
sabinene	0.78	0.83	11.62	13.00	0.34	14.65	17.00	10.22	–	–	10.60
β-pinene	0.86	–	0.22	0.75	0.87	0.35	0.13	–	0.66	0.32	0.09
myrcene	1.90	0.65	5.68	6.53	3.05	10.03	7.92	8.72	8.64	13.76	9.63
α-terpinene	–	0.02	–	1.39	0.11	0.68	0.04	0.31	0.62	0.28	4.47
p-cymene	0.31	0.23	–	–	0.16	0.02	–	0.34	0.22	0.11	–
limonene	14.60	15.24	16.97	17.71	7.39	1.34	1.83	10.22	0.82	1.91	1.21
β-phellandrene	3.67	3.66	14.15	18.79	3.33	27.96	22.52	27.34	18.66	37.63	25.50
1,8-cineole	0.33	–	0.04	–	13.04	0.24	0.45	0.03	0.79	–	–
(E)-β-ocimene	–	0.09	0.61	–	0.11	–	–	–	0.03	0.06	–
γ-terpinene	0.51	0.18	0.11	–	0.77	–	2.17	1.22	0.78	–	–
cis-linalool oxide	0.45	–	0.66	0.02	1.00	0.50	–	0.67	–	0.05	0.78
trans-linalool oxide	0.21	0.44	–	0.03	0.31	0.10	0.25	0.32	–	–	–
α-terpinolene	0.05	–	–	–	–	–	–	–	–	0.55	–
linalool	46.50	42.66	13.39	9.76	10.40	15.46	14.83	20.52	22.36	13.00	33.44
nonanal	–	–	0.32	–	–	0.21	–	–	–	0.94	–
terpinen-4-ol	2.30	–	12.21	0.66	2.02	0.02	2.17	–	1.21	0.33	0.54
cryptone	–	0.58	0.29	–	2.59	–	–	–	0.50	0.07	–
α-terpineol	1.10	0.23	0.22	1.31	1.18	0.03	1.22	0.34	0.37	0.27	0.07
piperitone	0.30	–	4.35	–	–	0.11	0.22	–	–	–	–
geraniol	0.40	–	–	–	0.02	0.34	–	–	0.11	–	–
phellandral	–	–	0.11	0.72	4.21	0.52	1.43	–	0.89	0.46	–
2-undecanone	0.45	4.10	0.30	–	10.70	4.10	6.60	0.16	7.26	6.66	2.11
E-methyl cinnamate	15.70	1.77	2.12	3.27	2.92	2.22	3.33	–	1.26	–	2.34
trans-caryophyllene	0.40	10.66	4.51	10.62	2.05	4.64	1.84	5.14	10.37	8.63	0.04
Allo-aromadendrene	–	0.08	0.22	–	–	1.76	–	–	0.56	0.73	0.33
germacrene D	–	0.15	–	–	–	–	–	–	0.04	–	0.19
β-selinene	0.78	0.09	–	–	0.25	0.04	–	0.08	0.13	–	–
2-tridecanone	0.21	0.39	1.06	–	4.79	2.21	–	1.69	0.77	0.67	–
caryophyllene oxide	0.56	0.26	0.30	0.45	1.96	0.98	–	0.35	0.33	1.22	–
β-eudesmol	–	–	–	0.25	0.03	–	0.02	0.33	0.71	0.08	–

α-eudesmol	0.77	–	0.24	0.23	0.34	–	0.67	–	–	–	–
α-cadinol	0.09	0.41	–	–	–	0.27	0.02	–	0.11	–	0.39
tetradecanoic acid	0.34	–	–	–	11.17	0.67	2.56	–	0.69	0.79	0.22
phytol	0.60	–	0.22	–	1.81	0.89	0.21	0.56	0.49	0.47	0.45
hexadecanoic acid	0.03	0.34	–	4.01	0.45	1.11	0.55	0.77	2.68	0.28	0.03
oleic acid	–	0.19	–	0.67	0.34	0.47	0.78	0.21	0.31	–	–
monoterpene hydrocarbons	23.8	28.81	51.76	61.41	18.38	59.01	54.61	61.17	39.53	56.61	55.67
oxygenated monoterpenes	67.29	45.1	33.1	15.77	35.1	19.54	23.9	21.88	26.99	14.11	37.17
sesquiterpene hydrocarbons	1.18	10.98	4.73	10.62	2.3	6.44	1.84	5.22	11.1	9.36	0.56
oxygenated sesquiterpenes	1.42	0.67	0.54	0.93	2.33	1.25	0.71	0.68	1.15	1.3	0.39
oxygenated diterpenes	0.60	–	0.22	–	1.81	0.89	0.21	0.56	0.49	0.47	0.45
others	1.03	5.89	2.32	4.68	31.38	9.55	11.09	3.52	12.64	10.73	3.06
Total identified (%)	**95.32**	**91.45**	**92.67**	**93.41**	**91.30**	**96.68**	**92.36**	**93.03**	**91.90**	**92.58**	**97.30**
Oil yield (%)	1.9	3.8	1.6	2.1	3.0	1.9	2.4	2.8	2.0	1.7	2.7

To characterize and verify the variation of the essential oils and to identify the different possible chemotypes in the populations of *Z. alatum*, their compositions were analysed by cluster analysis. Cluster analysis allowed separating the nineteen populations into five groups, each representing a chemotype. The five different chemotypes characterized were Chemotype I (limonene), Chemotype II (linalool/ sabinene/ 2-undecanone), Chemotype III (linalool), Chemotype IV (limonene/ β-phellandrene/ linalool) and Chemotype V (β-phellandrene/ linalool/ sabinene). The oils of the chemotype I (P3, P8, P9, P11, P15) and chemotype III (P4, P13) were characterised by the domination of limonene (23.29–41.48%) and linalool (42.66–46.50%), respectively. The chemotype II (P1, P7, P17) also possessed linalool rich oil (32.36–40.12%) along with sabinene (7.21–13.00%) and 2-undecanone (0.21–18.15%). On the other hand, β-phellandrene (18.66–37.63%) was characterized as the first main constituent followed by linalool and sabinene in chemotype V (P2, P5, P10, P14, P16, P18), while in the oil of chemotype IV (P6, P12, P19), limonene was slightly more abundant than β-phellandrene and linalool.

Taking into account our present and previous studies on the composition of *Z. alatum* oils (seed and fruit pericarp) from India, it has been noted that most of the oils contained linalool in higher amounts, such as 70.6% in the seed oil from northern India (Jain et al., 2001) and 71.2-72% in fruit pericarp oil from North-western Himalaya (Shah et al., 1991; Mohan et al., 2012). In another study from this region, linalool (45.2%) was the most abundant compound, however, limonene (19.6%) and β-phellandrene (14.4%) were also detected in appreciable amounts (Kumar et al., 2014). A seed oil sample from Spain also reported the presence of linalool to an extent of 87.7% (Ramidi et al., 1998). In contrast, compared with different studies, the present study has shown the domination of limonene and β-phellandrene chemotypes along with linalool rich seed oils. So, the populations having β-phellandrene and limonene rich seed oils might be considered as new chemotypes in the *Z. alatum*.

CONCLUSION

Although the populations of *Z. alatum* have shown a clear chemical variability in the seed essential oils but no correlation was found between the chemical composition of the oils and altitudes of the collection sites. The variations may be due to the influence of edaphic factors or genetic variability; nevertheless, the study has provided noteworthy data on the presence of different chemotypes in seed essential oil of *Z. alatum* populations.

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CONFLICTS OF INTEREST

The author(s) declare(s) no conflicts of interest.

DECLARATION

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