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Volatile Compound Profile Analysis of Seasonal Flower, Fruit, Leaf, and Stem of *Zanthoxylum armatum* DC. from Manipur Using HS-SPME-GC-MS

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Abstract: In the present study, GC-MS analyses were performed with powder samples of flower, fruit, leaf, and stem of *Zanthoxylum armatum* DC. collected from Thambalkhong, Imphal-East district of Manipur, a north-eastern region of India, based on the season and growth stage of the plant using the extraction method headspace solid-phase microextraction (HS-SPME) to study the total profile of volatile compounds. Variations were discovered in the volatile compound profiles. HS-SPME-GC-MS analyses of the plant parts detected and identified 16 to 36 compounds and found a total area percentage composition of 96.81 to 98.63%. The analysis showed that nine common compounds were detected in the studied plant parts and seasons, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, and caryophyllene. The monoterpenoid eucalyptol (1,8-cineole) was revealed to be the principal component with an area percentage composition of 31.02% in spring leaf to 73.16% in monsoon stem. The extraction method used in this investigation was very fast and feasible for the analysis, and the findings of the present study will help understand the mechanism behind the changes in the plant's volatile organic compound profile and future research work for selecting aroma-rich accessions for targeted improvement of this plant.

Keywords: *Z. armatum* DC.; HS-SPME; GC-MS; volatile compounds; profile; eucalyptol

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1. Introduction

Zanthoxylum armatum DC. (synonyms: *Z. alatum* Hemsl., *Z. alatum* Roxb.) is an important medicinal plant which belongs to the Rutaceae family. It is a thorny shrub or small tree that is extensively used in the traditional systems of medicine in different parts and throughout the north-east region of India. The plant parts such as leaves, stems, fruits, seeds and bark are traditionally used for treating stomachache, toothache, cold, fever, headache, asthma, abdominal pain, etc. Its common name includes winged prickly ash, prickly ash, and toothache tree and other local names are Tejphal (Hindi), Mukthrubi (Manipuri), Konda-Kasimi (Telugu), Timur (Nepali) etc. It is widely distributed in India occurring throughout the north-eastern part of the country and at altitudes up to 2500 m from Kashmir to Bhutan. It is found at an altitude of 1300–1500 m in China, Taiwan, Nepal, Philippines, Malaysia, Pakistan, and Japan [1]. It is used as a spice in Indian, Chinese, Nepali, Sichuan, and Tibetan cuisine [2].

Studies showed the extract, fraction, and essential oil of the plant possessed biological activities. An anti-inflammatory activity was observed with the ethyl acetate fraction of

95% ethanolic extract of stems and roots of the plant when tested in mice [3], aqueous extracts of leaves of *Z. armatum* demonstrated an anti-diabetic property in both in vivo and in vitro condition [4], the leaves' essential oil of the plant showed antinociceptive and anticonvulsant activities when experimented in NMRI mice [5], and the seeds' essential oil of *Z. armatum* DC. were revealed to have antioxidant, in vitro anti-inflammatory, and antibacterial activity against *E. coli* and *S. aureus* [6]. These findings revealed the importance of the plant and potential for further studies.

The literature showed that a GC-MS analysis of different parts of *Z. armatum*, such as leaves, fruits, seeds, pericarp, stem bark, branches, and aerial parts, has been reported using essential oils or extracts. Reports from countries such as India, China, Pakistan, Nepal, and Vietnam revealed the extraction of essential oil by hydrodistillation and extraction of compounds using solvents and injected the liquid sample into the instrument for analysis [7–14]. Different studies using essential oils have identified linalool [15–17], α-pinene [10], 2-undecanone [18–20], bornyl acetate [21], 3-borneol [12], β-terpinene [11], (*Z*)-β-ocimene [6], β-phellandrene [22], and 1,8-cineole [14] as major constituents. Numerous studies on the chemical makeup of *Z. armatum* essential oil have been published from different parts of India, such as Uttarakhand, Himachal Pradesh, Uttar Pradesh, etc., predominantly from Uttarakhand [6,10,16,19,21,23,24].

The present study aims to investigate the volatile chemical composition profile of *Z. armatum* DC. plant parts, namely, the flower, fruit, leaf, and stem, which were collected based on season and growth stage of the plant from Manipur, a north-eastern state of India (part of the Indo-Burma biodiversity hotspot) to find variation in the volatile chemical composition profile in the samples under study and to find out the principal component. The present study used dry-powder sample materials for the extraction of volatile compounds using the extraction method headspace solid-phase microextraction (HS-SPME) instead of hydrodistillation method used in earlier studies. The HS-SPME method is an easy and less time-consuming method for sample preparation, which requires a small amount of sample for the extraction of volatile compounds compared to hydro-distillation method to extract essential oil.

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

Z. armatum DC. (female plant, sample code Zan 7) was collected from Thambalkhong, Imphal East District of Manipur (latitude—N 24°47.645′, longitude—E 093°57.719′ ± 14 ft, elevation—2499 ft msl). The collections were performed in four different phases based on the season and its growth stage. During the flowering stage in spring, the plant parts, flower, leaf, and stem, were collected. With the start of summer, green young fruit, leaf, and stem samples were collected, and during the following monsoon, red, mature fruit, leaf, and stem samples were collected. Finally, after the fruiting stage was completed, leaf and stem were collected during winter. The collected plant materials were thoroughly checked for any possible contamination such as small insects, dust, and injured or torn parts, and those were removed if any from the collected materials. The materials were then properly cleaned with distilled water and wiped using a delicate task wiper (Kimwipes), then dried in shade at ambient temperatures between 20 and 25 °C, and later ground into a powder with a grinder prior to the extraction.

The identification of the plant was done at the Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, and also identified by plant taxonomist Dr Biseshwori Thongam of the Institute of Bioresources and Sustainable Development (IBSD), Imphal. The herbarium of the specimen was deposited at IBSD, and the voucher number (IBSD/M-268) was provided. A molecular identification of the plant was also conducted using barcoding genes or regions such as ITS, matK, rbcL, psbA-trnH, and trnL-trnF, and their corresponding sequence accession numbers provided by the National Center for Biotechnology Information (NCBI) were MW362848, MW518006, MW518014, MW517998, and MW517990, respectively.

2.2. Headspace Solid-Phase Microextraction (HS-SPME)

Solid-phase microextraction (SPME) fibre coated with 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) from Supelco (57344-U, fused silica, black plain) was used for the experiment, which is compatible for the extraction of gasses and low-molecular-weight compounds (MW 30-225) as volatile organic compounds are low-molecular-weight (50–200 daltons) small molecules having a high vapour pressure under ambient conditions [25]. Conditioning was carried out prior to every injection for 10 min at 250 °C. All the extractions of the powdered samples were performed in 15 mL septum-sealed clear glass vials (Supelco, 27159). The extraction vial was filled, with the sample occupying one-third of the vial. The SPME fibre was exposed to the sample for one hour for extraction and then immediately transferred to the GC-MS instrument for injection.

2.3. Gas Chromatography—Mass Spectrometry (GC-MS) Analysis

A GC-MS from Thermo Scientific (Trace 1300-TSQ DUO) with a triple quadrupole detector was used for the analysis of the chemical composition of the samples. A TG-5MS column having a 0.25 µm film thickness, 0.25 mm I.D., and 30 m length (Thermo Scientific™ TraceGOLD™ TG-5MS GC Column, 26098-1420) was used for the study. The ionization energy was set at 70 eV for the GC-MS detection. The inlet injector and mass transfer line temperatures were set at 250 °C and 280 °C, respectively. The ion source temperature was maintained at 240 °C. The initial column temperature was programmed from 40 °C for 1 min to 250 °C with a heating ramp at a rate of 5 °C/min and then held at 250 °C for 20 min. Helium was used as a carrier gas at a flow rate of 1 mL/min with a split ratio of 1:20. The SPME fibre was exposed to the sample and was injected for 2 min into the GC-MS inlet injector, and the analysis was performed for one hour. Data acquisition and processing were performed using Xcalibur software. The chromatograms are presented in Figure 1.

The chemical compounds detected were then identified based on the scores of the NIST match factor or similarity index (SI) and reverse match factors or reverse search index (RSI) of the mass spectra present in the National Institute of Standards and Technology (NIST) GC-MS Library 2017, following the NIST library guidelines for match factor (SI) and reverse match factor (RSI) thresholds of mass spectral match (>900—excellent match, 800–900—good match, 700–800—fair match and <600—poor match) and also referred to previous literature. An area percentage composition greater than 0.09% was used to analyse the results.

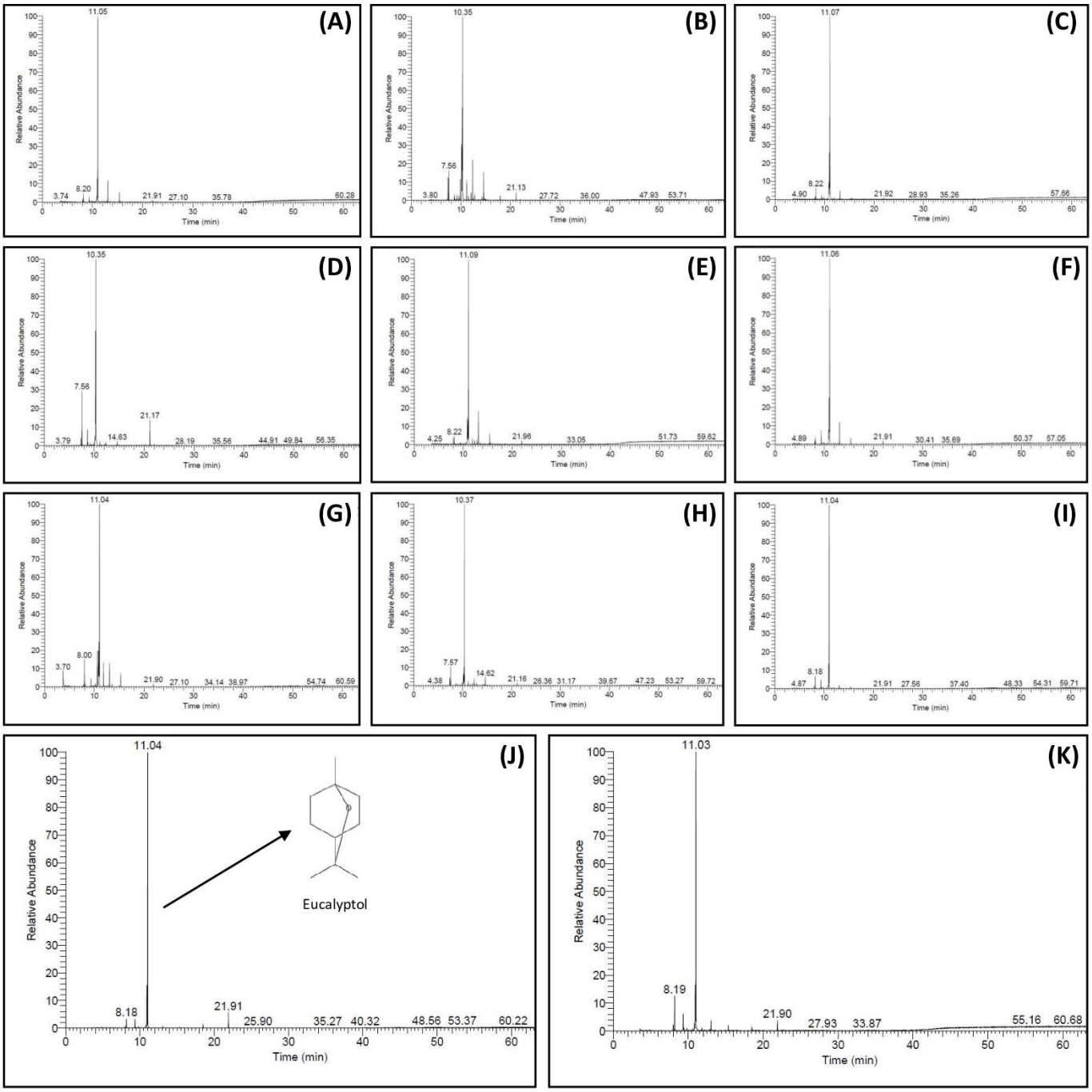

Figure 1. Chromatograms from the GC-MS analysis of (**A**) flower in spring, (**B**) leaf in spring, (**C**) stem in spring, (**D**) fruit in summer, (**E**) leaf in summer, (**F**) stem in summer, (**G**) fruit in monsoon, (**H**) leaf in monsoon, (**I**) stem in monsoon, (**J**) leaf in winter, and (**K**) stem in winter of *Z. armatum* DC.

3. Results

Variations were found in the volatile compound profiles of the HS-SPME-GC-MS analysis of the plant parts, which showed the detection and identification of compounds ranging from 16 to 36 and a total percentage composition of 96.81 to 98.63%. The number of compounds identified and the total area percentage composition of plant parts collected in different seasons were as follows: flowers in spring, 22 and 96.81% (Table 1); fruits in summer, 23 and 97.77% (Table 2); fruits in monsoon, 23 and 98.63% (Table 3); leaves in spring, 30 and 98.26% (Table 4); leaves in summer, 36 and 97.15% (Table 5); leaves in monsoon, 28 and 98.13% (Table 6); leaves in winter, 17 and 98.58% (Table 7); stems in spring,

22 and 97.80% (Table 8); stems in summer, 22 and 98.14% (Table 9); stems in monsoon, 16 and 98.42% (Table 10); and stems in winter, 25 and 97.47% (Table 11).

Table 1. Compounds identified in *Z. armatum* DC. flower in spring.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	5.31	Propane	430	837	0.12
2.	8.02	α-Thujene	897	916	1.01
3.	8.20	α-Pinene	930	942	2.75
4.	9.35	Sabinene	888	900	1.89
5.	9.44	β-Pinene	900	922	0.47
6.	9.85	β-Myrcene	823	836	0.97
7.	10.24	β-Thujene	710	884	0.15
8.	10.60	Terpinolene	869	923	0.37
9.	10.85	o-Cymene	937	962	2.22
10.	10.96	Sylvestrene	847	889	9.51
11.	11.05	Eucalyptol	931	936	62.13
12.	11.85	4-Carene, (1S,3S,6R)-(-)-	804	866	0.36
13.	12.30	cis-Linalool oxide	833	856	0.49
14.	12.77	Thujaketone	229	927	0.62
15.	12.98	Decane	775	870	0.24
16.	13.07	Linalool	879	884	7.49
17.	13.58	β-Thujone	764	911	0.23
18.	14.27	Isopinocarveol	785	851	0.31
19.	15.36	Terpinen-4-ol	863	864	4.22
20.	15.55	1H-Indene, 1-methylene-	840	950	0.29
21.	15.93	Sarohornene	481	935	0.17
22.	21.91	Caryophyllene	874	890	0.80
		Total area percentage: 96.81			

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 2. Compounds identified in *Z. armatum* DC. fruit in summer.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	7.39	α-Thujene	902	915	1.73
2.	7.56	α-Pinene	926	933	10.30
3.	7.98	Camphene	796	892	0.10
4.	8.68	Sabinene	909	922	3.14
5.	8.77	β-Pinene	868	878	0.50
6.	9.20	L-β-Pinene	835	846	0.73
7.	9.57	α-Phellandrene	816	863	0.32
8.	9.93	Terpinolene	889	912	0.69
9.	10.17	o-Cymene	926	946	2.82
10.	10.28	Sylvestrene	870	895	26.88
11.	10.35	Eucalyptol	904	912	39.25

Table 2. *Cont.*

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
12.	10.59	1,5-Cyclooctadiene, 1,5 dimethyl-	829	901	0.18
13.	11.17	γ-Terpinene	881	885	0.95
14.	11.43	trans-p-Menth-2-ene-1-ol	775	827	0.10
15.	12.05	Cyclohexene,1,5,5-trimethyl-3-methylene-	799	900	1.03
16.	12.31	Decane	787	890	0.38
17.	12.37	Linalool	864	881	0.91
18.	14.63	Terpinen-4-ol	856	860	1.04
19.	14.80	1H-Indene, 1-methylene-	799	903	0.16
20.	20.25	Methyl cis-cinnamate	832	900	0.30
21.	21.18	Caryophyllene	911	916	5.73
22.	21.39	5,10-Pentadecadiyne, 1-chloro-	262	911	0.10
23.	22.03	Humulene	823	859	0.43
Total area percentage: 97.77					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 3. Compounds identified in *Z. armatum* DC. fruit in monsoon.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	7.79	Anisole	940	954	0.53
2.	8.00	α-Thujene	905	914	5.18
3.	8.19	α-Pinene	927	942	0.59
4.	9.33	Sabinene	917	929	1.54
5.	9.42	β-Pinene	889	919	0.13
6.	9.84	L-β-Pinene	792	813	0.57
7.	10.23	α-Phellandrene	878	887	0.75
8.	10.45	Anisole, o-methyl-	855	874	0.23
9.	10.58	Terpinolene	929	941	8.41
10.	10.73	trans-Isolimonene	737	843	0.13
11.	10.83	o-Cymene	942	961	11.47
12.	10.95	Sylvestrene	793	850	13.14
13.	11.04	Eucalyptol	924	933	42.45
14.	11.84	γ-Terpinene	909	910	4.48
15.	12.12	cis-β-Terpineol	841	879	0.10
16.	12.28	cis-Linalool oxide	858	872	0.23
17.	12.73	Cyclohexene,1,5,5-trimethyl-3-methylene-	812	906	0.64
18.	13.06	Linalool	909	911	4.24
19.	13.25	β-Thujone	807	900	0.11
20.	13.56	Tanacetone	849	893	0.54
21.	14.97	Benzene, p-dimethoxy-	943	956	0.36
22.	15.34	Terpinen-4-ol	866	866	2.29

Table 3. *Cont.*

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
23.	21.90	Caryophyllene	884	885	0.52
Total area percentage: 98.63					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 4. Compounds identified in *Z. armatum* DC. leaf in spring.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	7.17	Anisole	933	941	0.33
2.	7.39	α-Thujene	927	931	3.23
3.	7.56	α-Pinene	925	928	4.36
4.	8.67	Sabinene	936	941	0.72
5.	8.75	β-Pinene	873	879	0.28
6.	9.19	β-Myrcene	882	882	1.07
7.	9.55	α-Phellandrene	901	904	0.90
8.	9.77	Anisole, o-methyl-	865	883	0.14
9.	9.91	Terpinolene	897	903	3.61
10.	10.15	o-Cymene	901	910	11.87
11.	10.27	Sylvestrene	864	883	18.36
12.	10.35	Eucalyptol	924	928	31.02
13.	10.54	β-Ocimene	846	864	0.12
14.	10.84	3-Carene	870	910	0.12
15.	11.14	γ-Terpinene	922	922	3.37
16.	11.55	cis-Linalool oxide	929	935	0.69
17.	12.02	Cyclohexene,1,5,5-trimethyl-3-methylene-	775	887	1.60
18.	12.35	Linalool	930	930	6.29
19.	12.51	β-Thujone	820	897	0.30
20.	12.82	Thujone	906	912	1.06
21.	14.23	Benzene, p-dimethoxy-	950	956	0.68
22.	14.50	Isocamphopinone	825	882	0.19
23.	14.59	Terpinen-4-ol	902	902	4.74
24.	14.76	1H-Indene, 1-methylene-	916	944	0.35
25.	14.84	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	671	887	0.15
26.	14.98	α-Terpineol	891	931	0.12
27.	15.14	4-Cyclopropylnorcarane	520	855	0.18
28.	17.89	Dodecane	876	898	0.87
29.	21.13	Caryophyllene	940	942	1.42
30.	21.98	Humulene	860	889	0.12
Total area percentage: 98.26					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 5. Compounds identified in *Z. armatum* DC. leaf in summer.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	3.75	Isopentyl alcohol	836	899	0.12
2.	3.80	sec-Butylcarbinol	819	874	0.16
3.	4.25	Cyclobutene, 2-propenylidene-	874	905	0.38
4.	4.41	2-Ethyl-oxetane	675	840	0.11
5.	4.50	4-Hexen-3-ol	751	949	0.14
6.	4.73	Crotonaldehyde, 3-methyl-	568	846	0.10
7.	4.88	n-Caproaldehyde	826	850	0.31
8.	6.28	3-Hexen-1-ol	758	907	0.12
9.	6.59	Hydroperoxide, hexyl	799	869	0.16
10.	7.82	Anisole	873	925	0.14
11.	8.03	α-Thujene	904	923	1.75
12.	8.22	α-Pinene	918	927	1.96
13.	9.08	Benzaldehyde	840	878	0.19
14.	9.37	Sabinene	906	920	0.42
15.	9.45	β-Pinene	903	911	0.29
16.	9.82	Sulcatone	788	880	0.64
17.	10.26	α-Phellandrene	831	848	0.27
18.	10.62	Terpinolene	903	923	0.64
19.	10.87	o-Cymene	928	939	8.94
20.	10.99	Sylvestrene	834	881	8.04
21.	11.09	Eucalyptol	928	931	53.05
22.	11.88	γ-Terpinene	914	917	1.40
23.	12.33	cis-Linalool oxide	904	916	1.08
24.	12.81	trans-Linalool oxide (furanoid)	794	877	1.49
25.	13.11	Linalool	926	929	8.70
26.	13.24	(E)-9-Hydroxylinalool	374	816	0.10
27.	13.31	β-Thujone	750	867	0.16
28.	13.62	Thujone	837	864	0.49
29.	14.31	Isopinocarveol	838	865	0.35
30.	15.19	trans-Linalool 3,7-oxide	845	884	0.10
31.	15.40	Terpinen-4-ol	896	896	3.29
32.	15.59	1H-Indene, 1-methylene-	911	949	0.45
33.	15.79	α-Terpineol	873	918	0.14
34.	15.97	Sarohornene	456	946	0.25
35.	21.96	Caryophyllene	919	924	1.07
36.	43.59	Phthalic acid, di(oct-3-yl) ester	531	856	0.15
Total area percentage: 97.15					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 6. Compounds identified in *Z. armatum* DC. leaf in monsoon.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	7.18	Anisole	892	933	0.20
2.	7.39	α-Thujene	913	920	2.73
3.	7.57	α-Pinene	943	947	6.09
4.	7.98	Camphene	878	938	0.11
5.	8.68	Sabinene	920	926	0.63
6.	8.77	β-Pinene	885	892	0.36
7.	9.19	β-Myrcene	865	872	0.51
8.	9.56	α-Phellandrene	872	878	0.42
9.	9.92	Terpinolene	904	914	1.30
10.	10.16	o-Cymene	935	946	5.72
11.	10.28	Sylvestrene	846	876	8.02
12.	10.36	Eucalyptol	919	922	58.73
13.	11.16	γ-Terpinene	903	904	1.85
14.	11.57	cis-Linalool oxide	900	914	0.45
15.	12.04	Cyclohexene,1,5,5-trimethyl-3-methylene-	780	898	1.09
16.	12.37	Linalool	937	939	2.81
17.	12.53	β-Thujone	789	893	0.14
18.	12.84	Thujone	898	908	0.86
19.	13.47	(+)-Nopinone	834	901	0.23
20.	14.26	Benzene, p-dimethoxy-	952	963	0.65
21.	14.53	Isocamphopinone	844	916	0.19
22.	14.62	Terpinen-4-ol	893	893	3.27
23.	14.79	1H-Indene, 1-methylene-	873	941	0.21
24.	14.87	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran	638	870	0.10
25.	15.17	4-Cyclopropylnorcarane	499	852	0.18
26.	15.38	Heptane, 3,4,5-trimethyl-	560	852	0.15
27.	21.16	Caryophyllene	923	927	1.02
28.	22.01	Humulene	789	837	0.11
		Total area percentage: 98.13			

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 7. Compounds identified in *Z. armatum* DC. leaf in winter.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	6.54	Hydroperoxide, hexyl	809	887	0.16
2.	8.00	α-Thujene	870	896	0.61
3.	8.18	α-Pinene	935	946	2.25
4.	9.32	Sabinene	896	907	2.13
5.	9.41	β-Pinene	893	909	0.23
6.	9.83	β-Myrcene	828	836	0.62
7.	10.58	Terpinolene	858	905	0.18
8.	10.83	o-Cymene	926	954	1.28

Table 7. *Cont.*

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
9.	10.94	Sylvestrene	866	897	12.95
10.	11.04	Eucalyptol	934	937	71.96
11.	11.84	4-Carene, (1S,3S,6R)-(-)-	810	870	0.14
12.	12.77	m-Cymene	611	870	0.11
13.	13.06	Linalool, formate	812	852	0.45
14.	15.36	2-Cyclopenten-1-one, 2,3,5-trimethyl-4-methylene-	497	867	0.25
15.	18.50	2-Undecanone	866	870	1.08
16.	21.91	Caryophyllene	909	914	4.03
17.	22.76	cis-α-Bisabolene	831	869	0.15
Total area percentage: 98.58					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 8. Compounds identified in *Z. armatum* DC. stem in spring.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	3.82	sec-Butylcarbinol	771	852	0.24
2.	4.90	Hexanal or n-Caproaldehyde	846	873	0.37
3.	7.83	Anisole	899	941	0.12
4.	8.04	α-Thujene	887	902	0.83
5.	8.22	α-Pinene	937	947	3.12
6.	9.36	Sabinene	914	925	1.22
7.	9.45	β-Pinene	923	934	0.46
8.	9.87	α-Myrcene	853	858	0.76
9.	10.26	β-Thujene	748	893	0.11
10.	10.62	Terpinolene	855	908	0.20
11.	10.86	o-Cymene	945	962	6.98
12.	10.98	Sylvestrene	851	892	17.35
13.	11.07	Eucalyptol	930	936	61.02
14.	11.87	γ-Terpinene	867	871	0.52
15.	12.32	cis-Linaloloxide	786	925	0.11
16.	12.79	m-Cymene	648	903	0.37
17.	12.99	Decane	749	875	0.18
18.	13.08	Linalool	889	894	2.41
19.	15.03	Benzene, p-dimethoxy-	832	915	0.11
20.	15.37	Terpinen-4-ol	855	859	0.48
21.	15.56	1H-Indene, 1-methylene-	921	957	0.37
22.	21.92	Caryophyllene	889	902	0.47
Total area percentage: 97.80					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 9. Compounds identified in *Z. armatum* DC. stem in summer.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	6.60	Hydroperoxide, hexyl	782	872	0.12
2.	8.03	α-Thujene	906	924	1.25
3.	8.21	α-Pinene	932	942	1.81
4.	9.07	Benzaldehyde	883	927	0.18
5.	9.35	Sabinene	917	928	3.56
6.	9.44	β-Pinene	906	916	0.40
7.	9.86	β-Myrcene	844	856	1.25
8.	10.25	β-Thujene	795	908	0.14
9.	10.61	Terpinolene	880	927	0.27
10.	10.85	o-Cymene	940	959	5.39
11.	10.97	Sylvestrene	860	898	17.36
12.	11.06	Eucalyptol	936	942	55.75
13.	11.86	γ-Terpinene	883	886	0.37
14.	12.30	cis-Linalool oxide	865	879	0.38
15.	12.77	m-Cymene	463	860	0.55
16.	13.07	Linalool	903	907	5.79
17.	13.58	β-Thujone	817	914	0.15
18.	14.28	Isopinocarveol	819	862	0.13
19.	15.36	Terpinen-4-ol	885	885	2.05
20.	15.55	1H-Indene, 1-methylene-	874	959	0.15
21.	18.51	2-Undecanone	874	891	0.17
22.	21.91	Caryophyllene	914	921	0.92
		Total area percentage: 98.14			

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 10. Compounds identified in *Z. armatum* DC. stem in monsoon.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	8.00	α-Thujene	899	915	1.10
2.	8.18	α-Pinene	933	941	4.15
3.	9.33	Sabinene	923	933	2.92
4.	9.41	β-Pinene	935	940	0.50
5.	9.83	β-Myrcene	858	864	0.59
6.	10.23	β-Thujene	792	887	0.10
7.	10.59	Terpinolene	882	918	0.21
8.	10.83	o-Cymene	942	964	1.97
9.	10.95	Sylvestrene	867	901	10.85
10.	11.04	Eucalyptol	941	944	73.16
11.	11.84	γ-Terpinene	864	867	0.30
12.	12.29	cis-Linalool oxide	820	849	0.10
13.	12.75	m-Cymene	452	841	0.20
14.	13.06	Linalool	887	891	1.30

Table 10. *Cont.*

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
15.	15.35	Terpinen-4-ol	870	870	0.73
16.	21.91	Caryophyllene	881	896	0.24
Total area percentage: 98.42					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

Table 11. Compounds identified in *Z. armatum* DC. stem in winter.

Sl. No.	RT [a]	Compound	SI [b]	RSI [c]	RA [d] (%)
1.	4.87	n-Caproaldehyde	809	870	0.26
2.	8.01	α-Thujene	897	912	1.51
3.	8.19	α-Pinene	944	952	6.77
4.	9.33	Sabinene	911	921	3.28
5.	9.42	β-Pinene	919	932	0.71
6.	9.84	β-Myrcene	840	858	0.73
7.	10.23	β-Thujene	775	890	0.15
8.	10.59	Terpinolene	874	913	0.42
9.	10.83	o-Cymene	942	962	1.90
10.	10.95	Sylvestrene	875	906	11.09
11.	11.03	Eucalyptol	940	943	61.30
12.	11.84	γ-Terpinene	881	887	0.66
13.	12.13	cis-β-Terpineol	836	904	0.11
14.	12.29	cis-Linalool oxide	798	866	0.16
15.	12.76	2-Cyclopenten-1-one, 2,3,5-trimethyl-4-methylene-	389	838	0.31
16.	12.97	Decane	816	901	0.28
17.	13.06	Linalool	883	890	2.41
18.	13.57	β-Thujone	744	907	0.14
19.	14.26	Isopinocarveol	725	854	0.10
20.	15.35	Terpinen-4-ol	879	883	1.35
21.	15.54	1H-Indene, 1-methylene-	779	955	0.16
22.	15.92	Sarohornene	455	934	0.10
23.	18.50	2-Undecanone	894	910	1.00
24.	21.90	Caryophyllene	925	933	2.38
25.	22.76	Humulene	822	872	0.19
Total area percentage: 97.47					

Note: [a] Retention time. [b] Match factor or similarity index on TG-5MS capillary column. [c] Reverse match factor or reverse search index on TG-5MS capillary column. [d] Relative area (peak area relative to the total peak area).

The analysis of the spring flower and summer and monsoon fruits detected and identified 22, 23, and 23 compounds, constituting 96.81, 97.77, and 98.63% of total composition, respectively, and 11 common compounds were found among them, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, linalool, terpinen-4-ol, and caryophyllene. The analysis of leaf samples collected in spring, summer, during the monsoon, and in winter revealed the detection and identification of 30, 36, 28,

and 17 compounds covering 98.26, 97.15, 98.13, and 98.58% of the total composition, respectively, and the number of common compounds found was 9, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, and caryophyllene. The analysis of stem samples collected in spring, summer, during the monsoon, and winter revealed the detection and identification of 22, 22, 16, and 25 compounds accounting for 97.80, 98.14, 98.42 and 97.47% of the total composition, respectively, and 13 common compounds were detected, namely, α-thujene, α-pinene, sabinene, β-pinene, β-thujene, terpinolene, o-cymene, sylvestrene, eucalyptol, γ-terpinene, linalool, terpinen-4-ol, and caryophyllene. The number of common compounds found in flower, leaf, and stem samples collected in spring was 12, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, linalool, terpinen-4-ol, 1H-indene, 1-methylene-, and caryophyllene; for fruit, leaf, and stem samples collected in summer, it was 13, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, γ-terpinene, linalool, terpinen-4-ol, 1H-indene, 1-methylene-, and caryophyllene; for fruit, leaf, and stem samples collected in monsoon was 13, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, γ-terpinene, cis-linalool oxide, linalool, terpinen-4-ol, and caryophyllene and for leaf and stem samples collected in winter, it was 11, namely, α-thujene, α-pinene, sabinene, β-pinene, β-myrcene, terpinolene, o-cymene, sylvestrene, eucalyptol, 2-undecanone, and caryophyllene. Overall, the number of common compounds found in the examined plant parts and seasons was nine, namely, α-thujene, α-pinene, sabinene, β-pinene, terpinolene, o-cymene, sylvestrene, eucalyptol, and caryophyllene. The number of common compounds are presented in a Venn diagram prepared by using the web-based tool InteractiVenn [26] (Figure 2). A comparison was made between spr-flower with sum-fruit and mon-fruit in Figure 2A because the sample collection was based on season and growth stage, and flowers and fruits are not available in all the seasons as the flower transforms into fruit and produces seeds, whereas leaf and stem parts are available throughout the year.

Five major compounds discovered based on the order of their relative area percentages in the plant parts and seasons were eucalyptol (62.13%), sylvestrene (9.51%), linalool (7.49%), terpinen-4-ol (4.22%), and α-pinene (2.75%) for flowers in spring; eucalyptol (39.25%), sylvestrene (26.88%), α-pinene (10.30%), caryophyllene (5.73%), and sabinene (3.14%) for fruits in summer; eucalyptol (42.45%), sylvestrene (13.14%), o-cymene (11.47%), terpinolene (8.41%), and α-thujene (5.18%) for fruits during the monsoon; eucalyptol (31.02%), sylvestrene (18.36%), o-cymene (11.87%), linalool (6.29%), and terpinen-4-ol (4.74%) for leaves in spring; eucalyptol (53.05%), o-cymene (8.94%), linalool (8.70%), sylvestrene (8.04%), and terpinen-4-ol (3.29%) for leaves in summer; eucalyptol (58.73%), sylvestrene (8.02%), α-pinene (6.09%), o-cymene (5.72%), and terpinen-4-ol (3.27%) for leaves in monsoon; eucalyptol (71.96%), sylvestrene (12.95%), caryophyllene (4.03%), α-pinene (2.25%), and sabinene (2.13%) for leaves in winter; eucalyptol (61.02%), sylvestrene (17.35%), o-cymene (6.98%), α-pinene (3.12%), and linalool (2.41%) for stems in spring; eucalyptol (55.75%), sylvestrene (17.36%), linalool (5.79%), o-cymene (5.39%), and sabinene (3.56%) for stems in summer; eucalyptol (73.16%), sylvestrene (10.85%), α-pinene (4.15%), sabinene (2.92%), and o-cymene (1.97%) for stems during the monsoon and eucalyptol (61.30%), sylvestrene (11.09%), α-pinene (6.77%), sabinene (3.28%), and linalool (2.41%) for stems in winter.

Eucalyptol occupied the highest area percentage composition (31.02% in spring leaves to 73.16% in monsoon stems) in the analysis, which was followed by sylvestrene (8.02% in monsoon leaves to 26.88% in summer fruits) (Figure 3), except in leaves in summer, where sylvestrene (8.04%) came after o-cymene (8.94%) and linalool (8.70%).

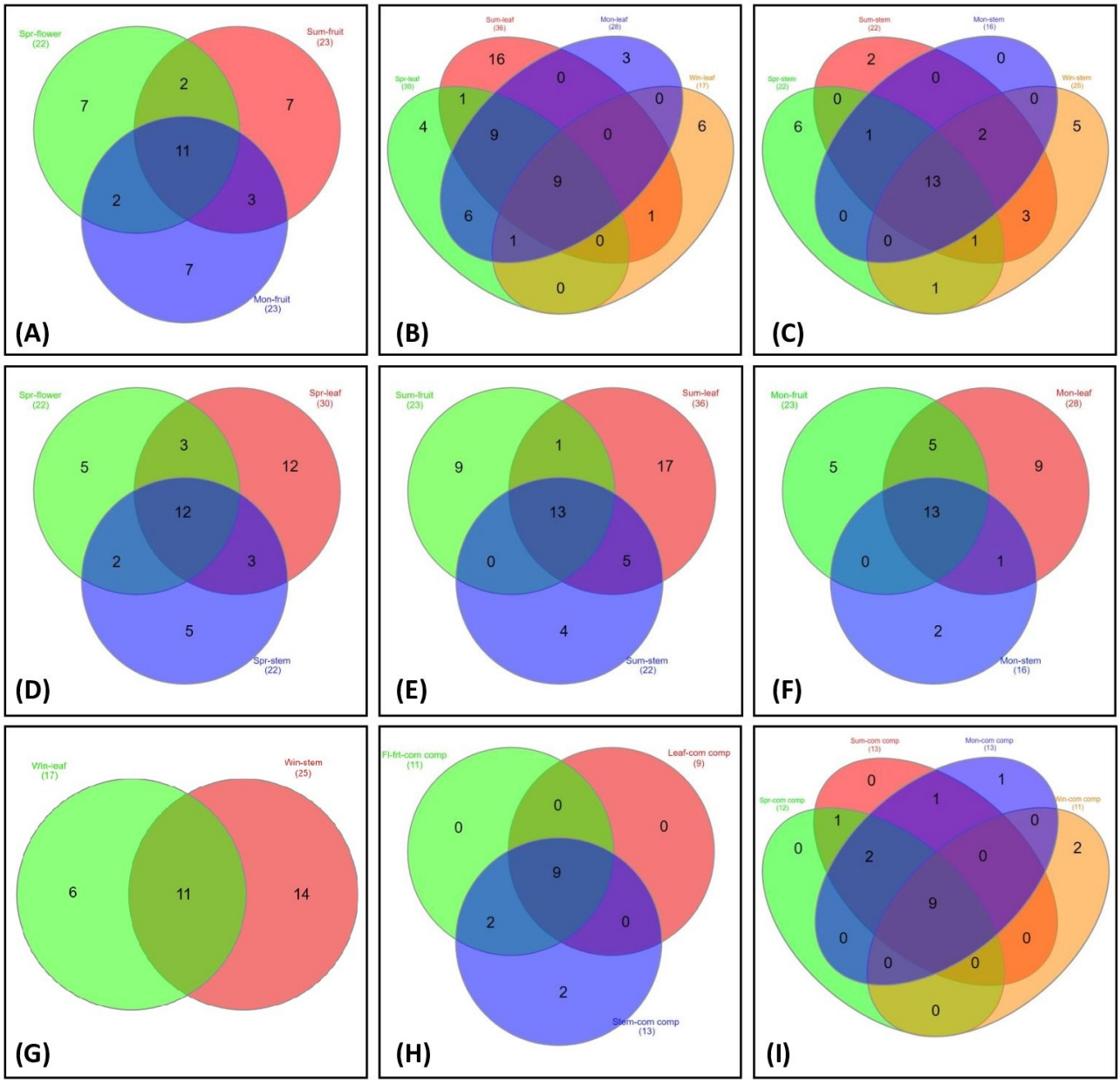

Figure 2. Venn diagram to find the number of common compounds found in (**A**) flowers in spring and fruits in summer and during the monsoon (e.g., 7 in green circle: compounds present only in spr-flower; 2 between green and pink circles: compounds present in spr-flower and sum-fruit; 7 in pink circle: compounds present only in sum-fruit; 3 in between pink and blue circles: compounds present in sum-fruit and mon-fruit; 7 in blue circle: compounds present only in mon-fruit; 2 between blue and green circles: compounds present in mon-frt and spr-flower; 11 between green, pink, and blue circles: compounds present in spr-flower, sum-fruit, and mon-fruit); (**B**) leaves in spring, summer, during the monsoon, and in winter; (**C**) stems in spring, summer, during the monsoon, and in winter; (**D**) flowers, leaves, and stems in spring; (**E**) fruits, leaves, and stems in summer; (**F**) fruits, leaves, and stems during the monsoon; (**G**) leaves and stems in winter; (**H**) the plant parts in spring, summer, during the monsoon, and in winter; and (**I**) the different seasons of plant parts in *Z. armatum* DC.

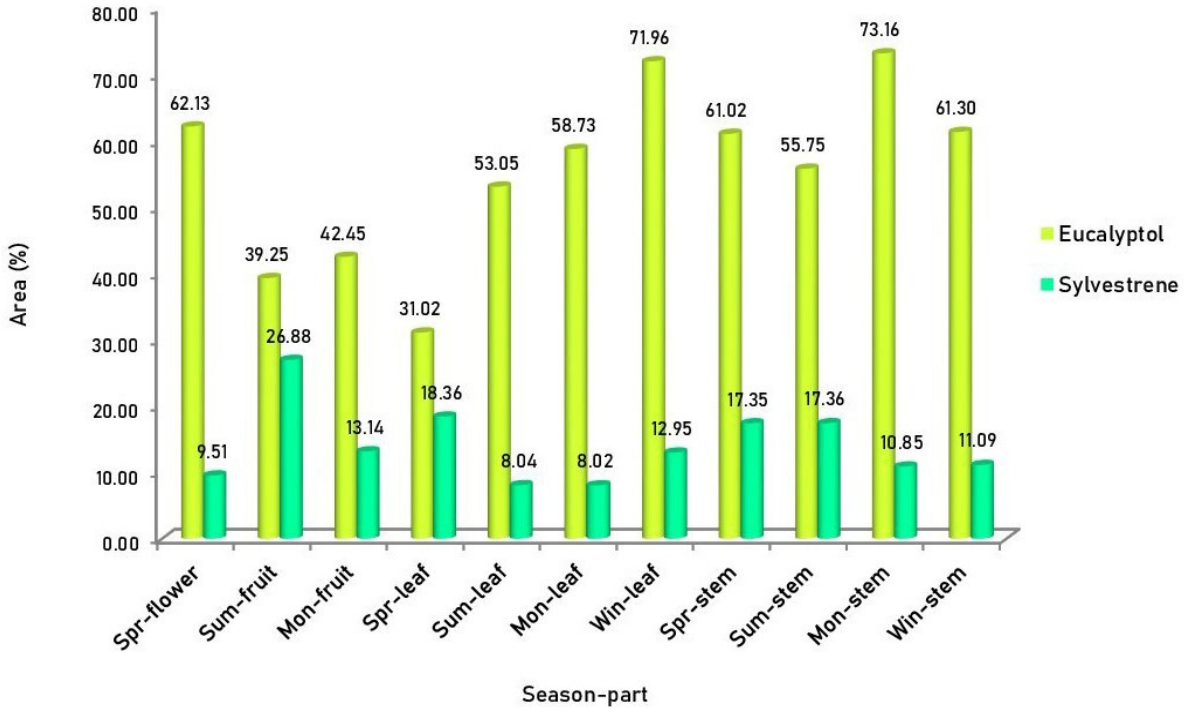

Figure 3. Area percentage composition of eucalyptol and sylvestrene in flowers, fruits, leaves, and stems with their season of collection of *Z. armatum* DC.

4. Discussion

In several earlier studies, the chemical composition analysis of various *Z. armatum* parts identified linalool as the primary compound. Linalool (72%), methyl cinnamate (12.2%), limonene (6.2%), and β-phellandrene (5.3%) were the major constituents of the pericarp essential oil of the fruit of *Z. armatum* DC. growing in the wild in the U.P. hills [27]. In another study, *Z. armatum* collected from Munshiyari (Pithoragarh district, Uttar Pradesh Hills of the Central Hi malayas) found that linalool (55.30%), limonene (22.46%), methyl cinnamate (8.82%), and myrcene (3.55%) were the major constituents in the fruit essential oil of *Z. armatum* [28]. Similarly, as previously stated, the major components in the dried seeds of *Z. alatum* purchased from a local market in Lucknow (Uttar Pradesh, India) were identified as linalool (71%), limonene (8.2%), β-phellandrene (5.7%), and (Z)-methylcinnamate (4.9%) [9]. The GC-MS analysis of the essential oil of seeds purchased from Gorakhpur's local market (India), found it to contain linalool (62%) and limonene (18.1%) as the major components [8]. Study findings from the samples collected or purchased from different places in Uttar Pradesh revealed linalool as the main compound with a high percentage composition.

Again, linalool (57%) and limonene (19.8%) were the major components in the seeds essential oil of *Z. armatum* DC. purchased from a local market in Delhi [15]. Leaves' essential oil of *Z. armatum* collected from north-western Himalaya (India) showed that the major components were linalool (30.58%), 2-decanone (20.85%), β-fenchol (9.43%), 2-tridecanone (8.86%), β-phellandrene (5.99%), sabinene (4.82%), and α-pinene (4.11%) [17]. In another study, a GC-MS analysis of leaves' essential oil of *Z. armatum* collected from different elevations and populations in the Salyan district of Nepal revealed that, on average, linalool (38.73%), limonene (19.82%), and undecan-2-one (22.75%) were the three major components [13]. Linalool was discovered to be the most abundant component in all the aforementioned investigations, with varying percentage composition in the GC-MS analysis of the essential oils of pericarp, fruits, seeds, and leaves.

Other researchers have identified different compounds as major components in *Z. armatum* instead of linalool. The leaf essential oils of the plant from Kumaon (India) had 2-undecanone and 2-tridecanone as the major components [18]. The essential oils

of the bark of *Z. armatum* collected from three different altitudes of the Kumaun region revealed the presence of α-pinene (33.9%, 28.9%, 35.9%) and 2-undecanone (3.9%, 16.2%, 10.1%) as the major constituents [10]. The compound 2-hydroxy cyclopentadecanone with a percentage composition of 27.37% was discovered as a prominent constituent in hexane extract of *Z. armatum* fruits obtained from the Bobang Village Development Committee of the Dhorpatan Hunting Reserve area of Nepal [29]. According to a study from Pakistan, 3-borneol (9.718%), iso-bornylacetate (9.574%), and dihydro carveol (8.816%) were identified as major components of seeds essential oil in *Z. armatum* collected from Balakot Mansehra (N.W.F.P., Pakistan) [12] and bornyl acetate (16.61–22.66%) was the main component in essential oil of leaves collected from Mandal forest (Uttarakhand, India) [21]. A study from China revealed β-terpinene (45.56%), piperitone (33.47%), and 3-carene (8.88%) as the main components of branch and leaf essential oil of plants collected from Wen County (Gansu province, China) [11]. β-phellandrene was discovered to be the predominant component in unripe fruit essential oil of *Z. armatum* collected from Thala village of (Palampur, India) [22]. The seed essential oil of the plant collected from Pithoragarh (Kumaun region of Uttarakhand, India) showed (Z)-β-ocimene (28.1%) as the main component [6].

In our study, the monoterpenoid eucalyptol (1,8-cineole) was revealed to be the main component in the GC-MS analysis of the flower, fruit, leaf, and stem of *Z. armatum* DC. In a previous study, 1,8-cineole was found as a major component with 41% in leaves' essential oil followed by 2-undecanone (9.6%), sabinene (8.4%), terpinen-4-ol (5.2%), linalool (4.5%), and α-terpineol (4.1%) out of 54 components separated by GC, and 50 were identified by a GC-MS analysis from a plant collected from the Hop Tien village, Trieu Son district, Thanh Hoa province of Vietnam [14], and its eucalyptol percentage composition was similar with those of the summer and monsoon fruits in the present study, which were 39.25 and 42.45%, respectively. However, the amount of the compound present in the samples may differ as it is determined by the peak area. The area percentage composition (area%) of a compound is directly related to the individual area of each peak. For any compound, it is calculated by dividing the individual area by the total area, which is the sum of all the peak areas in the chromatogram, multiplied by 100.

Eucalyptol was found as a component in numerous other research studies. Aerial essential oil of *Z. armatum* collected from the Dauladhar hills near Palampur, Himachal Pradesh, contained 1,8-cineole (15.7%) [30]. It was found in traces in seed essential oil [15]. Different percentage compositions of 1,8-cineole were reported from the leaf essential oils whose samples were collected in the first year (2008) and the second year (2009) and revealed to be 0.5% and 4.7% of the composition, respectively. It was also reported that the difference in percentage composition of the compound was an impact of the long distillation time of day 1 and day 2 which resulted in 4.3% and 0.5% of the composition, respectively [18]. Eucalyptol was identified as a compound in hexane extracts of *Z. armatum* fruit with a percentage composition of 3.02% [29]. It was found present at 0.25% in fruits' essential oil of *Z. armatum* [28].

The extraction technique and exposure time applied in the present study successfully extracted the volatile compounds and produced desirable results that were comparable with the previous study reports of volatile compounds detected and identified from essential oils of different parts of *Z. armatum* DC. but different in the main compounds identified (Table 12). A similar extraction method was used in the GC-MS analysis of dried powdered leaves of *Mikania glomerata* Sprengel, and the authors suggest it as a potential analytical tool for *M. glomerata*'s volatile and semivolatile compound analysis, which is faster and requires a smaller sample than the hydrodistillation of essential oils [31]. The SPME technique reduces the amount of sample and the time required for sample preparation. It is portable, sensitive, and easy to perform. As the principle of direct injection of liquid samples into the instrument and the SPME technique are different, it is expected that there will be some difference in the results of the volatile and semivolatile compound profiles.

Table 12. Major constituents of *Z. armatum* DC. (*Z. alatum* Roxb.) in earlier studies.

Sl. No.	Plant Part	Sample Type	Main Constituents, Number of Compounds Detected or Identified with Total Area Percentage Composition	Collection Site (Country)	Ref.
1.	Seed	E.O.	Linalool (57%), limonene (19.8%), E-methyl cinnamate (5.7%); 28 (97.60%)	Local market in Delhi (India)	[15]
2.	Seed	E.O.	Linalool (70.6%), limonene (8.2%), β-phellandrene (5.7%); 56 (99.50%)	Local market in Lucknow (India)	[9]
3.	Seed	E.O.	Linalool (62.0%), limonene (18.1%), *Trans*-methyl cinnamate (6.5%); 38 (99.80%)	Local market of Gorakhpur (India)	[8]
4.	Seed	E.O.	Linalool (87.7%), β-phellandrene (4.1%), (E)-methyl-cinnamate (1.2%), β-caryophyllene (1.2%); 31(99.2%)	Local market (India)	[32]
5.	Pericarp	E.O.	Linalool (72.00%), methyl cinnamate (12.20%), limonene (6.20%); 25 (99.61%)	Near Munsiyari in U.P. hills (India)	[27]
6.	Leaf	E.O.	Female leaf—linalool (34.06, 27.19, 11.67%), limonene (1.59, 2.43, 6.46%); not found Male leaf—linalool (35.57, 19.80, 10.00%), limonene (2.76, 4.52, 4.00%); not found	Gwar, Semi, Kumud in Uttarakhand (India)	[16]
7.	Fruit	E.O.	Linalool (55.30%), limonene (22.46%), methyl cinnamate (8.82%); 17 (96.17%)	Munshiyari, Pithoragarh district of Uttar Pradesh Hills (India)	[28]
8.	Aerial	E.O	Linalool (18.8%), undecan-2-one (17.0%), 1,8-cineole (15.7%); 52 (97.40%)	Dauladhar hills near Palampur, Himachal Pradesh (India)	[30]
9.	Leaf, aerial, unripe, and ripe fruit	E.O.	Leaf—linalool (32.4%), undecan-2-one (15.2%), *β*-phellandrene (10.0%); 14 (82.3%) Aerial—limonene (12.5%), 1,8-cineole (11.6%), undecan-2-one (9.8%); 16 (83.1%) Unripe fruit—*β*-phellandrene (40.6%), sabinene (16.6%), *β*-myrcene (8.6%); 15 (96.6%) Ripe fruit—sabinene (18.5%), *β*-phellandrene (14.9%), terpinen-4-ol (12.9%); 17 (89.7%)	Thala village of Palampur (India)	[22]
10.	Leaf	E.O.	Summer—2-undecanone (5.1–80.1%), linalool (0.8–67.9%), *β*-phellandrene (1.3–36.5%); 14–26 and (94.0–99.2%) Winter—2-undecanone (33.0–61.5%), linalool (0.2–26.3%), *β*-phellandrene (2.2–16.2%); 13–22 and (93.1–96.6%)	Himachal Pradesh (India)	[20]
11.	Leaf	E.O.	Linalool (30.58%), 2-decanone (20.85%), β-fenchol (9.43%); 14 (98.4%)	North-western Himalaya (India)	[17]
12.	Stem bark	E.O.	Bhimtal—α-pinene (33.9%), germacrene-D (8.9%), *E*-caryophyllene (7.9%); 47 (95.2%) Dharchula—α-pinene (28.9%) undecanone (16.2%), linalool (6.2%); 72 (95.8%) Pithoragarh—α-pinene (35.9%), 2-undecanone (10.1%), β-copaene (6.1%); 44 (92.4%)	Bhimtal, Dharchula, Pithoragarh in Uttarakhand (India)	[10]
13.	Leaf	E.O.	ZA1, ZA2, day 1, day 2—2-undecanone (48.4, 51.8, 55.7, 46.0%), 2-tridecanone (13.5, 5.0, 3.5, 27.1%), linalool (8.4, 6.7, 11.5, 1.8%); 35, 35, 35, 35 (84.2, 96.1, 91.7, 89.9%)	Jones Estate in Kumaon (India)	[18]
14.	Seed	E.O.	Dharchula—linalool (54.3%), cinnamic acid (18.2%), sylvestrene (15.4%,); 32 (99.1%) Pithoragarh—(*Z*)-*β*-ocimene (28.1%), β-myrcene-(11.6%), β-phellandrene (8.7%); 53(93.1%)	Dharchula and Pithoragarh of Kumaun region of Uttarakhand (India)	[6]
15.	Leaf	E.O.	6 am, 12 noon, 6 pm—bornyl acetate (16.61, 17.82, 22.66%), cymene (8.25, 8.35, 12.50%), α-copaene (7.54, 7.54, 7.59%); 11, 11, 11 (61.16, 59.23, 75.10%)	Mandal forest of Uttarakhand (India)	[21]
16.	Aerial	E.O.	Leaves—2-undecanone (65.6%), 2-tridecanone (16.6%), *cis*-fernesol (6.3%); 23 (99.5%) Seeded leaves—linalool (39.4%), 2-undecanone (30.9%), 2-tridecanone (8.8%); 21(99.3%) Pericarp—linalool (71.2%), methyl cinnamate (10.4%), limonene (7.6%); 24 (99.2%)	Joshimath, Garhwal region of Uttarakhand (India)	[33]
17.	Leaf	E.O.	2-undecanone (44.58%), linalool (14.53%), 2-tridecanene (7.98%); 26 (92.18%)	Bhimtal in Uttarkhand (India)	[19]
18.	Leaf and bark	Methanolic and chloroform extract	Leaves methanolic—fargsin (21.9%), eudesmin (15.4%), sesamin (6.9%); 78 (79.4%) Leaves chloroform—fargsin (18.9%), eudesmin (12.8%), *cis*-5,8,11-eicosatrienoic acid, trimethylsilyl ester (6.4%); 62 (82.2%) Bark methanolic—t-butylamine (23.1%), 1-[(trimethylsilyl) oxy] propan-2-ol (7.5%), propylene glycol, 2-TMS derivative (5.8%); 56 (92.2%) Bark chloroform—benzoxazole, 2-(isobutyl-amino) (42.7%), (Z,Z)-6,9-*cis*-3,4-epoxy-nonadecadiene (22.0%), eudesmin (5.4%); 82 (3.0%)	Aadi Kailash region (Bhimtal), Nainital, Uttarakhand (India)	[23]

Table 12. *Cont.*

Sl. No.	Plant Part	Sample Type	Main Constituents, Number of Compounds Detected or Identified with Total Area Percentage Composition	Collection Site (Country)	Ref.
19.	Leaf	E.O.	Fresh leaves—2-undecane (30.0%), linalool (15.9%), (*E*)-β-ocimene (14.9%); 26 (90.6%) Dry leaves—β-phellandrene (35.5%),undecanal (22.5%), myrcene (7.6%); 30 (96.9%)	Lohaghat in Champawat district, Uttarakhand (India)	[24]
20.	Not specified	E.O.	Linalool (41.73%), D-limonene (13.24%), β-phellandrene (7.53%); 66 (97.88%)	Xiluyuan Market, Liangxiang (China)	[34]
21.	Branch and leaf	E.O.	β-Terpinene (45.56%), piperitone (33.47%), 3-carene (8.88%); 7 (98.09%)	Wen County of Gansum province (China)	[11]
22.	Seed	E.O.	3-Borneol (9.718%), iso-bornylacetate (9.574%), dihydro carveol (8.816%); 22 (68.36%)	Balakot Mansehra N.W.F.P. (Pakistan)	[12]
23.	Leaf	E.O.	beta-Linalool (53.05%), bergamot mint oil (12.73%), alpha-limonene diepoxide (11.39%); 34 (100%)	Not mentioned	[35]
24.	Fruit	E.O.	Linalool (75.31%), E-methyl cinnamate (11.73%), limonene (9.45%); 6 (99.27%)	Lalitpur district (Nepal)	[36]
25.	Fruit	Hexane extract	2-Hydroxy cyclopentadecanone (27.37%), palmitic acid (6.99%), piperitone (6.71%); 36 (70.3%)	Bobang Village Development Committee (Nepal)	[29]
26.	Leaf	E.O.	Average—linalool (38.73%), undecan-2-one (22.75%), limonene (19.82%); 9.3 (97.62%)	Salyan district (Nepal)	[13]
27.	Fruit	E.O.	Average—linalool (59.37%), methyl cinnamate (17.57%), limonene (16.95%); 6.9 (98.09%)	Salyan district (Nepal)	[7]
28.	Leaf	E.O.	1,8–cineole (41.0%), 2-undecanone (9.6%), sabinene (8.4%); 50 (98.2%)	Hop Tien village, Trieu Son district, Thanh Hoa province (Vietnam)	[14]

Our results revealed that some compounds from the same plant parts changed in different seasons, and some compounds remained the same, which were described as common compounds in individual plant parts, but their amount (peak area) changed. Changes were also observed in the number of compounds identified and area percentage composition (area%). There were compounds which were detected in one season and not detected in another, although coming from the same plant part. For example, in Tables 1–3 for the flower–fruit part, the common compound o-cymene was detected in spring, summer, and during the monsoon with percent compositions of 2.22, 2.82, and 11.47%, respectively. Decane was detected in two seasons with percent compositions of 0.24 and 0.38% in spring and summer, respectively, while humulene was detected only in summer with a percent composition of 0.43%. The compounds of the leaf part listed in Tables 4–7 showed the presence of common compounds in all the seasons, sometimes in three seasons, sometimes in two seasons, and compounds which were present in one season only. For example, α-pinene with percent compositions of 4.36, 1.96, 6.09, and 2.25% was detected in spring, summer, during the monsoon, and in winter, respectively. β-Thujone was detected in three seasons with percent compositions of 0.30, 0.16, and 0.14% in spring, summer, and during the monsoon, respectively. α-Terpineol was detected in two seasons with percent compositions of 0.12 and 0.14% in spring and summer, respectively, while 2-undecanone, with a percent composition of 1.08%, was detected in winter only. Likewise, for the stem part in Tables 8–11, for example, sabinene, with percent compositions of 1.22, 3.56, 2.92, and 3.28%, was detected in spring, summer, during the monsoon, and in winter, respectively. 1H-Indene, 1-methylene- was detected in three seasons with percent compositions of 0.37, 0.15, and 0.16% in spring, summer, and winter, respectively. Isopinocarveol was detected in two seasons with percent compositions of 0.13 and 0.10% in summer and winter, respectively, and benzene, p-dimethoxy-, with a percent composition of 0.11%, was detected in spring only.

The changes in the compounds and their amounts (peak areas) and area percentage compositions (area%) could be explained from the point of view of gene expression and their regulation leading to the production of the enzymes involved in the biosynthetic pathway of the compounds. The factor(s) which trigger gene regulation could be one or

a combination of signals or stimuli due to seasonal environmental variations and other climatic and edaphic factors. Authors from previous studies reported that altitude, genetic and agroclimatic conditions, growing conditions, edaphic factors, other environmental factors, and genetic predisposition affected the production and distribution of phytochemical constituents of not only the plant under study [7,9,13] but other plants as well.

Differences observed in the major components and percentage compositions of the compounds reported from other places in India and other countries such as China, Pakistan, Nepal, and Vietnam could be due to a variation in geographical conditions, agroclimatic conditions, soil chemistry, and other environmental factors that provide different growing conditions for the plants. The main compound in the current study, eucalyptol, is similar to the main compound in a previous study that was published from Vietnam [14], and both the collection sites come under the Indo-Burma biodiversity hotspot, where similar seasonal climatic conditions might have some role to play with the expression level of compounds present in the plant. The variation observed in the chemical and percentage composition of the compounds of the same plant collected at different seasons and growth stages could be due to physiological changes, ontogeny of the plant, phenological shifts, and other environmental factors.

5. Conclusions

Variations were observed in the studied volatile compound profiles, and the extraction method used in this study was very efficient for the analysis, which simplified and sped up the sample preparation method. In the HS-SPME-GC-MS analysis of *Zanthoxylum armatum* DC. collected from Thambalkhong, Imphal East District of Manipur, the monoterpenoid eucalyptol was revealed to be the principal component. The findings of the present study can help understand the mechanism behind the changes in the plant volatile organic compound profile due to the plant parts studied or due to seasonal variation. The volatile compounds which were identified in the present study and earlier studies will help in the classification and identification of the species through chemotaxonomy. Overall, this study provides useful information for deciphering the volatile compounds from different parts of *Z. armatum* in different seasons, provides a reference point for future breeding and selection for the improvement of this plant, and can help guide further research on *Zanthoxylum armatum* DC.

The increase in research findings regarding the phytochemicals and biological activities which connect with the traditional uses of the plant further enhances the importance of the plant. In the future, we aim to study the transcriptome of *Zanthoxylum armatum* DC. and identify the genes and their expression analysis related to volatile compounds in order to understand the regulation mechanism.

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