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Research Article

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Chemical Composition of the Essential Oils of *Monarda didyma* L. During Different Phenological Stages

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ABSTRACT

The aim of the present study was to investigate the essential oil composition of Monarda didyma L. during different phenological stages i.e. pre flowering, full flowering and seed setting. Hydrodistilled essential oils obtained from the aerial parts of the plant were analyzed by GC and GC/MS. Total twenty compounds were identified in the oils representing 98.53%, 98.13% and 97.19%, respectively in pre flowering, full flowering and seed setting stages. In all the oils collected at different phenological stages, linalool was found as the major constituent which ranged from 47.12-61.89% followed by γ-terpinene (15.20-24.11%), thymol methyl ether (4.35-9.53%), p-cymene (2.28-6.09%), thymol (3.09-5.15%) and 1-octen-3-ol (1.75-3.75%). The maximum content of linalool (61.89%) was found at pre-flowering stage, γ-terpenene (24.11%) at flowering and thymol methyl ether (9.53%) at seed setting stages. All the oils were dominated by oxygenated monoterpenes (60.83-75.54%) followed by monoterpenes hydrocarbons (19.92-35.83%), while sesquiterpene hydrocarbons ranged from only 1.47-3.07%. The study concluded that however the oils were qualitatively similar but differed quantitatively during different growth stages.

Keywords: *Monarda didyma* L; Lamiaceae; Essential oil composition; Linalool; Thymol

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INTRODUCTION

The genus *Monarda* commonly known as bergamot, Oswego tea, horsemint, beebalm, belongs to family Lamiaceae. It is native to North America [1] and globally distributed in Italy, France, California, Canada, Poland [2,3]. The genus comprises about 15-30 species all over the world [1,4-7]. Out of them, the most known species are *M. didyma*, *M. fistulosa*, *M. citriodora*, *M. punctata* [4,7], which are commonly distributed in North America, Europe, Canada, United States, Florida, America [3,4,8-11]. Species of this genus are erect, aromatic, herbaceous, annual or perennial

[5]. *M. didyma* and *M. fistulosa* are ornamental and flowering plants and extensively used for medicinal purpose by Native Americans [12,13]. *M. didyma* is a perennial, herbaceous plant that grows to an average height of 1 meter depending on the cultivar [6]. The plant possesses secondary metabolites such as terpenes, phenols, alcohols and flavanoids [14,15]. Reports indicate use of *M. didyma* in folk medicines as the flowers of *M. didyma* are drunk as an infusion called "the rough" or "Oswego tea" [3,16]. From ancient times the genus is used as traditional medicine for the cure of many diseases such as anthelmintic, carminative, diuretic, rube-facient and digestive disorders curing agent [3,4,17]. *M. didyma* has shown many biological activities such as antimicrobial, antifungal, antioxidant, phytotoxic, anti-inflammatory and antibacterial activity [3,4,13,18,19]. The essential oil of *M. didyma* can also be used as bioactive agent for plant protection because it has been found to possess the strong nematotoxic activity against phytoparasitic nematodes *Meloidogyne incognita* and *Pratylenchus vulnus* was also proved [20]. In addition to their ornamental demand, *Monarda* species are highly valued for their essential oil content. The most common components in the essential oil of *M. didyma* include thymol, carvacrol, geraniol, linalool, 1,8-cineole and others. [5]. The present study aims to investigate the detailed essential oil profiling of *M. didyma* at different stages of plant growth.

MATERIALS AND METHODS

Collection of Plant Material

The aerial parts (400 g each) of *M. didyma* were harvested at three phenological stages; pre flowering stage (T1), full flowering stage (T2) and seed setting (T3) from the experimental field of Centre for Aromatic Plants (CAP) Selaqui, Uttarakhand, India in the month of April, May and June, 2017, respectively (Figure 1).

Figure 1. *Monarda didyma* crop at flowering stage

Isolation of Essential Oils

Fresh aerial parts of *M. didyma* of different growth stages (Phenological stages) were separately hydrodistilled for 4 hours using a Clevenger apparatus. The oil yield (%v/w) was estimated on a fresh weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and kept in cool and dark place (at 4°C) until analysis.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography analysis of the oils were performed by Agilent Technology (6890 model) gas chromatograph equipped with HP-5 fused silica column (30 m × 0.32 mm, 0.25 μm film thickness) and flame ionization detector

(FID). Nitrogen was used as a carrier gas. The injector and detector temperatures were maintained at 210°C and 230°C, respectively. The column oven temperature was programmed at 60°C to 220°C with an increase at the rate of 3°/min. The injection volume was taken as 0.2 µL.

GC-MS analysis of the oils were carried out on a Perkin Elmer mass spectrometer (Claurus 500) coupled to a Perkin Elmer Claurus 500 gas chromatograph with a 60 m × 0.32 mm, 0.25 µm film thickness column (Rtx5). Helium was used as the carrier gas (flow rate 1 mL/min). The oven temperature program was ranged from 60° to 220° at the rate of 3°/min. Other conditions kept same as described under GC.

Identification of Compounds

The identification of constituents was done on the basis of retention index (RI), determined with reference to the homologous series of n-alkanes (C_9–C_{24}) under the same experimental conditions, co-injection with standards (Sigma Aldrich USA), MS library search (Wiley/NIST/Pfleger) and by comparing with the MS literature data [21].

RESULTS AND DISCUSSION

The yield of essential oils (%) from fresh aerial parts of *M. didyma* was 0.71%, 0.72% and 0.60% (v/w) at pre flowering, full flowering and seed setting stages, respectively. The percent composition of the essential oils is presented in Table 1. A reference gas chromatogram of *M. didyma* essential oil at pre flowering stage is shown in Figure 2.

Table 1. Essential oil composition (%) of *Monarda didyma* during different phenological stages

S. No.	Compounds	RILit.	Phenological Stages			Identification methods*
			Pre Flowering	Full Flowering	Seed Setting	
1	α-Thujene	924	0.36	1.18	0.7	RI, MS
2	Sabinene	969	0.11	0.13	0.15	RI, MS, Co-inj
3	1-Octen-3-ol	974	3.34	3.75	1.75	RI, MS
4	α-Pinene	984	0.19	0.44	0.28	RI, MS, Co-inj
5	β-Myrcene	988	0.84	1.19	1.25	RI, MS
6	3-Octanol	991	0.89	0.85	0.89	RI, MS
7	α-Phellandrene	1002	0.17	0.23	0.21	RI, MS
8	δ-3-Carene	1008	0.4		0.46	RI, MS
9	α-Terpinene	1014	-	2.68		RI, MS
10	p-Cymene	1020	2.28	4.95	6.09	RI, MS
11	Limonene	1024	0.37	0.92	0.5	RI, MS, Co-inj
12	γ-Terpinene	1054	15.2	24.11	18.33	RI, MS
13	Linalool	1095	61.89	47.12	47.74	RI, MS, Co-inj
14	Thymol methyl ether	1232	5.12	4.35	9.53	RI, MS
15	Carvacrol methyl ether	1241	0.94	0.88	1.39	RI, MS
16	Thymol	1289	3.19	3.09	5.15	RI, MS, Co-inj
17	Carvacrol	1298	0.17	0.79	0.38	RI, MS
18	Trans-caryophyllene	1417	0.82	0.41	0.68	RI, MS
19	Germacrene-D	1484	1.85	0.71	1.31	RI, MS
20	α-Farnesene	1505	0.4	0.35	0.4	RI, MS

Monoterpene hydrocarbons		19.92	35.83	27.97	
Oxygenated monoterpenes		75.54	60.83	66.83	
Sesquiterpene hydrocarbons		3.07	1.47	2.39	
Total		98.53	98.13	97.19	
Oil yield (%)		0.71	0.72	0.6	

Note: *Retention Indices, comparision with RILit.; MS, comparision with mass spectra from libraries and litratures; Co-inj., comparision with retention time of standard compounds; RILit., Retention Indices (Literature).

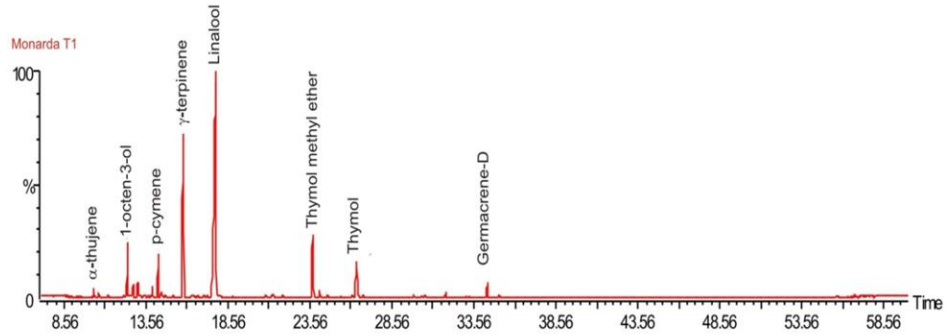

Figure 2. Reference gas chromatogram of *Monarda didyma* essential oil

A total of 20 components were identified, representing 97.19-98.53% of the whole composition. In all the oils monoterpenoids i.e. oxygenated monoterpenes,(60.83-75.54%) and monoterpenes hydrocarbons (19.92-35.83%) were reported as the major class of compounds, while sesquiterpene hydrocarbons were found in the lesser amount (1.47-3.07%). In all the oils, linalool (47.12-61.89%) was found as the major constituent. Linalool detected in maximum amount at pre flowering stage (61.89%), while it deceased at full flowering (47.12%) and seed setting stages (47.74%). γ-Terpinene, the second major compound was detected in appreciable amounts, ranged from 15.20-24.11%. The amount of γ-terpinene was maximum (24.11%) at full flowering stage as compared with pre flowering (15.20%) and seed setting stage (18.33%). On the contrary, the amount of thymol methyl ether in the oil of *M. didyma* was observed to be maximum (9.53%) when harvested at seed setting stage. Similarly here maximum amount of p-cymene and thymol was noticed at seed setting stages i.e. 6.09% and 5.15%, respectively. The other minor compounds such as 1-octen-3-ol (1.75-3.75%), germacrene-D (0.71-1.85%), carvacrol methyl ether (0.88-1.39%), limonene (0.37-0.92%), 3-octanol (0.85-0.89%) and trans-caryophyllene (0.41-0.82%) were also detected in the oils. Main compounds reported in the previous studies on *M. didyma* essential oils from different countries are shown in Table 2.

Table 2: Main constituents (>5%) in the essential oils of *Monarda didyma* from different countries

Main compounds (%)	Plant parts/country	References
Thymol (41.17%)		
γ-Terpinene (15.88%)		
Carvacrol (15.2%)		
Myrcene (12.58%)	Aerial parts/Canada	[18]
Thymol (63.73%)	Aerial parts/Italy	[12]

p-Cymene (10.57%)		
γ-Tepinolene (9.26%)		
γ-Terpinene (22.15%)		
Carvacrol (13.8%)		
Cymene (13.42%)		
Linalool (8.01%)		
Thymol (5.87%)	Aerial parts/Italy	[20]
Thymol (64.32%)		
p-Cymene (10.51%)		
γ-Tepinolene (9.26%)	Aerial parts/Italy	[4]
Thymol (59.3%)		
p-Cymene (10.3%)		
Tepinolene (9.2%)	Aerial parts/Italy	[19]
Thymol (57.3%)		
p-Cymene (10.5%)		
γ-Terpinene (9.3%)	Stem with leaves/Italy	[3]
Thymol (51.7%)		
γ-Terpinene (14.3%)		
p-Cymene (9.7%)		
δ-3-Carene (6.2%)		
Camphene (5.6%)	Stem with flowers/Italy	[3]
Linalool (64.5%)		
p-Cymene (11.0%)		
γ-Terpinene (5.3%)		
Sabinene (5.0%)	Flowers/France	[16]
Linalool (74.2%)		
Bornyl acetate (5.7%)		
Germacrene-D (5.3%)	Leaves/France	[16]
1,8-Cineole (27.36%)		
Limonene (12.93%)		
p-Cymene (12.24%)		
Bornyl acetate (7.74%)		
Linalyl acetate (5.06%)	Leaves/California	[1]
Linalool (46.91%)		
Thymol (17.72%)		
p-Cymene (9.66%)		
Thymol methyl ether (6.4%)	Leaves/Poland	[13]

A previous study on *M. didyma* from France reported that the leaf and flower oils were characterized by higher amount of linalool i.e. 72.2% and 64.5%, respectively [16]. Similarly, report on leaf essential oil of *M. didyma* from Poland also indicated higher contribution of linalool (46.91%) while p-cymene (9.66%) and thymol methyl ether

(6.40%) were also present in an appreciable quantity [13]. In contrast to our study, thymol was communicated as the major compound in some reports carried out in different countries [3,4,12,18,19]. The essential oil of *M. didyma* obtained from the aerial parts from Italy contained maximum amount of thymol 59.31-64.32% [4]. Here γ-terpinene, reported as the second major component in our study, was not reported in some previous studies [4,12,19], or it was noted in scanty amount only 0.9% in leaf oil from France [16], 2.10% from California [1] and 2.95% from Poland isolates [13]. Two previous studies from Italy and Canada reported higher content of γ-terpinene in the oil of the aerial parts of the plant, viz 22.15% [20] and 15.88% [18]. Thymol methyl ether was also not detected in the essential oils of *M. didyma* grown in Italy, France and California [1,3,4,16], while the leaf oil from Poland informed 6.40% thymol methyl ether as constituent [13]. In some other investigations, the same compound was reported in small portions, ranging from 0.11-0.60% [12,13,18-21]. Linalool, the major compound found in the oil of *M. didyma* is widely used as main composition in almost all types of perfumes. It has a weird creamy-floral taste, but not particularly sweet. It is pleasant only in combination with other flavors and when it is used in low concentrations [22].

CONCLUSION

The current study concluded that the composition of the essential oils of *Monarda didyma* at pre flowering, full flowering and seed setting stages were qualitatively similar but differed quantitatively during different growth stages. Harvesting of the crop at pre flowering or at the time of initiation of flowering is optimum time to get better oil yield (%) and higher linalool content. Our findings will be very useful for the aromatic crop cultivators for promoting *M. didyma* as an essential oil crop for cultivation in Uttarakhand region of India.

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