

# Diurnal Effects on Chinese Wild *Ledum palustre* L. Essential Oil Yields and Composition

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## Abstract

This study was conducted to evaluate the diurnal effect on essential oil yield and composition of *Ledum palustre* L. grown in northern Inner Mongolia, China. Essential oil content and composition were determined and compared as a function of different harvesting times viz. 7:00 AM, 11:00 AM, 3:00 PM, 7:00 PM, and 11:00 PM within a day. The essential oil obtained by hydrodistillation was investigated by gas chromatography-mass spectrometry (GC-MS). The yield of essential oil was varied from 1.21% to 1.62%; the maximum oil yield was obtained at 3:00 PM and the minimum at 7:00 PM. Similar to oil yield, qualitative difference in essential oil composition of *L. palustre* was observed. For the best essential oil yields, *L. palustre* should be harvested during 11:00 AM to 3:00 PM.

## Keywords

*Ledum palustre* L., Diurnal Changes, Essential Oil Yield, Essential Oil Composition, Alpha-Thujenal

## 1. Introduction

*Leum palustre* L., also known as wild rosemary or marsh tea, is an evergreen low shrub growing wild in northern China and America, northern and central Europe. The plant grows in peaty soils, shrubby areas, moss and lichen tundra. Its leaves and flowers have a strong smell causing a headache in some people. All parts of the plant contain poisonous terpenes that affect the central nervous system, causing aggressive behaviour. *L. palustre* is widely used in folk medicine and homoeopathy for the treatment of rheumatism, arthrosis, and insect bites [1]. The expectorant and antitussive effect of the marsh tea is due to the ledol contained in the plant's essential oil [1]. The composition of the essential oil from *L. palustre* varies considerably with habitat [2] [3]. The major components

present in the essential oil of *L. palustre* are (+)-ledol [4], (-)-palustrol [4] [5], (-)-cyclocolorenone [4], myrcene, *p*-cymene and limonene [4] [6]. Essential oils were obtained from different parts of *L. palustre* (all overground parts, shoots and leaves) plants. The content of oils in young leaves and shoot was higher than in the corresponding aged parts [4]. Ledol was determined in the leaf essential oil and three compounds (ledol, palustrol and germacron) in shoot oil [4]. The composition of the essential oil varied in a wide range in different localities. In recent years, the well-known repellent properties of the essential oil of wild *L. palustre* against bedbugs, clothing moths and other insects are valued [7] [8]. The *L. palustre* has been the focus of many scientific researches investigating its shoots and the essential oil for different bioactivities [9]. The promising antimicrobial, antioxidant and antidiabetic properties were reported [1].

Although the biosynthesis of secondary metabolites is controlled by genetic processes, it is also strongly affected by climatic conditions such as light, temperature, irrigation, soil and nutrition as well as the season and the time plant material is harvested [10]-[15]. Previous studies found significant impact of diurnal changes on essential oil yield and composition on a number of crops such as basil (*Ocimum gratissimum* L.) [16], Pelargonium sp. [17], oil-bearing rose (*Rosa damascene* Mill.) [18], dill Cistaceae (*Cistus monspeliensis*) [19], lavender (*Lavandula angustifolia* Mill.) [20], *Eucalyptus* spp. [21], and spearmint (*Mentha spicata* L.) [10]. However, diurnal changes in Chinese wild *L. palustre* L. essential oil yield and composition are not known. The objective of this study was to evaluate the effect of diurnal variation on yield and composition, of the essential oil from the aerial parts of *L. palustre* L. grown wild in northeastern China.

## 2. Experimental Procedures

### 2.1. Materials

The aerial parts of *L. palustre* were collected in Northern Inner Mongolia, China, in August 2016. All samples were obtained within a 24 h period. *L. palustre* was at flowering stage at the time of harvest, to ensure the best essential oil content and composition. The plant was harvested every 4 h: 7:00 AM, 11:00 AM, 3:00 PM, 7:00 PM, and 11:00 PM, each harvest in three replicates, resulting in 15 plant samples. Each plant sample was around 2 kg of fresh weight. The fresh *L. palustre* samples were dried in a well-ventilated barn at shade. Voucher specimens (No. CAF20160001) were deposited at the Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry.

### 2.2. Extraction of Essential Oil

The ground powders of all 15 *L. palustre* samples (each around 1.5 kg) were subjected to hydrodistillation using a modified Clevenger-type apparatus (Senco, SENCO Technology Co., Ltd.) for 5 h. The beginning of each distillation was measured when the first drop of essential oil was out of the condenser and in the separator. At the end of the each distillation, the power was turned off; the oil and the water were decanted from the separator into glass vials. The oil was se-

parated from the water and anhydrous sodium sulphate was used to remove water after extraction. The essential oil was stored in an airtight container in a refrigerator at 4°C. The essential oil content (yield) was calculated as grams of oil per 100 g of dry herbage.

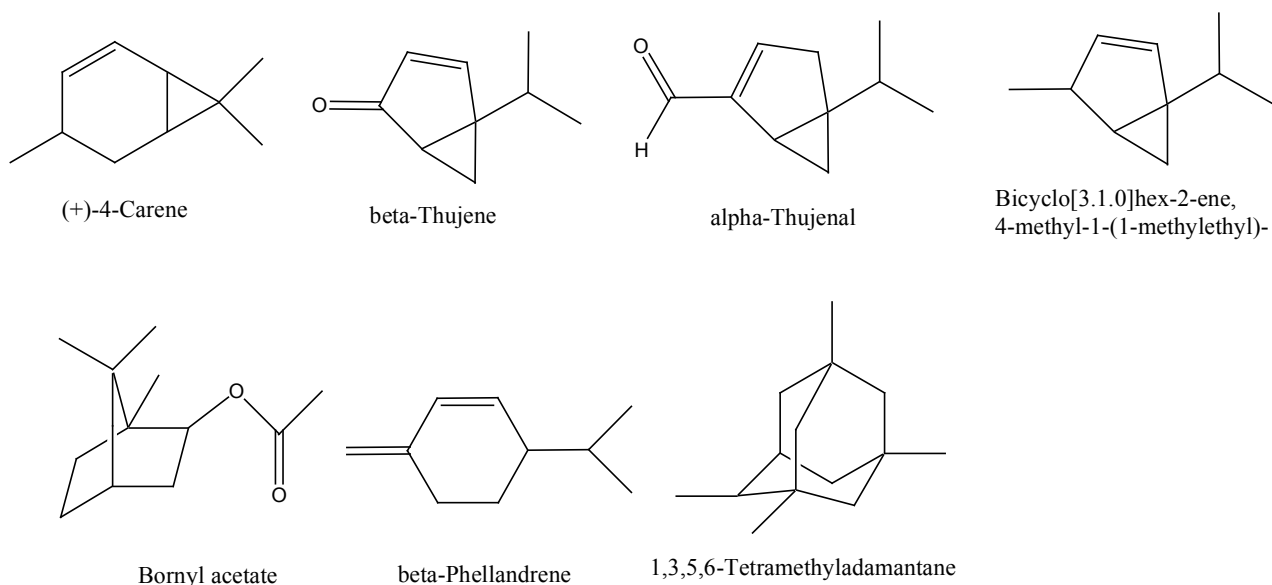
### 2.3. Analysis of Essential Oil

The samples were analysed on a Varian Saturn 2000 System using a 1079 injector that had been fitted with the Chromato Probe kit. This kit allows the thermal desorption of small amounts of solids or liquids contained in quartz microvials, or in our case the thermal desorption of the trapped volatiles. The adsorbent tube was loaded into the probe, which was then inserted into the modified GC injector. The injector split vent was opened (1/20) and the injector heated to 40°C to flush any air from the system. The split vent was closed after 2 minutes and the injector was heated at 200°C/minute, and then held at 200°C for 4.2 minutes, after which the split vent opened (1/10) and the injector cooled down.

A ZB-5 column (5% phenyl polysiloxane) was used for the analyses (60 m long, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex). Electronic flow control (EFC) was used to maintain a constant helium carrier gas flow of 1.8 mL/minute. The GC oven temperature was held for 7 minutes at 40°C, then increased by 6°C per minute to 250°C and held for 1 minute. The MS interface was 260°C and the ion trap worked at 175°C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of 1 scan<sup>-1</sup> from m/z 30 to 350. The GC-MS data were processed using the Saturn Software package 5.2.1. Component identification was carried out using the NIST 08 mass spectral data base (NIST algorithm).

## 3. Results and Discussion

The seven major compounds alpha-thujenal, bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)-, beta-thujene, 4-carene, bornyl acetate, beta-phellandrene and 1,3,5,6-tetramethyladamantane were identified and quantified in the essential oil of *L. palustre* (Figure 1). The oil and the main components yields (content) were significantly affected by diurnal variation (Table 1). It can be seen from the table, essential oil yield varied from 1.21 to 1.62 g of oil per 100 g of dry herbage. The highest essential oil yield was obtained at 3:00 PM and 11:00 AM, and the lowest at 7:00 PM and 7:00 AM. Concentration (%) of the main components of the *L. palustre* essential oil extract at the five harvest times has been showed in Table 2. The concentration of 4-carene in the oil varied from 1.59% (at 7:00 AM) to 1.95% (at 11:00 AM) of the total oil and was generally high from 11:00 AM to 11:00 PM. Similar to 4-carene concentration, the yield of 4-carene (a function of oil yield and 4-carene concentration in the oil) was also the highest at 11:00 AM and 3:00 PM and the lowest at 7:00 AM. The concentration and yield of bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)- (9.46% - 12.73% of the total oil, and 116.37 - 206.72 mg/100 g dry material) were the highest at 3:00 PM



**Figure 1.** Chemical structure of the major components of the *L. palustre* essential oil extract.

**Table 1.** Mean oil yield (oil content g/100 g dry herbage) and the yield (mg/100 g dry material) of some main components at the five harvest times.

Harvest time	Oil yield	alpha-Thujenal yield	Bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)- yield	beta-Thujene yield	1,3,5,6-Tetramethyladamantane yield	(+)-4-Carene yield	Bornyl acetate yield	beta-Phellandrene
7:00 AM	1.23a	342.10	116.37	57.69	24.60	19.56	24.48	11.81
11:00 AM	1.56c	411.60	188.04	85.57	36.45	30.51	35.67	17.36
3:00 PM	1.62c	478.56	206.72	52.61	36.86	27.77	37.02	15.10
7:00 PM	1.21a	343.20	143.15	28.02	21.59	20.74	27.42	11.04
11:00 PM	1.39b	308.11	165.04	32.40	28.78	23.36	21.55	12.93

Within a column, means followed by the same letter are not significantly different.

and the lowest at 7:00 AM. The highest concentration and yield of beta-thujene were found at 11:00 AM and the lowest at 7:00 PM and 11:00 PM. The concentration and yield of alpha-thujenal were the highest at 3:00 PM and the lowest at 11:00 PM. The concentration of bornyl acetate was generally high from 11:00 AM to 7:00 PM and the lowest at 11:00 PM, whereas the yield of bornyl acetate was highest at 3:00 PM. The concentrations of beta-phellandrene and 1,3,5,6-tetramethyladamantane range from 0.91% to 1.11%, and 1.78% to 2.33%, respectively. Furthermore, there is high concentration of 2-carene (3.97% - 3.14%) from 11:00 AM to 11:00 PM, but no 2-carene was detected at 7:00 AM.

Predominant presence of alpha-thujene, beta-thujene and bornyl acetate in *L. palustre* essential oil was also reported in previous studies [3] [8] [22] [23] [24]. Recently, Zhao *et al.* [8] compared the essential oil compositions from wild *L. palustre* stems, leaves, and flowers in bloom and non-bloom periods from Northeast China. The study reported the predominant constituents in all oils were 4-thujene, 5-(1-methylethyl)-bicyclo[3.1.0]hex-3-en-2-one, alpha-thujenal

**Table 2.** Concentration (%) of the main components of the *L. palustre* essential oil extract at the five harvest times.

Retention time/min	Components	Concentration (%)				
		7:00 AM	11:00 AM	3:00 PM	7:00 PM	11:00 PM
1.495	Furan, tetrahydro-3-methyl-	2.52	2.22	2.03	2.04	1.93
6.9	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	1.04	1.42	1.01	0.72	0.71
7.05	alpha-pinene	0.46	0.55	0.41	0.26	0.23
7.44	camphene	0.56	0.73	0.57	0.4	0.33
8.17	beta-thujene	4.69	5.47	3.24	2.31	2.33
8.25	beta-pinene	0.27	0.27	0.26	0.22	0.19
8.59	furan, 2-pentyl-	0.25	0.29	0.22	0.2	0.15
8.87	alpha-phellandrene	0.66	0.89	0.75	0.74	0.75
9.22	(+)-2-carene		3.97	3.36	3.27	3.14
9.49	benzene, 1,2,4,5-tetramethyl-	5.82	7.25	7.09	5.84	5.62
9.55	beta-phellandrene	0.96	1.11	0.93	0.91	0.93
9.69	1,3,6-Octatriene, 3,7-dimethyl-	0.25	0.33	0.3	0.24	0.2
10.19	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	4.26	5.16	4.27	4.21	4.02
10.296	beta-terpineol	0.11				
10.75	(+)-4-carene	1.59	1.95	1.71	1.71	1.68
11.55	bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)-	9.46	12.02	12.73	11.8	11.87
11.854	pinocarveol	1.17				
12.117	2,6-dimethylbicyclo[3.2.1]octane	1.37	1.12	2.12	1.78	0.9
12.292	bicyclo[3.1.0]hexan-2-one, 5-(1-methylethyl)-	2.77	0.19	1.2	0.94	1.41
12.836	alpha-Thujenal	27.81	26.31	29.47	28.29	22.16
13.055	bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	1.72	1.68	1.72	1.68	6.28
13.45	Phenol, 4-(1-methylethyl)-	1.11	1.18	1.01	1.09	0.96
13.755	Propanal, 2-methyl-3-phenyl-			5.38	6.86	6.47
14.33	Bornyl acetate	1.99	2.28	2.28	2.26	1.55
14.4	Benzenemethanol, 4-(1-methylethyl)	1.65	1.9	1.93	1.97	1.92
14.875	o-Isopropylphenetole	1.18	1.36	1.65	1.73	1.52
14.969	g-terpinene	1.85	2.17	2.13	2.35	2.23
16.896	(+)-aromadendrene	2.64	3.18	2.9	2.49	2.95
17.5	Shyobunone	0.5	0.51	0.99	0.87	0.95
18.747	Ledol				0.28	0.27
18.822	1,3,5,6-Tetramethyladamantane	2	2.33	2.27	1.78	2.07
19.517	4-Isopropyl-trans-bicyclo[4.3.0]-2-nonen-8-one	1.49	1.4	1.37	1.26	1.59
Total		82.15	89.24	95.3	90.5	87.31

and (-)-terpineol. Our study confirmed the significant difference in oil composition of *L. palustre* from different countries. In the previous reports, the main compositions of the essential oils from the mixture of stems and leaves of wild *L. palustre* from Da Hingan Mountains of Northeast China were identified as alpha-thujenal (17.13%), 5-(1-methylethyl)-bicyclo[3.1.0]hex-3-en-2-one (8.95%),

sabinaketone (4.96%), 4-thujene (3.28%) and  $\gamma$ -terpinene (2.45%) [22], while sabinene (25.9%), *p*-cymene (14.8%), myrtenal (14.1%), 4-terpineol (7.3%) and cuminaldehyde (5.3%) were identified as main compositions from wild *L. palustre* varangustum E. Busch of the same region [25]. In European varieties, ledol (21.0% - 32.2%) and palustrol (26.2% - 37.9%) were predominant constituents in Lithuania *L. palustre* oils [3].

Variations in terpenoid concentration in the essential oil of other species due to diurnal changes have been observed before. Variation of the terpenoid concentration has been known to be temperature dependent [26] and the concentration increases during the day, reaches maximum values at evening and decreases at night and then increases again in early morning [27]. Lopes *et al.* [28] reported that relative level of monoterpenes in *Virola surinamensis* essential oil was 28% at 6:00 AM, then dropped to approximately 15% at noon, and increased back to the same concentration at 9:00 PM. The same authors reported that, limonene, a major compound in *V. surinamensis* showed higher concentrations (19.29% - 19.85%) during early morning (6:00 - 9:00 AM) and dropped at 12:00-6:00 PM (12.59% - 11.87%).

Our results showed that higher essential oil yield, and alpha-thujenal and bicyclo[3.1.0]hex-3-en-2-one,5-(1-methylethyl)- yield yields, during 11:00 AM and 3:00 PM harvest times. The lowest essential oil yield was found during night and early morning harvest times. These results may be associated with diurnal variation of climatic conditions such as, light, temperature and relative humidity, and support previous reports on the effect of diurnal studies on essential oil yield of other aromatic plants. Diurnal changes in essential oil yield and composition, and hence optimal harvest time for various crops have been known for a long time. Our results are in agreement with the report by Rao *et al.* [17] who reported in geranium harvested during the day from 8:00 AM until 4:00 PM produced higher oil yield than that harvested at night or early morning. Similarly, Ayanoglu *et al.* [29] observed that essential oil content of lemon balm showed diurnal variation in two locations, oil yields were higher at noon in both locations. Bufalo *et al.* [10] reported the highest essential oil yield from spearmint (*Mentha spicata* L.) was obtained at 9:00 AM and the lowest at 7:00 PM. In experiments with *Cymbopogon winterianus*, Blank *et al.* [30] found that oil yields were higher at noon and lower at 5:00 PM. Shevchenko [31] found the diurnal maximum and minimum essential oil accumulation in *Salvia sclarea* to be at noon and at 3:00 AM, respectively. However, in a diurnal study with *Rosa damascena* Mill., Kumar *et al.* [15] found the highest essential oil content at 04:00 AM and the lowest at 2:00 PM. Angelopoulou *et al.* [19] reported that in *Cistus monspeliensis* L. leaves during the months of May, August, and February, oil yield was the highest at 6:00 PM, whereas in November, the oil yield was the highest at 12:00 PM. Indeed, Lawrence [32] stated that the effect of environment factors on the accumulation of essential oil depends on plant species. Plants have different behaviors during diurnal variation. The higher temperature and solar intensity usually occurs between 11:00 AM and 2:00 PM and may be optimum

for oil accumulation in some species [33] [34].

This study demonstrated that diurnal variation affects yield of the essential oil and composition of Chinese wild *L. palustre*. For best essential oil yields under northern Inner Mongolia conditions, the *L. palustre* aerial part should be harvested during 11:00 AM and 3:00 PM. The oil extracted from *L. palustre* harvested at these times contains high 4-carene, bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)-, beta-thujene, alpha-thujenal, bornyl acetate, and beta-phellandrene concentration. Harvests at 7:00 AM and 7:00 PM would result in low *L. palustre* oil yield, should be avoided.

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