Journal of Medicinal Plants and By-products (2020) 2: 207-214

Original Article

Effects of Drying Methods on the Essential Oil Content and Composition of *Nepeta binaludensis* Jamzad

Hosien Hashemi Moghaddam[1], Fatemeh Sefidkon[2], Ali Ashraf Jafari[2*] and Sepideh Kalate Jari[1]

[1]*Department of Horticultural Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran*

Article History: Received: 06 April 2020/Accepted in revised form: 27 May 2020

Abstract

Nepeta binaludensis Jamzad is an endemic and rare perennial plant belonging to the Lamiaceae family which grows in a limited area in Binaloud Mountain in northeast of Iran. In order to study the effect of drying methods on its essential oil content and composition, the aerial parts were collected in flowering stage from its habitat at altitude of 2650 m above sea level in July 2018. Seven drying methods as sun drying, shade drying, oven drying at 30^{o}C, 40^{o}C and 50^{o}C and microwave drying (180W and 360W) were applied in three replications. The essential oils of all samples were obtained using a Clevenger type apparatus during 3 h. The essential oils were analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). Data statistically analyzed using one way ANOVA (CRD) with three replications and mean comparison were made using the Tukey method. The highest oil yield was obtained in oven drying 50 °C (2.16%) followed by shade drying (1.96%). In contrast, the lowest values of essential oil content, 1.09 and 0.65%, were obtained in sun drying and microwave 360W, respectively. Twenty-four compounds were identified in the essential oils of *N. binaludensis* with various drying methods with neptalactone (23.1-54.0%), 1,8-cineol (25.5-51.0%) and myrcene (2.0-4.0%) as major components. The highest percentage of 1,8-cineole (51%) was obtained from sample dried in oven 50 °C, while, the highest amount of neptalactone (54.0%) was obtained from sample dried by the microwave 180 W. The results demonstrated that drying the aerial parts of *N. binaludensis* in the shade and oven 50^{o}C are the best optimal methods to obtain the highest oil yield, while drying in microwave at 180 W is recommended to enhance higher neptalactone content in the oil.

Keywords: *Nepeta binaludensis* Jamzad, Drying method, Essential Oil, 1,8-cineole, Nepetalactone.

Introduction

The genus *Nepeta* L. comprises approximately 300 species, widely distributed in Eurasia. It is one of the largest genera of subfamily *Nepetoideae*, family *Lamiaceae* in southwestern Asia [1]. Iran is one of the main origins of this genus with 79 species [2]. Most of them being endemics [3]. Many species of *Nepeta* contain considerable amounts of essential oils, and diverse amount iridoids and monoterpene neptalactones [4]. *Nepeta* species have various biological properties such as feline attractant, canine attractant, insect repellant, arthropod defense, antibacterial, antifungal and antiviral activities. Several *Nepeta* spp. are widely used in traditional medicine as diuretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue and sedative agents [5], and for antiseptic and astringent properties as a topical remedy in children with cutaneous eruptions, and for snake and scorpion bites [6,7].

Nepeta binaludensis Jamzad is an endemic and endangered herb that grows in limited areas of Binalud Mountain in Razavi Khorasan Province in the northeast of Iran. This species grows in places with elevations at the ranged from 2300 to 2700 m above the sea level, an annual precipitation rate of

*Corresponding author: Research Institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
Email Address: aajafari@rifr-ac.ir

area is 350-370 mm and mean annual temperature in the range of 6-7 ºC [8].

There are some reports for important components of *N. binaludensis* essential oil including 1,8-cineol (42.3-68.3%), neptalactone (19.0-25.2%) as well as other minor components such as β-cryopropylene, α-pinen, β-pinen, α-terepineol, linalool, and γ-terepinene [9, 10].

Nowadays, the demand for high quality medicinal herbs is in a high priority in the world. Herbs can be marketed as dried products. Drying is the most common and oldest method for post-harvest preservation and a fundamental requirement to achieve a high quality product [11]. The aim of drying products is to allow longer periods of storage, minimize packaging requirements, reduce shipping weights, and improve shelf life. Due to lower costs, natural drying and hot air drying are still among the most important methods used in the production of dry plant material. The natural drying methods (shade and sun) have some disadvantages, for example, the inability to relocate large amounts of plant material and achieve to standard quality [12].

There are many published reports on the effects of drying method of medicinal plants on their essential oils quality and quantity. Omidbiagi in roman chamomile suggested drying temperatures of 40-50 °C were optimal for plant parts containing essential oils. The use of higher temperatures can dramatically reduce the essential oil yield [13]. Ebadi *et al*., in *Lippia citriodora* Kunth found that the highest essential oil content using vacuum-dried (60 °C) and oven-dried (40 °C) samples [14]. Venskutonis suggested microwaves drying method due to high drying speed and low energy input, prevents essential oil loss rates [15]. Soysal *et al*. with peppermint revealed that the natural drying had greater preservation of essential oil than oven drying method [16]. Azizi *et al*. in chamomile (*Matricaria recutita*) found the highest essential oil yield in both oven (50 and 60 °C) and shade drying methods [17]. Díaz-Maroto *et al*. in *Mentha spicata* L. found oven drying (45 °C) and ambient temperatures, as the best drying methods [18]. Tarakemeh and Abutalebi in *Ocimum basilicum* L. found the highest essential oil content in shade drying [19]. Blanco *et al*. examined the effects of drying temperatures on the content and composition of rosemary essential oil. They found increasing temperatures led to decreases the essential oil content from 2.13% at oven 40 °C, to 1.09% at oven 80 °C [20]. Fathi *et al*. found no difference between sun and shade drying treatments on essential oil yield of *Eucalyptus largiflorens* leaf [21]. Kayhani *et al*. with *Saturja sahandica* Bormn, found lower essential oil content in oven drying in temperature (50 °C) than oven (30 and 40 °C) and shade drying [22]. Mohtashami *et al*. in *Dracocephalum moldavica* L. found the highest and the lowest essential oil yields in the shade and oven (40 and 50 °C) drying methods, respectively [23]. Mohammadizad *et al*. with *Nepeta cataria* L. found the highest essential oil yield in oven drying at 55 °C [24]. Ebadi *et al*., in *Lippia citriodora* Kunth found the maximum essential oil content in oven (40 °C) drying method [14].

The published data for essential compounds are less than the essential oil. Díaz-Maroto *et al*. in *Mentha spicata* L, found higher Monoterpens in both oven (45 °C) and drying at ambient temperatures [18]. Blanco *et al*. with rosemary found by increasing drying temperatures up to 80 °C, the myrcene, α-pinene, and camphor concentrations were decreased compared to oven drying at 40 °C [20]. Fathi *et al*. in *Eucalyptus largiflorens* found higher values of 1,8-cineol using oven (50 °C) treatment [21]. Kayhani *et al*. in *Saturja sahandica* Bormn found higher values of thymol content and total phenolic compounds in oven (30 °C and 40 °C) and shade drying methods [22]. Hadjibagher Kandi *et al*. in *Laurus nobilis* leaves found higher values of 1,8-cineole (61.0 %) and a-pinene (4.8%) in oven (40 °C) than that for shade (25 °C) and oven drying (30 °C and 50 °C) [25].

Conclusion from published data indicated that, there are significant effects of drying methods on quantitative and qualitative properties of herbal active substance. However, there are no reports published on the effectiveness of different drying methods on essential oil yield and composition *N. binaludensis*. This study, therefore, aimed to investigate the effects of different drying methods (in the shade, sun, oven, and microwave) on essential oil yield and its compounds in *N. binaludensis* collected from its natural habitat in Iran.

Material and Methods

In order to study the effect of drying methods on essential oil content and composition of *Nepeta* *binaludensis*, samples of flowering stage collected in its natural habitat in altitude of 2650 m above sea level in Zoshk habitat, Mashhad, Iran in July 2018.

Seven fresh samples (50 g) of *N. binaludensis* in flowering stages prepared for drying up to 90% dry weight. Drying treatments were seven methods in three replications as follows.

I) Natural drying, including shade drying at a 25±2 °C room temperature,

II) Sun drying at 35±2 °C,

III) Oven drying (30, 40, and 50 °C),

IV) Two microwave powers (180 and 360 W).

Samples were dispersed on the trays of oven and microwave to absorb uniform energy and preventing mold. The essential oils of all samples were obtained (expressed as w/w using a Clevenger type apparatus during 3h. Then the essential oils were analyzed using gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS). In the laboratory of science, Islamic Azad University, Neyshabour Branch, Iran.

The collected data of essential oil yield and their compounds from the seven treatments were subjected to one way ANOVA (CRD) with three replications and mean comparison were made using the Tukey method. Since the essential oil compounds were measured in only one replicate, the drying treatments were compared based on the standard error of the seven treatments.

Results

The result of analysis of variance showed significant difference between seven dying methods for essential oil yield. The highest value of essential oil content obtained in oven drying 50 °C (2.16%) followed by shade drying (1.96%). In contrast, the lowest values of essential oil content of 1.09 and 0.65% were obtained in sun drying and microwave 360W, respectively (Fig 1). There was no significant difference of essential oil between microwaves 180W (1.67%) and oven drying 50°C (1.61%) and both of them ranked in the mid class (b).

The effects of different drying treatments in all of compounds are shown in Table 1. In total 24 compounds were identified in the essential oils of *N. binaludensis* with various drying methods with neptalactone (23.1-54.0%), 1,8-cineol (25.5-51.0%) and myrcene (2.0-4.0%) as major components (Table 1 and Fig 2 and 3). The effects of treatments were not the similar for all of the compounds. So that, the identified compounds numbers of shade, sun, ovens (30, 40, and 50 °C), and microwaves (180 and 360W) treatments were 24, 23, 22, 22, 22, 24, and 23 numbers, respectively.

For nepetalactone content, the treatment ranked high to low as 54.0%, 45.5%, 42.4%, 42.0%, 38.4%, 35.4% and 33.1% for microwave (180W), microwave (360W), oven (40 °C) shade drying, oven (50 °C), sun drying and oven (30 °C), respectively. For 1, 8-cineol the obtained data were 51.0%, 40.2%, 39.3%, 35.6%, 34.1, 26.9%, 25.5%, for oven (30 °C), sun drying, oven (50 °C), oven (40 °C), shade drying, microwave (180W) and microwave 360W, respectively. For myrcene the obtained data were 4.0%, 3.5%, 3.2%, 2.9%, 2.8%, 2.1%, and 2.0% for oven (30 °C), shade drying, oven (50°C), oven (40 °C), sun drying, microwave (180W) and microwave (360W), respectively. For α-terpinene the obtained data were 2.33%, 2.20%, 1.99%, 1.69%, 1.43%, 0.53%, and 0.58%, for oven (40 °C), shade drying, oven (50 °C), oven (40 °C), sun drying, microwave (180W), microwave (360W) and oven (30 °C), respectively. For α-terpineol the obtained data were 3.34%, 3.11%, 2.80%, 2.77%, 2.50% and 2.43% for microwave (360W), oven (30 °C), shade drying, sun drying, oven (50 °C), oven (40 °C) and microwave (180W), respectively. For p-cymene the mean values were 3.65%, 3.05%, 1.99%, 1.83%, 1.62% and 1.50% and 0.93% for oven (30 °C), sun drying, microwave (360W), oven (50 °C), oven (40 °C) shade drying and microwave (180W), respectively.

In overall, the higher values of 13 compounds out of 24 were belonged to oven drying 30 °C treatment than that for others. For the major components, the highest percentage of 1,8-cineole (51%) was obtained from sample dried in oven 50 °C, while, the highest amount of neptalactone (54.0%) was obtained from sample dried by microwave 180 W.

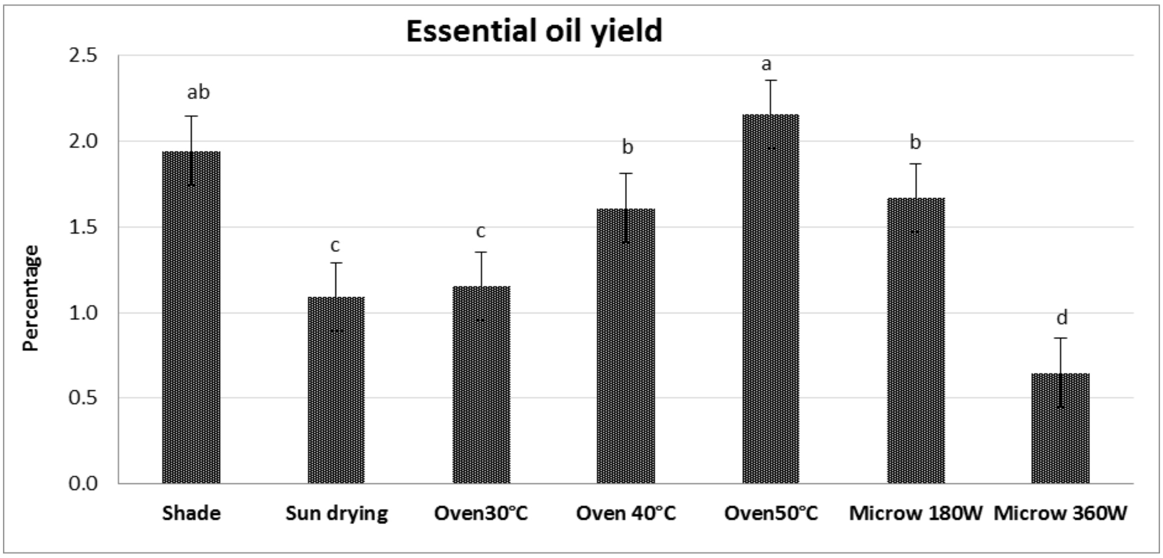

Fig. 1 Mean of Essential oil yield of seven drying methods of *Nepeta binaludensis* Jamzad for flowering shoot of *Nepeta binaludensis* Jamzad (Means of columns followed by the same letters are not significantly different at the 5% probability level by Tukey's test).

Table 1 Chemical compositions of *Nepeta binaludensis* Jamzad essential oils by different drying methods

Compound	RI	Shade drying	Sun drying	Oven 30 °C	Oven 40 °C	Oven 50 °C	Microwave 180W	Microwave 360W	Mean	SE*
α-thujene	928	0.4	0.2	0.52	0.42	0.35	0.28	0.20	0.3	0.05
α-pinene	939	1.1	0.8	1.25	0.96	1.03	0.67	0.61	0.9	0.09
Sabinene	975	1.1	0.6	1.13	0.93	0.98	0.72	0.49	0.9	0.09
β-pinene	978	0.6	0.3	0.61	0.48	0.55	0.35	0.2	0.5	0.06
Myrcene	1000	3.5	2.80	4.07	2.91	3.22	2.14	2.03	3.0	0.28
α-terpinene	1018	2.2	0.53	1.43	2.33	1.99	1.69	0.58	1.5	0.28
p-cymene	1026	1.5	3.05	3.65	1.62	1.83	0.93	1.99	2.1	0.36
Limonene	1029	0.5	0.65	0.52	0.44	0.41	0.27	0.26	0.4	0.05
β-phellandrene	1030	0.5	0.22	0.4	0.56	0.42	0.38	0.17	0.4	0.05
1,8-cineole	**1031**	**34.1**	**40.2**	**51.0**	**35.6**	**39.3**	**26.9**	**25.5**	**36.1**	**3.28**
γ-terpinene	1062	1.2	0.3	0.56	0.94	0.63	0.77	0.62	0.7	0.12
Terpinolene	1087	0.5	0.10	0.26	0.79	0.57	0.82	0.1	0.5	0.12
Linalool	1099	0.1	0.10	0.16	0.14	0.12	0.12	0.17	0.1	0.01
Trans-sabinene hydrate	1101	1.5	1.74	2.3	1.56	1.91	1.20	2.25	1.8	0.15
δ-tepineol	1168	1.5	1.55	1.64	1.24	1.33	1.16	1.52	1.4	0.07
Tepinene-4-ol	1179	1.6	2.35	2.34	1.48	1.55	1.19	2.77	1.9	0.22
α-terpineol	1190	2.8	2.77	3.11	2.5	2.59	2.43	3.34	2.8	0.13
Verbenone	1208	1.0	2.34	0.86	0.90	1.07	1.05	2.76	1.4	0.30
4aα,7α,7aα-nepetalactone	**1364**	**42.0**	**35.4**	**33.1**	**42.4**	**38.4**	**54.0**	**45.4**	**41.1**	**3.60**
E-caryophyllene	1424	0.2	0.25	0.24	0.3	0.29	0.27	0.38	0.3	0.02
δ-elemene	1439	0.4	0.48	0.19	0.44	0.52	0.48	0.59	0.5	0.05
Cis-β-farnesene	1445	0.1	0.2	-	-	-	0.1	0.2	0.15	0.02
Germacrene	1491	-	1.4	-	-	-	-	2.4	1.90	0.28
E-β-farnesene	1450	-	0.5	-	--	-	--	1.1	0.80	0.17
Total		98.4	98.8	99.5	99.1	99.1	98.0	95.8	98.4	0.50
Essential oil%		1.94	1.09	1.16	1.61	2.16	1.67	0.65	1.47	0.20

* Since the essential oils were measured in a single replicate, a comparison was made between the drying methods based on standard error (SE)

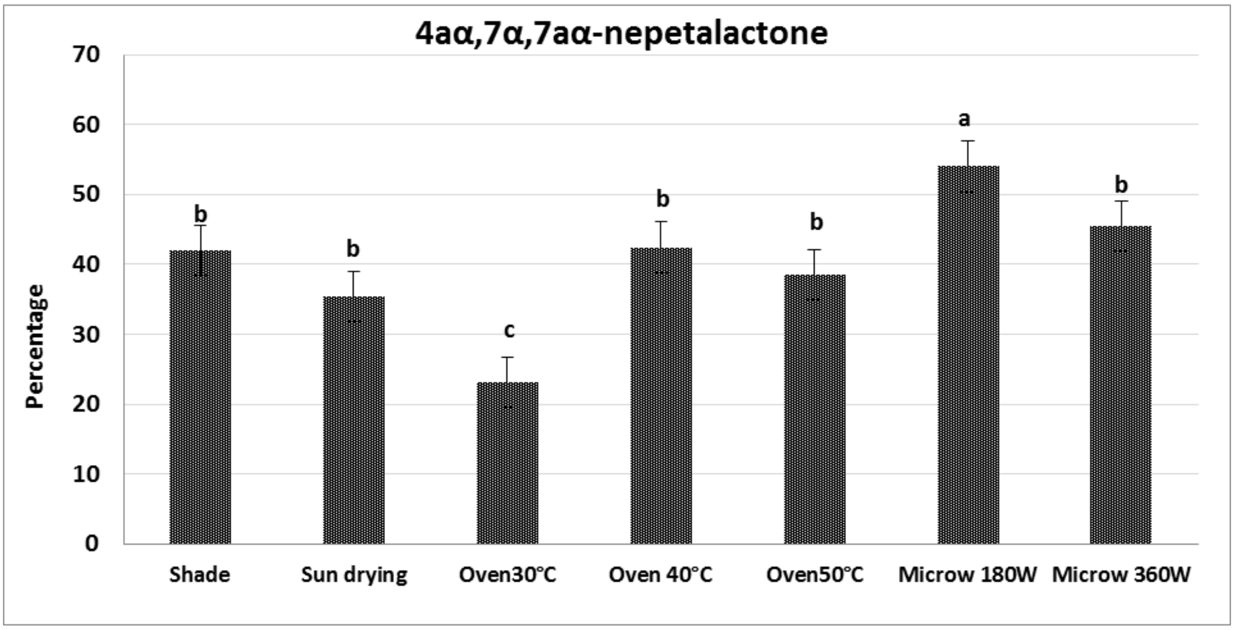

Fig. 2 Comparison of nepetalactone in the oils of *Nepeta binaludensis* Jamzad in 7 drying methods (Means of columns followed by the same letters are not significantly different at the 5% probability level by Tukey's test).

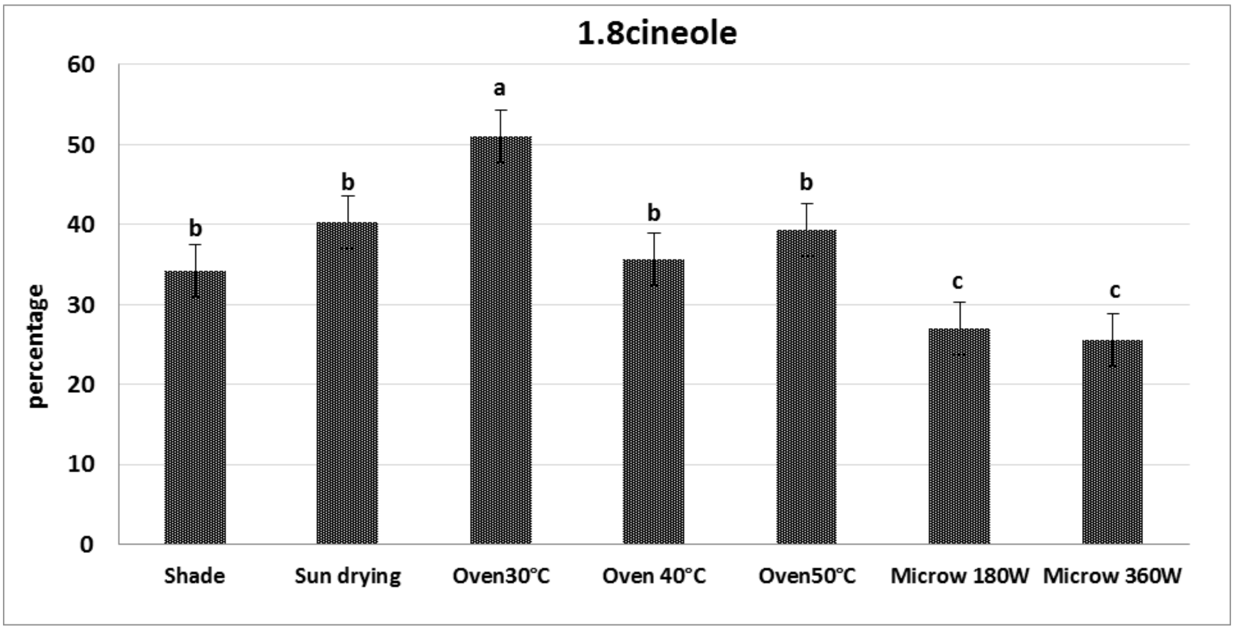

Fig. 3 Comparison of 1,8-cineole in the oils of *Nepeta binaludensis* Jamzad in 7 drying methods (Means of columns followed by the same letters are not significantly different at the 5% probability level by Tukey's test).

Discussion

After harvesting, the fresh plants normally contain high moisture content (60%-80%), which causes irreparable biological, bacterial, fungal, and enzymatic damages. In addition, changes occur in color, odor, taste, and overall crop quality due to oxidation reactions that effectively reduce or change these properties [23]. In drying process moisture of the plant will evaporate to reach a certain threshold in order to storage for a long period and inhibit the enzymatic activities of micro-organisms and yeasts therein.

In the present study the highest essential oil content was obtained in oven drying 50 °C (2.16%) followed by shade drying (1.96%). In contrast, the lowest values of essential oil content of 1.09 and 0.65% were obtained in sun drying and microwave

360W, respectively (Fig. 1). Similar to our result, Sefidkon *et al*., (2006) in savory found the highest essential oil contents of 1.06 and 0.94%, in oven (45 °C) and shade drying methods, respectively [26]. Ebadi *et al*. in *Lippia citriodora* Kunth found the highest essential oil content in oven drying (40 °C) [14] and in *Ocimum basilicum* L. they found the highest values of essential oil (1.3%) in shade drying [27]. Ahmed *et al*. in *Mentha pulegium* found the highest essential oil (1.78%) in shade drying method [28]. Omidbeigi *et al*., in Roman chamomile found the highest essential oil content (1.9%) in shade drying those for sun drying (0.4%) and oven 40 °C drying (0.9%) [13]. Rahim Malek and Goli in *Thymus daenensis* found the highest essential oils in oven 50 °C (1.46%) and sun drying (1.42%) [29]. Mir Mostafaie *et al*. in *Valeriana officinalis* L found the higher values of essential oil in oven 35 °C (0.26%) than microwave 450 W (0.11%). Ghani and Azizi in five species of *Achillea* found the highest essential oil yield in shade drying method [29].

Most of the mentioned references suggested the shade drying and oven drying (40° to 50 °C) were the best methods. The shade drying with or without of hot air is still widely used as a low cost method. However, it has many disadvantages due to the inability to control large quantities of crops and to achieve standards quality [31]. We obtained the highest essential oil in oven 50 °C method. There is some report indicating the negative effects of high temperature on essential oil quantity and quality [20]. Blanco *et al*., in rosemary reported that by increasing oven temperatures from 40 °C to 80 °C, the essential oil content decreased up to 50%. They stated to obtain the higher essential oil in *N. binaludensis* oven drying (50 °C) was recommended [20].

The lowest essential oil yield obtained from microwave 360W (0.65%), respectively. However, there was no significant difference of essential oil between microwaves 180W (1.67%) and oven drying 50°C (1.61%) and both of them ranked in the mid class (b). Similarly, Rezvani Moghaddam *et al*. in *Artemisia dracunculus* L., found higher essential oil yield in microwave 180 W than that for other powers [32].

In total, 24 compounds were identified in the essential oils of *N. binaludensis* with various drying methods with neptalactone (23.1-54.0%), 1,8-cineol (25.5-51.0%) and myrcene (2.0-4.0%) as major components (Table 1 and Fig 2 and 3). The effects of treatments were not the similar for all of the compounds. The identified compounds numbers of shade, sun, oven (30, 40, and 50 °C), and microwave (180 and 360W) treatments were 24, 23, 22, 22, 22, 24, and 23 numbers, respectively. For many of compounds,

Result of mean comparisons between drying method showed that for nepetalactone and α-terpineol content the microwave drying, For 1, 8-cineol, myrcene and p-cymene the oven (30 °C) drying and for α-terpinene the oven (40 °C) drying had the highest values. In overall, the higher values of 13 compounds out of 24 were belonged to oven drying 30 °C treatment than that for others. For the major components, the highest percentage of 1,8-cineole (51%) was obtained from sample dried in oven 50 °C, while, the highest amount of neptalactone (54.0%) was obtained from sample dried by microwave 180 W.

Our result was in agreement with some published data. Asekun *et al*. in *Mentha longifolia* L. obtained higher value limonen (40.8%) in oven (40 °C) drying [33]. Blanco *et al*. with rosemary found higher values of myrcene, α-pinene in oven (40 °C) drying [20]. Rahimmalek and Goli in *Thymus daenensis* obtained higher essential oil in oven drying (40-50 °C) but found higher values of thymol, carvacrol, and β-cryopropylene in oven (70 °C) method [29].

There are many references suggested shade drying method. Ahmed *et al*. in *Rosa damascene* Mill, found the higher values of citronellol and geraniol contents in shade drying method [28]. Ebadi *et al*. in *Ocimum basilicum* L. found shade drying as the best method for preservation of gerainal and methyl cavicol [27], Similarly, Asekun *et al*. in *Mentha longifolia* L. obtained a higher value of menthone (47.9%), in shade drying method [33]. Rahimi and Farrokhi in *Origanum vulgare* L. suggested shade drying as the best method for preserving marjoram. However, for detection and increasing concentration of major compounds required specific drying method [34].

The results of the present study demonstrated that drying the aerial parts of *N. binaludensis* in the shade and oven 50 °C are the best optimal methods to obtain the highest oil yield, while drying in the microwave at 180 W is recommended to enhance higher neptalactone content and oven 30 °C for enhancing higher 1,8-cineole content plus many minor compounds in the oil.

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