

# Phytochemical Characteristics of Sage (*Salvia officinalis* L.) under Various LED Light Spectra

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## Summary

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Light influences secondary metabolite biosynthesis and quality, with varying effects depending on spectral composition. Therefore, more investigations into species-specific reactions to various light spectra are essential. In this investigation, the phytochemical characteristics of sage (*Salvia officinalis* L.) were investigated under six different light treatments. The plants were exposed to various light levels, such as white, blue, red, and mixtures of red and blue light (R70:B30, R50:B50, R30:B70) at the same intensity. Three replications were implemented in a completely randomized design. The variety of light spectra substantially affected the phytochemical characteristics of sage. The total flavonoid content decreased when red and blue lights were used separately, compared to when they were used simultaneously. The R70:B30 light treatment resulted in the maximum essential oil percentage (1.75% v/w). In the sage essential oil, 52 compounds were identified in total. The R70:B30 light treatment also greatly increased the production of important essential oil compounds, including camphor,  $\alpha$ -pinene, camphene, borneol, and 1,8-cineole. The results of this study suggest that a ratio of 70% red and 30% blue is the most effective for improving phytochemical indices in the commercial production of sage in controlled conditions and advanced agricultural practices.

## Key words

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light-emitting diodes (LEDs), essential oil composition, secondary metabolites, sage

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## Introduction

Plant cultivation under controlled environmental conditions is considered a highly efficient and sustainable alternative for consistently achieving high yields and high quality in crop production (Heo et al., 2013). This approach also enables the strategic utilization of elicitors to activate metabolic pathways for the biosynthesis of target compounds (Goto, 2012; Kozai, 2013; Joshi et al., 2017). Determining the optimal conditions for the production of particular plant metabolites results in a significant enhancement in their synthesis (Narimani et al., 2017). A critical component of controlled environment systems is artificial light source. Currently, these environments frequently employ light-emitting diodes (LEDs), known for their effectiveness and energy efficiency (Goto, 2012). This is due to their numerous advantages over traditional illumination methods, such as their compact size, durability, low heat emission, and customizable wavelength selection (Zheng et al., 2019). The properties of light, including quality, duration, and intensity, are a key regulator in morphogenesis, differentiation, and plant growth (Bian et al., 2015). Photosynthetic performance and physiological processes are both affected by light quality (Liao et al., 2014; Batista et al., 2018; Kitazaki et al., 2018). Red and blue wavelengths typically have substantial impacts on the photosynthetic capacity and development of plants, although the specific responses vary among different plant types (Kasajima et al., 2008). Red light affects traits including chlorophyll content, leaf expansion, photosynthetic performance, and stem elongation (Rabara et al., 2017; Szopa et al., 2018). Various processes are affected by blue light, including leaf development, growth and opening of stomata, and secondary metabolite biosynthesis (Hogewoning et al., 2010; Jensen et al., 2018). Numerous experiments demonstrate that the light requirements of plants cannot be completely satisfied by single-spectrum red or blue light (Hernández & Kubota, 2016; Kim et al., 2021). For example, Cucumber (*Cucumis sativus* L.) plants exhibited symptoms of red light syndrome when they were cultivated under red light, as their leaf photosynthesis was significantly impaired (Trouwborst et al., 2016). Therefore, in addition to blue and red lighting, the effect of combining these two spectra should also be investigated (Kim et al., 2021). Olle and Virsile (2013) thoroughly reviewed the impact of light-emitting diode lights on secondary metabolite synthesis and plant growth. The concentration of antioxidants is significantly increased by red light, as demonstrated by Wu et al. (2007), with a twofold increase. Little is known about the effect of light-emitting diode (LED) lighting on the production of secondary metabolites in plants, particularly essential oils. Different research has demonstrated that using blue light supplements can increase levels of carotenoids, vitamin C, and polyphenols (Lefsrud et al., 2008; Q. Li & Kubota, 2009; Stutte et al., 2009; H. Li et al., 2013). According to studies by combining red and blue LED lights, the essential oil content of certain medicinal plants can be enhanced (Sabzalian et al., 2014). Peppermint had a greater amount of essential oil components when LED light sources were used in comparison to natural light under field conditions (Heidarizadeh et al., 2014). The chemical composition of two basil (*Ocimum basilicum* L.) ecotypes, Mobarake (green) and Ardestan (purple), under various light wavelengths was investigated by Hosseini et al. (2018). According to their findings, the red:blue (70:30) light treatment yielded the highest total phenol content,

antioxidant activity, and anthocyanin levels in both ecotypes. Zotov et al. (2020) demonstrated that the same spectral light components can have varying effects on phytochemical profiles and plant growth parameters. Therefore, more extensive studies on plant species and their specific responses to different light spectrum compositions are required. In this context, the model plant employed in this study was sage, scientifically known as *Salvia officinalis* L. (Lamiaceae). Sage is a perennial herb. The aerial part of the plant, particularly the leaves, is rich in essential oils, and contains between 1% and 2.5%. Sage essential oil mostly contains  $\alpha$ -thujone,  $\beta$ -thujone, 1,8-cineole, borneol, camphor, and  $\alpha$ -pinene. Bitter compounds, tannins, flavonoids, glycosides, and resins are also present in the plant (Omidbeigi, 2002). Recently, there has been an emphasis on the cultivation of sage in controlled conditions, e.g., greenhouses, besides open-field cultivation, due to its extensive use and significance in the pharmaceutical and food industries. Investigating the effects of different light spectra on the phytochemical properties of sage could provide useful information for improving cultivation conditions. Therefore, the impact of various LED light spectra in a controlled environment was investigated to identify optimal lighting conditions for achieving maximum phytochemical indices in sage.

## Materials and Methods

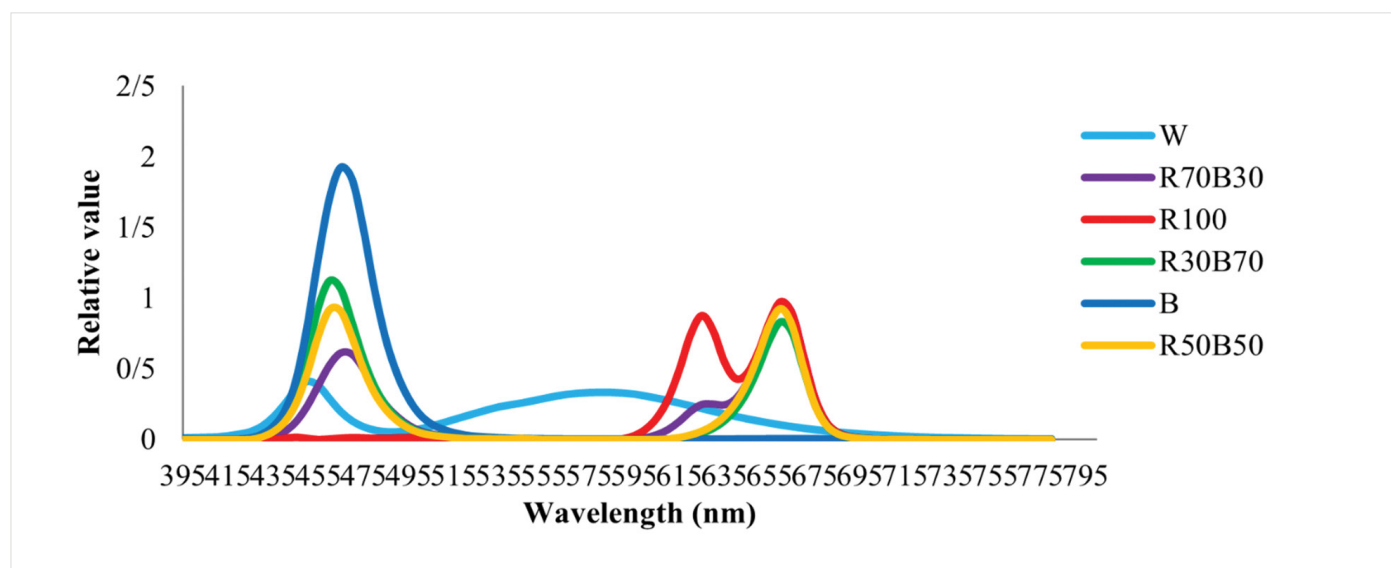
### Plant Materials and Environmental Conditions

In this investigation, six light treatments were applied: white light (W), red light (R), blue light (B), a combination of 70% red and 30% blue (R70:B30), an equal mixture of red and blue (R50:B50), and a blend of 30% red with 70% blue (R30:B70). The light treatments' spectra are illustrated in Fig. 1 in relation to each other. In a growth chamber with soilless cultivation conditions, a completely randomized design was implemented, consisting of six treatments and three replications. Each replicate included five pots. The plants were subjected to the light treatments continuously for 90 days. The sample collection was performed at the end of the treatment period. The control was a white light. In all of the plans, the photosynthetic photon flux density (PPFD) was kept at  $250 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$ , with 14 hours of light and 10 hours of darkness. The intensity of PPFD and a Sekonic C-7000 light meter, manufactured in Japan, were used to measure the light spectrum.

The temperature was kept at 25 °C during the day whereas overnight it was reduced to 20 °C. The relative humidity of the environment was kept at  $60 \pm 5\%$ . Seedling trays were used for the planting of sage seeds and subsequently positioned in six designated chambers within the growth room, each subjected to varying light treatments. After the seedlings reached the four-leaf stage, they were re-subjected to the various light treatments and transferred to larger containers. A complete Hoagland's nutrient solution was used to feed the plants, until the end of the experiment.

### Measurement of Total Flavonoids

The total flavonoid content was determined by carefully following the method described by Chang et al. (2002). 50  $\mu\text{L}$  of methanolic leaf extract was combined with 1 mL of 1 M



**Figure 1.** Light spectra of blue (B), red (R), red 50 and blue 50 (R50:B50), red 70 and blue 30 (R70:B30), red 30 and blue 70 (R30:B70) and white (W) lighting environments measured at plant level in the growth chambers

potassium acetate, 0.1 mL of 10% aluminum chloride solution, and 2.8 mL of distilled water. The samples were analyzed using a spectrophotometer at 415 nm after a 30-minute incubation at room temperature. The outcomes were reported in terms of quercetin equivalents, calculated per gram of dry biomass.

#### Extraction and Determination of Essential Oil Components

Using a steam distillation apparatus, the essential oil was extracted. In each replication, 15 grams of dried leaves were measured, and essential oil extraction was performed for 4 hours using a Clevenger apparatus (British Pharmacopoeia model). The components of the essential oils were identified using a GC/MS system manufactured by Agilent Technologies 5975 fitted with an HP-5ms column, featuring a 30-meter length, a stationary phase thickness of 0.25  $\mu\text{m}$ , and an internal diameter of 0.25 mm. The temperature program for the column was set to a range of 50  $^{\circ}\text{C}$  to 250  $^{\circ}\text{C}$ , and the heating rate was 5  $^{\circ}\text{C}\cdot\text{min}^{-1}$ . The Wiley software was utilized to operate the system on a computer. The injection volume was set at 0.1  $\mu\text{L}$ , and the temperature of the injection port was kept at 250  $^{\circ}\text{C}$ . The procedure used a split injection ratio of 1:20. A flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$  was employed with helium as the carrier gas. The mass spectrometer could detect masses from 35 to 465 mui, with an ionization current of 150 microamperes, an ionization potential of 70 electron volts, and an ion source temperature of 280  $^{\circ}\text{C}$ . Essential oil components were identified by comparing mass spectral patterns, retention index, and fragmentation patterns with standards found in the GC-MS library and the Wiley reference database (Adams, 2007).

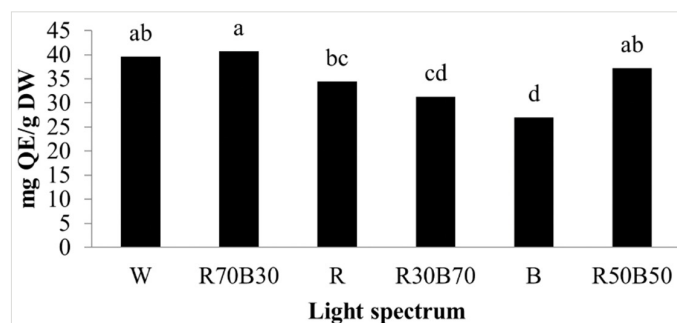
#### Statistical Data Analysis

A statistical analysis was performed on the experimental data using SAS software version 9.1. To compare means, the Duncan multiple range test was employed ( $P < 0.05$ ).

## Results

### Total Flavonoid Content

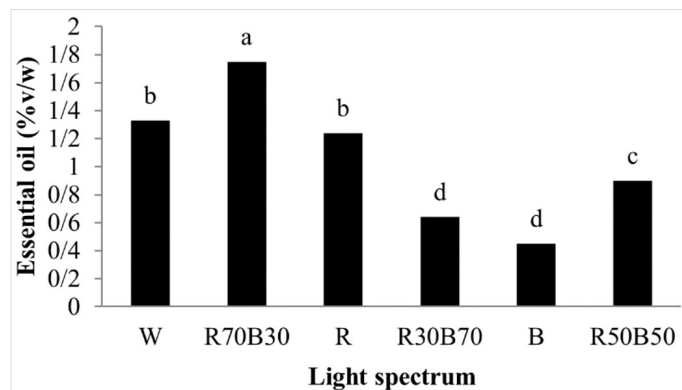
The study's findings demonstrated that the amount of flavonoids in *S. officinalis* was greatly affected by the quality of the light. The flavonoid content across all applied light treatments ranged from 27 to 40.66 mg per gram of dry weight. The maximum flavonoid content (40.66  $\text{mg}\cdot\text{g}^{-1}$  dry weight) was recorded under the R70:B30 light treatment. The observed difference was not statistically significant compared to the R50:B50 or white light. The lowest flavonoid content (27  $\text{mg}\cdot\text{g}^{-1}$  dry weight) was measured under blue light and no significant variation was observed relative to the R30:B70 light treatment (Fig. 2). The total flavonoid content was evaluated to be 33.6% lower under the blue light and 15.47% lower under the red light, in comparison to the R70:B30 light treatment (Fig. 2).



**Figure 2.** Total flavonoid content of Sage plants grown under different light spectra with same intensity. blue (B), red (R), red 50 and blue 50 (R50:B50), red 70 and blue 30 (R70:B30), red 30 and blue 70 (R30:B70) and white (W). Different letters above bars indicate significant differences ( $P < 0.05$ )

### Essential Oil Content

The quality of the light had a significant impact on the concentration of essential oil in the plant. The amount of essential oil evaluated in this study varied from 0.45% to 1.75%. Essential oil concentration was significantly the highest (1.75%) under the R70:B30 treatment, and the lowest (0.45%) under the blue light. There was no significant difference between the blue light treatment and the R30:B70 treatment. The comparison of mean values for light treatments revealed that the R70:B30 resulted in 32.33% increase in essential oil yield when compared with white light treatment (Fig. 3).



**Figure 3.** The content of essential oil of sage plants grown under different light spectra with same intensity. blue (B), red (R), red 50 and blue 50 (R50:B50), red 70 and blue 30 (R70:B30), red 30 and blue 70 (R30:B70) and white (W). Different letters above bars indicate significant differences ( $P < 0.05$ ).

### Essential Oil Component Analysis

In total, 52 constituents were found in the sage essential oil. The quantitative percentages of each compound are presented in Table 1. Under the R70:B30 light exposure, the  $\alpha$ -pinene and camphene concentrations were found to be the highest, while the blue light was shown to be the lowest. The concentration of 1,8-cineole was highest in the white and R70:B30 light treatments, while it was lowest in the blue light treatment. Under the white light, the percentage of  $\alpha$ -thujone was found to be the highest, while under the red light, it was found to be the lowest. The amount of camphor was the greatest when exposed to the red light and the lowest when exposed to the blue light. Furthermore, the red light had the lowest amount of borneol, while the R70:B30 light had the highest amount. According to the results, in all light treatments, the majority of the essential oil consisted of oxygenated monoterpenes such as camphor, 1,8-cineole, borneol,  $\alpha$ - and  $\beta$ -thujone. These were followed by hydrocarbon monoterpenes like  $\alpha$ - and  $\beta$ -pinene, camphene, and myrcene. Maximum quantities of hydrocarbon monoterpenes (19.5%) and oxygenated monoterpenes (57.4%), were measured under the R70:B30. The maximum and minimum amount of hydrocarbon sesquiterpenes, including humulene, was measured to be 9.6% and 6.6% under red and blue light conditions, respectively. In addition, the highest and lowest concentrations of viridiflorol (an oxygenated sesquiterpene) were measured to be 7.9% within the blue light spectrum and 3.2% within the red light spectrum, respectively. Plants under the white light exhibited the lowest concentration of diterpene compounds, including manool, while

under the blue light they exhibited the maximum concentration of these compounds.

### Discussion

The findings of this study suggest that a light ratio of 70% red and 30% blue significantly increase total flavonoid content in *S. officinalis*. This aligns with those reported by Sabzalian et al. (2014), who observed that the production of secondary metabolites enhanced under red:blue LED lighting. Chemicals with phenolic properties, like flavonoids, are secondary metabolites based on carbon (Zobayed et al., 2007). The framework of secondary metabolites can be synthesized by utilizing the surplus carbon generated by photosynthesis when it exceeds the plant's immediate needs (Heyworth et al., 1998). As a result, elements that limit photosynthesis, such red or blue light, can affect both the formation of primary metabolites and the amounts of secondary metabolites. Additionally, Chen et al. (2024) demonstrated that flavonoid accumulation in strawberry flesh was greatly increased by red light supplemented with blue light. The overexpression of transcription factors and structural genes involved in the flavonoid biosynthesis pathway was ascribed to this. Also, Son and Oh (2013) found that blue light increased the expression of phenylalanine ammonia-lyase, a key enzyme in the phenylpropanoid pathway. The biochemical properties of plants can be modified through exposure to varying light spectra, which influences the expression of genes responsible for enzymes associated with the secondary metabolite formation. Also, the activation of photoreceptors such as phytochromes and cryptochromes, which affect the expression of genes involved in phenolic biosynthesis, may also account for the rise in flavonoid concentration under mixed light (Feinbaum et al., 1991; Wade et al., 2001).

Environmental conditions considerably impact the concentration and composition of bioactive compounds in medicinal plants, alongside genetic factors (Omidbeigi, 2002). When comparing the effects of blue or red light on essential oil concentration, this study found that the optimal combination of red and blue light had a greater effect. This is likely due to the increased synthesis of primary metabolites under this light condition. Research has shown that plants produce less essential oil when exposed to short-wavelength (blue) light (Palvov & Ilieva, 1972; Saleh, 1973). If the percentage of red light is increased, it can cause a faster rate of photosynthesis, growth, and biomass, which could subsequently result in a higher production of essential oils (Nishioka et al., 2008). Red light increased the total essential oil concentration of some *Mentha* species (Dou et al., 2017). Blue light increased coumaric acid, ferulic acid, and vanillic acid levels in ginseng (Park et al., 2013). These studies collectively emphasize the role of light spectra in controlling secondary metabolite synthesis in plants. Theoretically, these wavelengths may stimulate the biosynthesis of secondary compounds by activating particular genes (Sabzalian et al., 2014).

Consistent with earlier results, this investigation found that the primary constituents of sage essential oil were camphor, 1,8-cineole,  $\alpha$ - and  $\beta$ -thujone,  $\alpha$ -humulene, borneol, and manool. The primary constituents of sage essential oil have been identified in various studies. Risaliti et al. (2019) and Russo et al. (2013) reported 1,8-cineole (eucalyptol) and sabinene, respectively, as major components.

**Table 1.** Chemical components of essential oil of Sage plants grown under different light spectra with same intensity

NO	Compound	RI	Percentage %					
			W	R70B30	R	R30B70	B	R50B50
1	tricyclene	925	0.3	0.4	0.3	0.2	t	0.3
2	$\alpha$ -thujene	932	0.4	0.4	0.3	0.4	t	0.3
3	$\alpha$ -pinene	939	4.3	4.5	4.5	3.3	1.2	4.0
4	camphene	954	5.0	6.2	4.9	3.9	1.8	5.0
5	sabinene	978	0.4	0.3	0.2	0.4	t	0.3
6	$\beta$ -pinene	980	4.5	4.2	3.9	3.3	1.2	3.4
7	1-octen-3-ol	986	T	0.1	t	t	t	t
8	myrcene	994	1.6	1.4	1.1	1.2	0.6	1.0
9	$\alpha$ -phellandrene	1005	0.1	0.1	0.1	0.1	t	0.1
10	$\alpha$ -terpinene	1019	0.4	0.3	0.3	0.3	t	0.2
11	o-cymene	1029	0.3	0.3	0.2	0.3	t	0.2
12	limonene	1033	T	t	t	1.7	1.0	1.8
13	1,8-cineol	1037	10.9	10.9	9.9	9.0	5.3	7.8
14	trans- $\beta$ -ocimene	1044	T	t	0.1	t	t	t
15	$\gamma$ -terpinene	1063	0.8	0.7	0.5	0.7	0.2	0.5
16	cis-sabinene hydrate	1073	0.2	0.3	0.2	0.3	0.2	0.2
17	cis-linalool oxide	1078	0.1	0.1	t	0.1	t	0.1
18	terpinolene	1090	0.9	0.7	0.8	0.7	0.3	0.5
19	trans-sabinene hydrate	1103	T	t	t	t	0.2	0.2
20	$\alpha$ -thujone	1114	17.9	16.9	14.3	21.4	20.5	15.5
21	$\beta$ -thujone	1122	3.4	2.6	3.3	3.6	2.8	3.2
22	iso-3-thujanol	1145	0.1	0.1	0.1	0.1	t	0.1
23	camphor	1154	14.4	15.6	18.0	13.9	10.9	14.0
24	isoborneol	1163	0.1	0.1	0.1	0.1	t	0.1
25	trans-pinocamphone	1166	0.1	0.1	0.2	0.1	t	0.1
26	borneol	1173	4.6	5.0	3.7	4.4	3.8	4.6
27	cis-pinocamphone	1179	0.1	t	0.1	t	t	t
28	terpinen-4-ol	1182	0.8	0.7	0.5	0.7	0.5	0.6
29	$\alpha$ -terpineol	1194	0.3	0.2	0.2	0.2	0.2	0.2
30	myrtenol	1200	0.2	0.2	0.3	0.1	t	0.1
31	trans-carveol	1225	0.1	0.1	0.1	0.1	t	0.1

Continued. Table 1

NO	Compound	RI	Percentage %					
			W	R70B30	R	R30B70	B	R50B50
32	cis-carveol	1237	T	t	t	t	t	t
33	neral	1246	T	t	t	t	t	t
34	(-)-carvone	1249	T	t	t	t	t	t
35	bornyl acetate	1291	3.3	3.2	2.7	2.1	1.4	1.4
36	trans-sabinyl acetate	1296	0.3	0.3	0.2	0.3	0.2	0.2
37	carvacrol	1311	0.1	t	t	0.1	t	0.1
38	myrtenyl acetate	1330	T	t	0.1	t	t	t
39	trans-carvyl acetate	1342	T	t	t	t	t	t
40	geranyl acetate	1387	T	t	t	t	t	t
41	$\beta$ -caryophyllene	1429	1.5	1.4	1.7	1.4	1.2	1.3
42	aromadendrene	1449	0.2	0.2	0.3	0.2	t	0.1
43	$\alpha$ -humulene	1468	5.4	4.9	7.3	5.2	5.4	4.9
44	trans- $\beta$ -ionene	1499	T	t	0.1	t	t	t
45	viridiflorene	1501	0.1	0.2	0.2	0.2	t	0.2
46	$\gamma$ -cadinene	1521	T	t	t	t	t	t
47	$\delta$ -cadinene	1530	0.1	0.1	0.1	t	t	0.1
48	spathulenol	1586	T	0.1	t	0.2	t	0.1
49	caryophyllene oxide	1591	0.1	0.2	0.1	0.2	0.2	0.2
50	viridiflorol	1603	4.9	4.8	3.2	6.8	7.9	5.4
51	isopimara-9-(11),15-diene	1920	0.1	0.1	t	0.1	t	0.2
52	manool	2067	11.2	12.0	15.6	12.7	33.2	21.5
Major Grouped Compounds								
	Monoterpene hydrocarbones		19.1	19.5	17.1	16.4	6.3	17.7
	Oxygenated monoterpenes		57.2	56.4	54.3	56.6	45.9	48.4
	Sesquiterpene hydrocarbones		7.3	6.8	9.6	7.0	6.6	6.5
	Oxygenated sesquiterpenes		5.1	5.2	3.4	7.2	8.0	5.7
	Diterpenoides		11.3	12.1	15.6	12.8	32.2	21.7

Note: RI - The Kovats retention indices relative to C8-C21 n-alkanes were determined on HP-5 ms capillary column; *t* = Trace, minor compounds less than 0.05%

The primary constituents of sage essential oil were determined by El Euch et al. (2019) to be camphor, 1,8-cineole, and  $\alpha$ -thujone. Essential oils from this plant mostly contain  $\alpha$ -thujone,  $\beta$ -thujone, 1,8-cineole, and viridiflorol, as reported by Taarit et al. (2009). Furthermore, the primary components of sage essential oil have been determined by several investigations to be 1,8-cineole, camphor,  $\alpha$ -thujone, borneol, and  $\alpha$ -humulene (Rhyu, 1979; Tsankova et al., 1994; Pino et al., 1997).

The present study demonstrated an increase in  $\alpha$ -pinene content under red light, aligning with previous findings. Noguchi and Amaki (2016) reported that the application of red light led to a higher abundance of monoterpenes in Mexican mint. Red light increased the concentration of  $\alpha$ -pinene and decreased the concentration of p-cymene in rosemary, as shown by Mulas et al. (2006). Monoterpenes are a significant component of sage essential oil, as previously mentioned.  $\alpha$ - and  $\beta$ -thujone, camphor, and 1,8-cineole are the primary oxygenated monoterpenes found in sage essential oil (Kulak et al., 2020). 1,8-cineole is well-known for its medicinal effects (Cegiełka et al., 2019). Sage essential oil exhibited both quantitative and qualitative enhancements under a red:blue light ratio of 70:30, with notable increases in monoterpenes including  $\alpha$ -pinene, camphene, borneol, 1,8-cineole, and camphor.

## Conclusion

According to the findings of this study, it appears that, when the R70:B30 light spectrum is used, flavonoids and important essential oil components like  $\alpha$ -pinene, camphene, borneol, 1,8-cineole, and camphor levels rise. This light spectrum is thus advised for the cultivation of sage in regulated conditions.

## CRedit Authorship Contribution Statement

**Mahdi Moradi:** Visualization, Conceptualization, Supervision, Investigation, Methodology, Data curation, Analysis, Writing – original draft. **Maryam Fallah:** Writing, including review and editing. **Mohammadnaser Modoodi:** Methodology, Investigation.

## Declaration of Competing Interests

There are no personal relationships between the writers or conflicts of interest that could have impacted the results of this study, as stated by the authors.

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