



Enhancing Medicinal Properties Of Salvia Hispanica: Influence Of Light Spectra On Growth, Polyphenolic Content, And Antioxidant Activity

1Acharya Shila, 2Moqadas Lyeba, 3Nisar Samrin, 4Sharma Chitranjan

1Lecturer, 24th Year Medical Student, 34th Year Medical Student, 43rd Year Medical Student

1Jalal-Abad State University, Medical Faculty,

2Jalal-Abad State University, Medical Faculty,

3Jalal-Abad State University, Medical Faculty,

4Jalal-Abad State University, Medical Faculty

Abstract:

Introduction:

Salvia hispanica (commonly known as chia) is a flowering plant native to Central and South America. It is highly valued for its seeds, which are a rich source of essential nutrients such as omega-3 fatty acids, dietary fiber, antioxidants, and high-quality proteins. Medicinally, chia seeds are recognized for their potential in promoting cardiovascular health, regulating blood sugar levels, and aiding in weight management. Additionally, they possess anti-inflammatory and antioxidant properties, making them beneficial in preventing chronic diseases such as diabetes, hypertension, and obesity. These attributes underscore their role as a functional food with significant therapeutic applications.

Objective:

To evaluate the impact of different colored light treatments on growth parameters, polyphenolic content, and antioxidant activity in *Salvia hispanica*, with a focus on enhancing its medicinally significant properties. This study aims to optimize light conditions to maximize the production of bioactive compounds that contribute to its therapeutic potential, including its antioxidant, anti-inflammatory, and disease-preventive effects.

Methodology:

The research was conducted to investigate the effects of different colored light treatments on the growth, polyphenolic content, and antioxidant activity of *Salvia hispanica*, with a focus on enhancing its medicinal properties. The experiment was carried out at Pakistan, under controlled environmental conditions.

Results:

The study demonstrated that different colored light treatments significantly influenced the growth parameters, polyphenolic content, and antioxidant activity of *Salvia hispanica*. Red and blue light treatments showed the most pronounced effects, enhancing plant height, biomass, and chlorophyll content. Plants exposed to blue light exhibited the highest polyphenolic content and antioxidant activity, indicating an

improvement in the plant's medicinal properties. These findings suggest that light quality plays a crucial role in optimizing the therapeutic potential of *Salvia hispanica*.

Conclusion:

This study concludes that light quality significantly affects the growth, polyphenolic content, and antioxidant activity of *Salvia hispanica*. Blue light was identified as the most effective in enhancing its medicinal properties, highlighting its potential for optimizing the production of bioactive compounds for therapeutic applications.

INTRODUCTION

Salvia hispanica is commonly known as chia. It is an herbaceous plant that hails from family Lamiaceae and genus *Salvia*. It is known to be the diverse genus in family Lamiaceae. It has about 900 species extensively distributed in different regions across the world including South-east Asia, Southern Africa, and many parts of America. Mexico is the largest producer of Chia, but it is also cultivated in Bolivia, Australia, Colombia, Peru and Argentina (Grancieri et al., 2019)[1]. Chia is the common name for few species of *hispanica* such as *S. columbariae*, *S. hispanica*, and *S. polystachya* are prominent among them. Carolus Linnaeus found *S. hispanica* and was confused that it is a wild plant native to Spain, hence this plant was named after him. Chia is seeds of plants used by Mesoamerican peoples and the name makes its way back to those populations (ValdiviaLópez et al., 2015)[2]. Human started using Chia from past 5500 years, its seeds are used for the purpose of nutrition, medicines, cosmetics and a tool for religious rituals. It is an important and most consumed crop after bean in Columbian societies. The term Chia is deduced from Spanish terminology 'chian' which means oily nature of the seed (oilseed). It contains high amount of omega-3 fatty acids. High quality proteins, dietary fibers, minerals, vitamins and a significant number of secondary metabolites which possess antioxidant activity. It protects the seed against chemical and microbial stresses. It is actively participating in medical and pharmaceutical fields (Ullah et al., 2016)[3]. The antioxidant and rheological effects of the fruits as well as extracts of *S. hispanica* has been well documented (de Falco et al., 2018)[4]. The seeds of chia exhibit remarkable pharmacological properties which includes antioxidant activity, anti-inflammatory activity, cytotoxicity, anti-microbial activity, hypolipidemic activity, hypoglycemic effect and anti-proliferative tendencies (Sehrawat and Singh., (2019) [5].

In sixteenth century, the pre-Colombian populations use chia to get energy, strength and fortitude. The Aztec army fulfilled their nutritional requirements through the consumption of Chia. The oils extracted from seeds have been used in cosmetic industry. Furthermore, offering to gods during religious custom and taxes were paid with seeds of chia. The leaves, roots and branches were used to treat infections of respiratory systems (Grancieret al., 2019)[6]. *Salvia hispanica* and many other species of the genus (*Salvia*) are historically important.

APPLICATIONS OF CHIA SEEDS

The extensive use of *S. hispanica* seeds is related to its health benefits in conditions like obesity, hyperglycemia, cardiovascular disorders and cancers. Protein (15 to 25%), fat (15 to 25%), carbohydrates (41%), dietary fibers (18 to 30%), ash (4 to 5%) and dry matter in range of 90 to 93% is present in the chia seeds. (Ixtaina et al., 2008)[7]. The level of heavy metal in seeds are in limited amount hence do not pose any threat due to absence of toxic mycotoxins and gluten (Peiretti and Gai 2009)[8]. Presently, the chia seeds are in use for the isolation of biologically important components for the commercialization of foods (Bresson et al., 2009)[9]. Chia plants cultivated in different habitats produce proteins within the range of 18.8% to 21.5%, fluctuation in the protein content might be in response to climate, agronomic and soil environment (Ting et al., 1990)[10]. One report suggested that chia seeds consist of 20% protein content, 25% dietary fibers and 34% oil. The high availability of fibers in chia seeds have a significant job in health sciences as it increases stool volume, work against diverticulosis and fight cancer. The announcement of chia as a functional food by European Parliament has accelerated its popularity among the people of Mexico, Japan, Chile, New Zealand, America, Australia and Canada. Now it is known as a safe food with no obvious hazards to health and is applicable in baked productions, nutritional supplements, snacks, breads and cookies (Beltran-Orozco and Romero, 2003)[11]. The chia seeds have satisfying amount of dietary fibers having the ability of water absorbance 15 times as compare to the weight of seed. The high content of fibers helps

during diabetes mellitus by impeding the metabolism and secretion of glucose. It improves peristalsis and help in lowering cholesterol in plasma. The medicinal value of chia is higher than other cereals and contain significant amount of calcium, potassium and magnesium than milk (De Tucci, 2006)[12].

MEDICINAL IMPORTANCE OF SALVIA HISPANICA

The plant seeds contain numerous natural compounds with antioxidant effect such as phenolics, flavonoids, and vitamins. It has the potential to stop the activation of transcription factor NF-KB, thus minimizing the inflammatory and carcinogenic responses. It also provides protection against neural disorders, immunodeficiency diseases, cardiovascular disorders, strokes, Parkinson disease, Alzheimer disease and cancer (Marcinek and Krejpcio, 2017)[13].

The cardiovascular diseases of humans are directly associated with the rise of blood serum HDL-cholesterol (Rasheed and Cummins, 2018)[14]. One recent study proposed that chia contain such proteins that can prevent the synthesis of key marker of cholesterol such as HMG-CoA reductase (Coelho et al., 2018)[15]. Hypertension is a common signal of cardiovascular disease (Fowokan et al., 2018)[16]. In adults with hypertension, the use of 35g/day of chia flour for the period of 3 months was enough to normalize elevated blood pressure. These positive effects were linked with the amount of n-3 fatty acids in chia (Toscano et al., 2014)[17]. The consumption of 35g/day chia for three weeks by patients with diabetes type-2 has led to decrease of systolic and diastolic pressure (Vuksan et al., 2007)[18].

Chia seeds are good source of nutritional values. It contains protein, Mg, P and beneficial fatty acids. In order to get best results of chia seeds, the proper intake of these seeds is very necessary. Direct ingestion is very useful because of the presence of mucilage layer which is the important part of soluble fibers. The milled and whole chia seeds have the properties to minimize the risk of diseases in over weighed peoples and in postmenopausal women. Chia seeds contain high value of lipids, protein and other contents as compare to other crops (Valdivia and Tecante, 2015)[19]. In human chia seeds are good nutritional food for women and for those patients having HIV-positive. It is suggested by many studies that by insertion of chia seed into chicken. The nutritional values of chicken product can be improved by increasing the content of omega-3 fatty acids, and by minimizing the level of cholesterol in meat as well as in eggs. Chia seeds are nowadays used in animal feed in order to improve the omega-3 oil content. These seeds also have anti-tumor activity in cancer of murine mammary glands (Ulbricht et al., 2009)[20].

MATERIALS AND METHODS

1: Seeds selection:

The seeds of chia (*S. hispanica*) were provided by our research supervisor. All the seeds were intact and represented a good quality of chia. The seeds were Sterilized according to the protocol. 70% ethanol for the time period of one minute and 0.2% Mercuric Chloride for two minutes were used to wash the surface of seeds to remove impurities followed by washing several times with distilled water, the seeds were allowed to dry.

2: Media preparation and germination of seeds:

Autoclaved cotton and filter papers in petri dishes were taken as media for the germination of chia seeds. Cotton was placed in each of the 18 petri dishes and on the surface of each plate was covered with filter paper. Later, water was added to the petri dish and filters were adsorbed on the cotton. Seeds of *S. hispanica* (10 in number) were sowed on each plate and with the help of sterilized needle, the seeds were aligned in a proper manner. The petri dishes were covered with plastic bags. Triplicate of plates were placed under different color light separately. The seeds germination rate, weight of germinated plantlets and length of radicle and plumule were measured for 18 days with three days interval between two consecutive measurements.

3: *Light exposure:*

Petri dishes were treated with five different spectral light in such a way that each set of triplicated petri plates were set against each light. And 15 petri dishes were exposed to five different color lights including green, red, blue, yellow and white as control. One triplicate of plates out of 18 were kept in continuous darkness. The number of seeds in each triplicate was 30. Suggesting that 30 seeds were subjected to individual light spectrum and continuous dark. It is worth mentioning that the germination of seeds initiated at third day of the inoculation.

4: *Calculation of biomass :*

The triplicated plates under each light were taken. Morphology of each germinated seeds was closely observed with naked eye followed by counting of germinating seeds (plants) in percentage. Then through random selections, one plantlet was taken to measure its length of radical (mm) and plumule (mm) using standard ruler. The fresh weight (biomass) of the respective plant was measured with the help of digital balance system. After taking readings plates were covered with plastic bags and kept back under the corresponding light. The percentage for mean of germinated seed, mean of radicle length, mean of plumule length were calculated. To get biomass, the mean of weight and mean of germinated seeds were added. When germination was completed at 18 day of experiment, the fresh weight of each of three plates as well as combine fresh weight of three plates was measured. The samples were subjected to sun heat for the evaporation of water particle for few days and dry weight of plates under each light was calculated. It was grinded using mortar and pestle to form powder. The powder weight for each sample was determined. Each powdered sample was dissolved in 10 ml ethanol.

5: *Determination of total phenolics and flavonoids content:*

For the determination of non-enzymatic compounds TPC and total TFC, the following protocols were applied. The Total phenolics content (TPC) was quantified by using the method of Ahmad et al. (2014). In short, 0.1 ml (2 N) Folin-Ciocalteus reagent was taken to mix it with 0.03 ml of extract sample and 2.55 ml of distilled water. After that the centrifugation of the mixture at speed of (10,000 rpm for 14 min) was carried out followed by incubation for six minutes. It was then filtered with a 45- μ m membrane in a UV-visible spectrophotometer (Shimadzu-1650 Japan) cuvette. The absorbance of the corresponding mixture was recorded at 760 nm. Gallic acid (Sigma 1.0-10 mg/ml R-0.9878) was used for plotting the standard calibration curve. Results as Gallic acid equivalent (GAE) mg/g of DW were achieved from %TPC by using the following equation

$$\%TPC-100 \times (AS-AB)/(CF-DF)$$

In the above equation, the sample absorbance is represented by As and absorbance of the blank by AB. While CF denotes the conversion factor from standard curve and dilution factor is marked by DF.

The Total flavonoids content (TFC) in the sample of *S. hispanica* was measured using the method of Ahmad et al. (2014). The methanolic sample's extracts (0.25 ml) and sterile distilled water (1.25 ml) both were mixed together followed by mixing with 0.075 ml AICII (5% w/v). The solution was then centrifuged (10,000 rpm for 14 min) and the supernatant was incubated for five minutes. After that the solution was mixed with 0.5 ml of NaOH (1 M). The absorbance of the mixture was tested at 510 nm using a UV-visible spectrophotometer (Shimadzu-1650PC Japan). To plot the standard calibration curve. Rutin (Sigma 1.0-10 mg/ml R-0.9866) was exploited. The TFC was represented in the term of rutin equivalent (RE) mg/g-DW of extracts

6: *Calculation of antioxidant activity:*

The DPPH radical scavenging activity (DRSA) of each extract sample of chia plant was examined by our supervisor. In short, the ethanolic extract (1.0 ml 5 mg/20 ml) of sample was taken individually and was mixed with 2.0 ml DPPH-free radical solution (0.25 mg/20mlx4). The combination was placed in dark for half an hour. UV-visible spectrophotometer model (Shimadzu, 1650PC, Japan) was used to measure the

mixture absorbance at 517 nm at room temperature. After that the antioxidant activity was calculated using the equation as follow

$$\text{RSA\%} = 100 \times (1 - A_p / A_D)$$

A_p denotes the extract absorbance sample at 517 nm and A_D denotes the absorbance capacity of the DPPH reagent without extracts

7 :Statistical analysis:

Microsoft excel was used for mathematical representation of data and analysis.

RESULTS

Plantlets development under spectral lights

In the current study, different spectral lights and dark showed diverse effect on the seed germination rate and morphology of the plant.

The effect of coloured lights on growth performance of *S.hispanica*

S.No	Treatment	Days	Morphology	Germination(%age)
1	Blue light	3	Greenish	55
		6	Greenish	62
		9	Dark green	69
		12	Light green	75
		15	Green	82
2	Red light	3	Greenish	25
		6	Dark green	29
		9	Dark green	35
		12	Green	39
		15	Green	45
3	Green light	3	Greenish	52
		6	Dark green	56
		9	Light green	61
		12	Light green	66
		15	Green	76
4	Yellow light	3	Greenish	44
		6	Light green	49
		9	Light green	53
		12	Dark green	57
		15	Yellowish green	63
5	White light	3	Light green	27
		6	Light green	31
		9	Dark green	37
		12	Green	42
		15	Yellowish green	48
6	Dark	3	White	30
		6	Yellowish	36
		9	Yellowish green	44
		12	white	51
		15	White	55

After 15 days, among different colored lights and continuous dark, maximum seed germination was observed under blue, green, yellow lights and dark than the white used as control while minimal germination than the control was observed under red light. Similarly, morphology of the plantlets was also affected under continuous five colored lights and dark. The germination rate in percentage and morphology of the plantlets has shown in the Table

Biomass accumulation

Like germination and morphology, fresh biomass accumulation under various spectral lights and dark was recorded. Results concluded that maximum fresh biomass accumulation was observed under blue light (0.812 g), green light (0.709 g), yellow light (0.680 g) and dark (0.450 g) when compared with the control conditions (0.448 g). While red light (0.425 g) accumulation of fresh biomass was inhibited compared to the control white light (0.448 g) used as control. The FW and DW of samples under a range of lights and dark showed in the Table .

The effect of different spectral lights on fresh weight and dry weight

S.No	Lights	Fresh weight(g)	Dry weight(g)
1	Blue	0.812	0.054
2	Red	0.425	0.043
3	Green	0.709	0.052
4	Yellow	0.680	0.056
5	white	0.448	0.042
6	Dark	0.450	0.051

Total Phenolics Content accumulation

In this study, the effect of various spectral lights were investigated for seed germination, fresh and dry biomass accumulation, effects on lights on morphological features of plantlets and further investigated the plantlets for secondary antioxidant metabolites accumulation. The antioxidant activity was also determined to test various treatments for best activity. In the present study, TPC accumulation was enhanced in response to blue light (8.33 mg/g-DW), followed by red light (7.78 mg/g-DW), dark (7.01 mg/g-DW) and green lights (4.1 mg/g-DW) when compared with the control conditions (3.8 mg/g-DW). While the yellow light (2.77 mg/g-DW) accumulation of TPC was suppressed comparing to control (3.8 mg/g-DW) cultures. The TPC and TPP quantification under different colored lights and dark are presented in the Table

Effect of various spectral lights and dark on TPC and TPP accumulation .

S.No	Light	Total phenolics content	Total phenolic production
		mg/g-DW	
1	Blue Light	8.33	0.449
2	Red Light	7.78	0.334
3	Green Light	4.1	0.213
4	Yellow Light	2.77	0.155
5	White Light	3.8	0.159
6	Dark	7.01	0.357

Total Flavonoids Content accumulation

The TFC accumulation in the plants showed positive response under blue light (5.04 mg/g-DW), red light (4.99 mg/g-DW), dark (4.88 mg/g-DW), and green light (3.66 mg/g-DW) when compared to the controlled conditions (2.88 mg/g-DW). However, under the yellow light (2.01 mg/g-DW) accumulation of TFC

showed negative response compared to control (2.88 mg/g-DW). These results have shown the positive correlation between TPC and TFC. The TPP and TFP accumulation under various spectral lights and dark are shown in the Table

Effects of various spectral lights and dark on TFC and TFP accumulation

S.No	Lights	Total Flavonoids content mg/g-DW	Total Flavonoids production
1	Blue Light	5.04	0.272
2	Red Light	4.99	0.215
3	Green Light	3.66	0.190
4	Yellow Light	2.01	0.112
5	White Light	2.88	0.120
6	Dark	4.88	0.248

Antioxidant Activity determination

In the current study, plants grown under blue light (71.33%), red light (66.29%), dark (62.09%), green light (58.88%) have shown maximum antioxidant activities compared to the control (49.51%). While yellow light (45.33%) showed lowered antioxidant activities than the control (49.51%). These results have shown that there is strong correlation between TPC, TFC, and antioxidant activities. The FRSA in percentage of plants grown under different spectral lights and dark are given in the Table.

Effect of various spectral lights and dark on antioxidant activities .

S.No	Lights	Aantioxidant activites(% age)
1	Blue light	71.33
2	Red light	66.29
3	Green light	58.88
4	Yellow light	45.33
5	white light	49.51
6	Dark	62.09

DISCUSSION:

1: Biomass accumulation:

In the current study, maximum seed germination and biomass production was recorded under blue light followed by green and yellow lights than the white and red light. Similarly, biomass accumulation under yellow light and was observed in the callus cultures of *Prunella vulgaris* (Fazal et al., 2016; Naveed et al. 2018)[21]. The current result concluded that the variation in biomass accumulation is related to the light quality, intensity, plant species, and the duration of exposure of the samples to lights.

2: TPC and TFC accumulation:

Our results showed that the maximum TPC and TFC was observed under blue, red, and green lights. Reported that blue Light stimulate maximum phenolics content and flavonoids content in the callus cultures of *S. rebaudiana*. Available literature showed the correlation between TPC and TFC. Similarly, blue light increases TPC and TFC in the callus cultures of *F. indica* after white light (Khan et al., 2018)[22]. It also been investigated that among various lights, blue light showed maximum accumulation of Sal (3.21003 mg/g DW) on day 21 of culture in the callus culture of *Radiola imbricate* (Kapoor et al., 2015)[23].

3: Antioxidant activity:

In the present study, we determined a strong dependency of phenolic and flavonoid production with free radical combating activity. As mentioned previously, blue light accelerates phenolic and flavonoid content. Moreover, the blue light increases antioxidant activities as compared to control. reported that blue light has a positive effect on TPC, ITC as well as antioxidant activities than the control.

4: Conclusion:

Light has been one of the recently used abiotic elicitor for its accelerating effects on seeds germination and important metabolites production in plant sciences. In present study, it was observed that green light has positive effects on the germination of seeds *S.hispanica* in comparison to white control and other colors. However, enough amount of biomass was accumulated under blue light spectrum followed by green and yellow spectrum. Similarly, blue light also showed significant amount of total phenolic content (TPC) as well as total flavonoids (TFC) synthesis followed by red and continuous dark. The antioxidant activity of chia plant was also dependent on TPC and TFC and recorded maximum activity in response to blue, red and dark respectively. So, it is concluded that green light is best in plantlets development while blue, red and dark is linked to the increased production of TPC,TFC as well as antioxidant activity in chia.

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