Research Article

Light Intensity Alters the Growth and Quality of Volatile Components from Hexane Extracts of *Hyptis suaveolens* (Family Lamiaceae): A Medicinal Plant

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ABSTRACT

Aim: This study was carried out to investigate the impact of light intensity on plant growth and n-hexane extracts of the leaves of *Hyptis suaveolens*. **Materials and Methods:** Plants were subjected to full sunlight (FS, 58753 lux), medium light intensity (MLI, 35880 lux) and low light intensity (LLI, 1250 lux) and were grown in loamy soil for 16 weeks. The parameters investigated include leaf area, shoot and root weight, number of leaves, Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Relative Growth Rate (RGR), type and amount of volatile components from n-hexane extract present in the leaves, using GC-MS. **Results:** There were mostly slight differences in the growth parameters but consistently the plants subjected to FS showed highest growth rate followed by those subjected to MLI. The dry weight roots of LLI plants, were significantly lower than the other treatments at $p = 0.05$ and these plants did not flower. Though there were only slight differences between the growth parameters of plants subjected to different light intensities, type and quantity of volatile compounds varied markedly. Volatile compounds like Ar-Abietatriene and 1-Phenanthrenemethanol were present under the three varying light intensities. Furthermore, LLI induced the highest concentration of 1-Phenanthrenemethanol than the other treatments, while FS induced the highest concentration of Ar-Abietatriene. Though full sunlight improved the growth of plants, the number of volatile compounds increased with decreasing light intensity from 7 to 12 compounds in the n-hexane extracts. **Conclusion:** This report shows that low light intensity increases the volatile compounds of *Hyptis suaveolens* markedly and this could enhance its antimicrobial activity.

Keywords: Hexane extract, Volatile compounds, Light intensity, *Hyptis suaveolens*.

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INTRODUCTION

Solar radiation is important in plant growth and development as it is expressly connected to photosynthesis and other physiological, biochemical and morphological processes.[1] For an effective photosynthetic process, intensity, quality, and duration of light as a source of energy to plants cannot be overemphasized.[2] Asides, light being a source of energy, it is a factor that monitors plant development.[3] Plants exposed to low light intensity tends to exhibit a markedly decrease in growth yield.[4] Effects of various light intensity on medicinal and aromatic plants invariably affects the production of secondary metabolites and essential oils.[1,5] Example is *Ocimum basilicum* grown under low light intensity had shown remarkable decrease in essential oil and chemical composition.[6] Essential oils also known as volatile oils play very important roles in plant defense and signaling processes.[7-9] For example, essential oils are involved in plant defense against microorganisms,

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insects, and herbivores, attraction of pollinating insects and fruit-dispersing animals, water regulation and allelopathic interactions.[7,10-12] A great diversity of biogenic Volatile Organic Compounds (VOCs) are emitted by plants from flowers, fruits, leaves, bark, and roots, as well as specialized storage structures.[13] Plants also use VOCs as a means of interacting with other plants and organisms.[14] VOCs consist of various organic classes such as isoprene, terpenes, fatty acid derivatives, alcohols, alkanes, alkenes, esters, and acids, among others. Many VOCs produced by plants, such as constituents of essential oils, are widely used commercially as flavourings and fragrances in the food and perfume industries.[15]

Hyptis suaveolens (Linn.) Poit. is a weed of world-wide distribution, originally native to Tropical America.[16] It has glandular trichomes that store the essential oils which contain an aromatic mixture of VOCs that imbue a minty smell to the plant when crushed.[17] Essential oils from this plant have been shown to have tumorigenic, antifertility and mycotoxic activities. *H. suaveolens* is widely used in developing countries in treatment of various diseases as it possesses therapeutic properties.[18] These diseases include Boils and gastric ulcer;[19] Cancer;[20] Gonorrhea;[21] stomach pain;[22] low sperm count;[23] Duodenal ulceration[24] and Headache, malaria and childbirth.[25-28] The objective of this study was to investigate the impact of light intensity on the growth and quality of volatile compounds from n-hexane extracts of *Hyptis suaveolens*.

MATERIALS AND METHODS

Study Area

The experiment was carried out at the Botanic Garden of the University of Lagos, Lagos State, Nigeria. A voucher herbarium specimen was deposited at the Botany herbarium under number 7478.

Collection of Materials and Planting Procedure

The seeds of *Hyptis suaveolens* were collected from the dried plants obtained from Mushin market in Lagos State, Nigeria. The seeds were planted by seed broadcast on planting beds which were prepared by mixing fresh garden soil and organic manure. Three batches of twenty plants, 6 weeks seedlings were subjected to full light (58,753 lux), medium light (35,880 lux) and low light (1,250 lux) intensities in a randomized complete block design. All throughout the experiment, photosynthetic photon flux density (PPFD) was supervised in full sun, at each light environment, using S LIA-M003 light radiation sensors which was attached to a Hobo Data Logging Micro Weather Station (Onset Computer, Massachusetts, USA). The data recorder was structured to take readings every 60 sec intervals and recorded at the average of ten-minute intervals. Samples of three replicates were harvested from each batch at 2, 4, 6, 8 and 10 weeks after treatment for growth analysis.

Growth Analysis

Plants (Figure 1) were harvested from the three batches at 8th, 10th, 12th, 14th and 16th weeks after planting in three replicates. Growth parameters estimated include plant height, stem girth, shoot fresh and dry weight, root fresh and dry weight, leaf area was determined as outlined by Ebenezer IO, *et al*.[29] The Leaf Area Ratio (LAR), Net Assimilation Rate (NAR) and Relative Growth Rate (RGR) were determined using the mathematical expression outlined by Olorunnisola OS, *et al*.[30]

Extraction of bioactive compounds using N-hexane from *Hyptis suaveolens*

Five (5) g of sample was weighed into a thimble. The thimble was placed in soxhlet which was connected to a round bottom flask attached to a condenser. About 30 ml of hexane was placed in the apparatus in a heating mantle. Then the sample was extracted for 4 hr. The extract was decanted and concentrated till further use.

Gas Chromatography Mass Spectrometry analysis of *Hyptis suaveolens*

The volatile oil of hexane extract of *Hyptis suaveolens* was separated and identified using Agilent Technologies 5975C MSD Gas Chromatography Mass Spectrometry with triple-axis detection using Helium as carrier gas (1 mi/min) and anfluent. The mass spectrometer with an attached MS detector interfaced with an Agilent technologies 7890A gas chromatograph with an HP5 column measuring 30 m × 0.32 mm id, 0.25 µm film thickness. The initial oven temperature was 80°C at 2 min to 120°C at 4.5°C/min, final temperature was held for 2 min at 220°C. The ion source was fixed at 240°C with an electron ionization. Hexane extract of 1.0 µL diluted in hexane injected manually into the GC/MS. The spectrums of the components identified were compared with the spectrum of known components in the GC-MS library database.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using Tukey's Honest Significant Difference Test (HSD) at p=0.05. (Results presented are expressed as means of three replicates).

Figure 1: (A-B): *Hyptis suaveolens* plant with and without flowers.

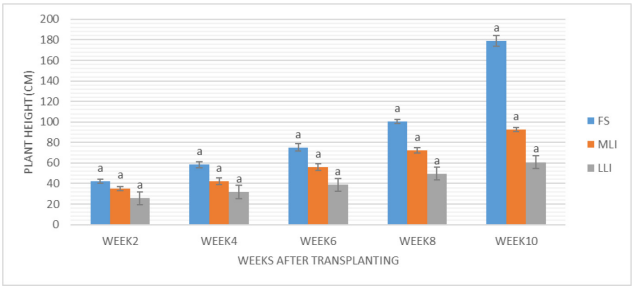

Figure 2: Mean height of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

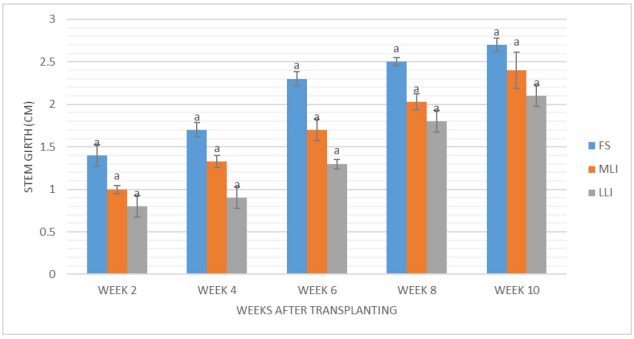

Figure 3: Mean stem girth of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

RESULTS

The seedling growth of *Hyptis suaveolens* under full sunlight (FS) had the highest growth followed in this order by plants treated with medium light intensity (MLI) and low light intensity (LLI). There were slight morphological differences between the FS, the MLI and LLI plants. The control plants produced long and broad stems, long adventitious roots, and large number of small leaves at the apex and large sized leaves at the base. Control plants flowered at week six, the MLI plants flowered at week eight, while LLI plants did not flower at all. Furthermore, the LLI plants produced

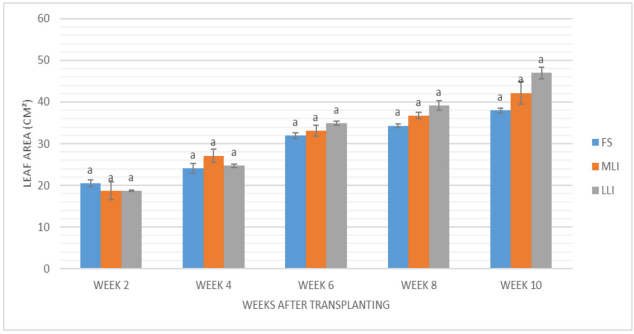

Figure 4: Mean leaf area of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

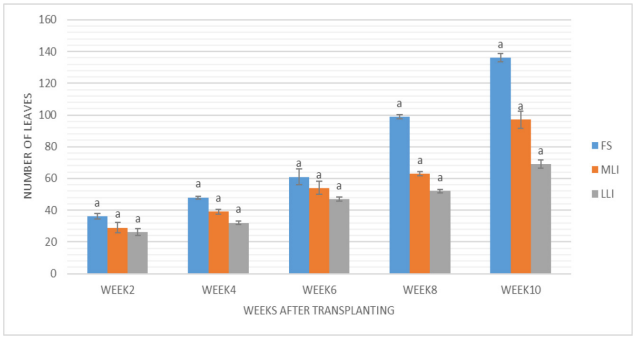

Figure 5: Mean number of leaves of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

short, slender stems and few number of leaves. The FS plants exhibited the highest height and stem girth followed by the MLI plants and LLI plants in this order, though the difference was not significant at $p = 0.05$ (Figure 2 and 3).

There was no significant difference at $p = 0.05$ among the treatments in the mean leaf area and number of leaves of plants subjected to different light intensity (Figure 4 and 5). The FS plants exhibited the highest mean fresh and dry shoot weight followed by the MLI plants and LLI plants with no significant difference at $p = 0.05$ (Figures 6 and 7).

The mean dry weight of the root of plants subjected to full sunlight (FS) was significantly higher at (p= 0.05) (Figure 8) than other treated plants (Figure 9). Table 1 shows the result of the growth parameters (Net Assimilation Rate, NAR; Leaf Area Ratio LAR and Relative Growth Rate, RGR) of *Hyptis suaveolens* of all the treated plants.

The GC-MS analysis of N- hexane extract of plants under full sunlight, medium light intensity and low light intensity revealed the presence of seven, nine and twelve bioactive compounds respectively. The concentration

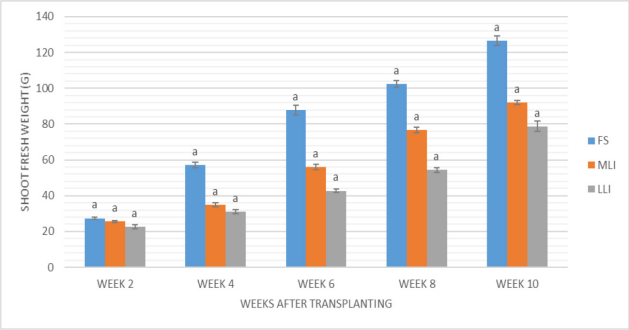

Figure 6: Mean fresh weight of shoot of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

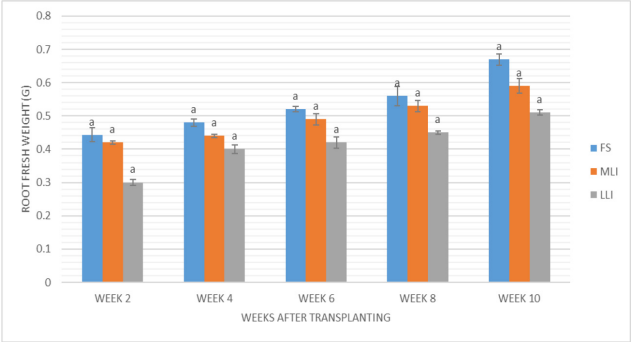

Figure 8: Mean fresh weight of the root of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

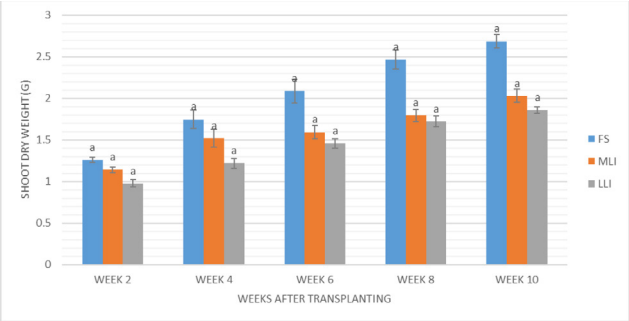

Figure 7: Mean dry weight of the shoot of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

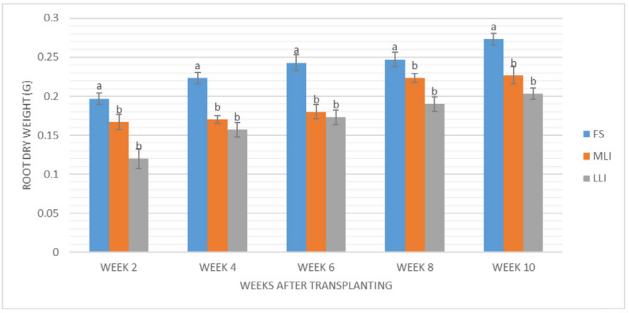

Figure 9: Mean dry weight of the root of *H. suaveolens* subjected to different light intensities in the course of growth. Columns with different letters shows that the difference in the treatment is significant at $p = 0.05$ using tukey's *post hoc* test.

of 1-Phenanthrenemethanol was commonly present in full sunlight, medium light intensity and low light intensity having a concentration of 27.00%, 26.11% and 36.99% respectively. LLI plants had the highest concentration of 1-Phenanthrenemethanol as shown in Table 2. Under FS plants has the least concentration of 1,3-Methano-5Bh-cyclobuta (5.11%). The FS plants produced less amount of bioactive compounds but exhibited greater growth. Medium light intensity had the least concentration of Bromoacetic acid, octadecyl ester (4.24%) in Table 3.

However, LLI plants exhibited the least concentration of Didodecyl phthalate (2.45%). The LLI plants produced the highest number of bioactive compounds (12) as compared to FS (7) and MLI (9) plants.

Table 5 shows the essential oil that are uniquely present at different light intensities. Three compounds were only present in plants subjected to full sunlight while five were present in MLI plants and 7 compounds present in LLI plants. Irrespective of light intensity, two volatile compounds (Ar-Abietatriene and 1-Phenanthrenemethanol) were present in all treated plants (Tables 2-4). Moreover, LLI enhanced the production of 1-Phenanthrenemethanol (36.99%). Plants subjected to LLI produced the highest number (12) of different volatile compounds; MLI plants produced 9 compounds while plants under FS produced only 7. Thus, increasing light intensity reduced the number of different volatile compounds present in plants. In addition, FS plants produced high concentration of the volatile compounds present.

Table 1: The Net Assimilation Rate (NAR); Leaf Area Ratio (LAR) and Relative Growth Rate of *Hyptis suaveolens* subjected to full sunlight (FS), medium sunlight (ML) and low sunlight (LLI).

Treatment	NAR	LAR	RGR
Control	0.0066327	13.440	0.0886012
Medium Sunlight	0.0041155	16.488	0.068
Low Sunlight	0.0039394	19.907	0.079

Table 2: Volatile compounds of n-hexane extract of *H. suaveolens* under low light intensity.

Sl. No	Retention time	Volatile compounds	% Volatile compound
1	15.687	Neophytadiene	4.75
2	16.259	Didodecyl phthalate	2.45
3	18.599	Ar-Abietatriene (Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-)	12.05
4	18.902	(4aS,4bR,10aS)-7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene	3.10
5	19.246	Phytol	9.07
6	20.218	Isothujopsene, 5-diethylboryl-dihydro-	5.09
7	20.413	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate	6.59
8	20.796	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	2.96
9	21.489	Sesquirosefuran	4.56
10	21.649	Phenanthrene, 1,2,3,4,4a,9,10,10a- octahydro-1,1,4a-trimethyl-	4.90
11	21.763	1-Phenanthrenemethanol, 1,2,3,4,4a,5,6,9, 10, 10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	7.50
12	22.301	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	36.99

Table 3: Volatile compounds of n-hexane extract of *H. suaveolens* under medium light intensity.

Sl. No	Retention time	Volatile compounds	% Volatile compound
1	15.818	2-Pentadecanone, 6,10,14-trimethyl	8.59
2	16.814	2,2,2-Trifluoroacetic acid octadecyl ester (Octacosyl trifluoroacetate)	4.76
3	16.888	Methyl palmitate (Hexadecanoic acid, methyl ester)	12.35
4	17.380	2H-Pyran, tetrahydro-2-(12-pentadecynyloxy)-	7.50
5	18.593	Ar-Abietatriene (Phenanthrene, 1,2,3,4,4a,9,10,10a-octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-)	19.99
6	19.583	Octadecylbromacetat (Bromoacetic acid, octadecyl ester)	4.24
7	20.407	Neophytadiene	7.25
8	21.500	3-(Trifluoromethoxy)benzonitrile	9.20
9	22.318	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-	26.11

Table 4: Volatile compounds of n-hexane extract of *H. suaveolens* under full sunlight.

Sl. No	Retention time	Volatile compounds	% Volatile compound
1	15.818	Hexahydrofarnesyl acetone (2-Pentadecanone, 6,10,14-trimethyl)	5.76
2	16.814	Pentatriacont-17-ene (17-Pentatriacontene)	9.29
3	18.599	Ar-Abietatriene (Phenanthrene, 1,2,3,4,4a,9,10,10a- octahydro-1,1,4a-trimethyl-7-(1-methylethyl)-, (4As-trans)-)	25.64
4	19.160	1,3-Methano-5Bh-cyclobuta[cd]penta len-5b-ol, octahydro-	5.11
5	19.583	2H-Azepin-2-one, 3-(dimethylamino) hexahydro-	7.62
6	21.483	Sesquirosefuran	19.57
7	22.301	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-	27.00

DISCUSSION

Light intensity like many other environmental factors affects the growth and composition of the major components of volatile compounds of n-hexane extracts of *H. suaveolens*. Slight differences were exhibited in plant height, leaf area, shoot dry weight, number of leaves, stem girth, Net Assimilation Rate (NAR), Leaf Area Ratio (LAR), Relative Growth Rate (RGR) of *H. suaveolens* subjected to high (FS), medium (MLI) and low (LLI) light intensities but consistently, the plants subjected to FS showed highest parameters followed by those subjected to MLI and LLI. Light intensity (58,753

Table 5: Essential oils uniquely present at different light intensities.

Full Sunlight	Medium Light Intensity	Low Light Intensity
17-Pentatriacontene	Octacosyl trifluoroacetate	Didodecyl phthalate
1,3-Methano-5Bh-cyclobuta[cd] penta len-5b-ol, octahydro-	Hexadecanoic acid, methyl ester	(4aS,4bR,10aS)-7-Isopropyl-1,1,4a- trimethyl-1,2,3,4,4a,4b,5,6,10,10a-decahydrophenanthrene
2H-Azepin-2-one, 3-(dimethylamino) hexahydro-	2H-Pyran, tetrahydro-2-(12-pentadecynyloxy)-	Phytol
	Bromoacetic acid, octadecyl ester	Isothujopsene, 5-diethylboryl-dihydro-
	3-(Trifluoromethoxy)benzonitrile	3,7,11,15-Tetramethylhexadec-2-en-1-yl acetate
		Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-
		1-Phenanthrenemethanol, 1,2,3,4,4a,5,6,9, 10, 10a-decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,10a.alpha.)]-

lux to 1,250 lux) affected both growth parameters and quality and quantity of essential oils in *Hyptis suaveolens* plants. Plants exhibited slightly reduced plant height, fresh weight, dry weight, stem girth, number of leaves, Net Assimilation Rate, Relative Growth Rate but caused a slight increase in leaf area and Leaf Area Ratio with decreasing light intensity. According to, Eze JMO, *et al*.[31] the reduction in dry stem biomass noticed under full light intensity could be as a result of transport of carbon from source to sink. However, a low output of PPFD levels on exposure to low light intensity can inhibit flowering in plants. During growth and development in plants, variation in solar radiation intensity suggests the differences in plant dry mass.[32] Plants subjected to low light intensity did not flower. Light intensity can affect plant form, flowering, leaf size, and colour in both herbaceous[33,34] and woody species.[35] There is variation within a species to response to light, this can infer the possibility of plant selection for development of cultivars with enhanced shade tolerance.[36,37]

Hyptis suaveolens is a chemotype, because there are differences in components and composition of its essential oils from several results of researches conducted in different areas and countries.[38] In Nigeria, some volatile compounds that are constituents of essential oils found in high percentages in *H. suaveolens* include 1-Phenanthrenemethanol (27%) Ar-Abietatriene (25.64%) Sesquirosefuran (19.57%) under full sunlight, Methyl palmitate (12.35%) Ar-Abietatriene (19.99%) 1-Phenanthrenemethanol (26.11%) under medium light intensity and 1-Phenanthrenemethanol (36.99%) and Ar-Abietatriene (12.05%) under low light intensity. Though volatile compounds like Phenanthrene and 1-Phenanthrenemethanol were present under the three light intensities, other compounds were either present or absent. Irrespective of light intensity, two components of essential oils (Ar-Abietatriene and 1-Phenanthrenemethanol) were present in *H. suaveolens*. However, low light intensity enhanced the production of 1-Phenanthrenemethanol (36.99%) while high light intensity induced high concentration of 1-Phenanthrenemethanol (27%). A study has shown the presence of Phenanthrenes from the Orchidaceae family. A few phenanthrenes have been found in the Hepaticae class and Dioscoreaceae, Combretaceae and Betulaceae families.[39,40] corroborated the presence of Phenanthrene (0.72%) in fresh oil of *H. suaveolens* and 1.89% when stored at 45°C while[41] reported high quantities of 1-Phenanthrenemethanol in *H. suaveolens*. Biological activities of Phenanthrenes include anticancer, anti-inflammatory, antialgal, antimicrobial and antiallergic.[39] This may likely infer some of the therapeutic properties of this plant.

Plants subjected to Low Light Intensity produced the highest number (12) of different essential oils; Medium Light Intensity plants produced the intermediate number of compounds (9) and plants under Full Sunlight produced the least number (7) of oil fractions. Thus, increasing light intensity reduces the number of different essential oils present in *H. suaveolens*. just as in the case of basil plant.[42] In contrast, FS plants produced this reduced variety of compounds in large quantities compared to the other two treatments. In a shading experiment, essential oil content of which plant decreased with shading level and Limonene content was not affected, whereas carvone content strongly decreased. As a consequence, the carvone/Limonene ratio decreased with a decrease in light intensity.[43]

Geographical position of the plant growing areas has also been shown to affect the composition of the major components of essential oils produced from *H. suaveolens*.[38,44] It was observed that monoterpene hydrocarbons are mainly produced by plants growing in sampling sites located at higher latitudes and altitudes, whereas sesquiterpenes are produced by plant samples grown at lower ones[16,45,46] which was in correlation to

results as active compounds like sesquiterpenes in form of sesquirosefuran were produced in both the Full Sunlight and Low Light Intensity.

The major constituents of essential oils from *H. suaveolens* in India were 1, 8-cineole (44.4%), β-caryophyllene, β-pinene and camphene.[47] An additional specimen from India was analysed with the major components being sabinene (41.0%), terpinen-4-ol (12.31%), β-pinene (10.0%) and β-caryophyllene (8.0%). In Malaysia,[48] reported β-caryophyllen, 1, 8-cineole, terpinen-4-o1, α-bergamotene, sabinene and α-copaene were the major components of the essential oil from *H. suaveolens*.[44] investigated chemical composition of that essential oil from Vietnam. The main constituent identified were eugenol and germacrene-D.[49] studied the constituent of *H. suaveolens* essential oil from Australia. They reported 8-cineole (32%) and β-caryophyllene (29%) as the major constituent in the oil. In this study, the major components of essential oil in the Nigerian *Hyptis suaveolens* was 1-Phenanthrenemethanol (27%) Ar-Abietatriene (25.64%) Sesquirosefuran (19.57%) under full sunlight, Methyl palmitate (12.35%) Ar-Abietatriene (19.99%) 1-Phenanthrenemethanol (26.11%) under medium light intensity and 1-Phenanthrenemethanol (36.99%) and Ar-Abietatriene (12.05%) under low light intensity.

In conclusion, plants subjected to low light intensity did not flower but exhibited the higher number of volatile compounds, this may be attributed to nutrients diversion to the formation of increased volatile compounds. Though, full light intensity induced fewer volatile compounds, they were highest in concentration compared to other treated plants. This research points to the fact that low light intensity alters the volatile compounds of *H. suaveolens* by increasing the number of volatile compounds but causing a marked reduction in their concentrations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NAR: Net Assimilation Rate; **LAR:** Leaf Area Ratio; **RGR:** Relative Growth Rate; **FS:** Full Sunlight; **MLI:** Medium Light Intensity; **LLI:** Low Light Intensity; **PPFD:** Photosynthetic Photon Flux Density.

SUMMARY

This study has shown that *Hyptis suaveolens* subjected to low light intensity did not flower and there is a remarkable increase in the volatile compounds and this could enhance its antimicrobial activity.

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