Essential oil of monkey-pepper (*Piper aduncum* L.) cultivated under different light environments

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Abstract

The monkey-pepper (*Piper aduncum* L.) is an herbaceous species and essential oil producer, with biological activities such as insecticidal, antimicrobial, larvacidal and others. The cultivation of the plant is still incipient, being necessary to know the influence of some factors, among them, the light. Thus, the effects of different lighting conditions on the production and profile of the constituents of essential oils from leaves and roots of *Piper aduncum* L. were evaluated. Plants grown for 150 days in five light conditions: 100%, 70% and 50% of natural irradiance and nets of blue and red colors, also blocking 50% of natural light irradiance. The essential oils obtained by hydrodistillation from leaves and roots and analyzed by GC / MS. Roots showed higher amount of essential oil (0.11% w/w), which had no influence on light conditions. On leaves the biggest oil content was observed in 50% brightness (0.085% w/w). The essential oil of leaves was characterized by a high content of sesquiterpenes and roots of phenylpropanoids. The leaves are rich in *E*-nerolidol, linalol (14.28-16.65%); α -humulene (8.45-10.62%); *cis*-cadin-4-en-7-ol (7.48-12.24%) and caryophyllene (7.93-12.24%). While the root is rich in apiol (16.27-29.51%). Most major compounds increased in the environment rich in blue light. The variation in the production and essential oil constituents of *P. aduncum* L. depends on the light conditions and can be related to phenotypic and genetic factors. Therefore control of the light environment in growing conditions makes it necessary for obtaining raw material quality.

Keywords: irradiances; medicinal plant; phytochemical constituents, apiole; *E*- nerolidol; environment

1.Introduction

The Piperaceae family has a pantropical distribution and is composed of approximately 2500 species that are mainly of the genus *Piper*. *Piper aduncum* L. is a shrub that is native to tropical regions of the Americas and was introduced in Asia during the 19th century (Hartemink, 2001). In medicine, the

essential oil of its leaves has been used for the direct and indirect combat several human diseases (Duarte et al., 2005; Lorenzi and Matos, 2008; Misni et al., 2011). In addition, the use of essential oil stands in agriculture by showing an insecticidal, larvicidal, leishmanicidal, molluscicide, antibacterial and antifungal activities (Moreira et al., 1998; Torres Santos et al., 1999; Lara Júnior et al., 2012).

The biological activities are related to the arylpropanoid dillapiole, which is a substance that has been reported by some authors to be the major essential oil compound found in the leaves of this species. However, literature data have shown that there is a chemical diversity for the essential oil composition (Vila et al., 2005; Potzernheim et al., 2006; Oliveira et al., 2013). According to Parmar et al. (1997) the biological properties attributed to the essential oil of the species may also be explained by the presence of other phenolic compounds, flavonoids and sesquiterpene which could be exploited by the chemical and pharmaceutical industry.

In agriculture, however, little is known about this species, which remain in the wild. In Brazil, for instance, most of the medicinal plant species that are used by the population are obtained from natural habitats (Oliveira et al., 2013). Additionally, the use of medicinal plants is growing promoting exploitation and loss of species considered. About 40% of the world's flora is in danger of extinction, mainly due to human activities such as the extraction and excessive collection of certain species promoting risk to their populations (Oliveira, 2010).

The ability to sustain production of raw vegetable materials that are of high quality under controlled conditions is important because of the need to produce standardized herbal medicines (Jannuzzi et al., 2010). There are a number of advantages of using cultivation instead of collect medicinal plants in a natural environment. According Jaafar and Rahmat (2008) the cultivation of medicinal plants where the light can be controlled microclimate is a promising alternative for the control of phytochemicals levels and high raw vegetable materials.

Plants grown in conditions that are different from those of their natural habitat may display qualitative and/or quantitative changes in their composition with regard to special metabolites. The chemical composition of essential oils, for example, is known to be altered depending on the exposure to different environmental conditions imposed during development (Maia et al., 2009). The light, both in terms of quality and in terms of quantity, it is one of the most important environmental factors affecting the synthesis of these compounds (Wang et al., 2007). The biosynthesis of phenolic compounds, for example, requires light or is enhanced by light (Ghasemzadeh and Ghasemzadeh, 2011). Previous studies

have demonstrated different light intensity and quality promoted an increase in the production of phenolic compounds in medicinal plants (Karimi et al., 2013). However, plants are interspecies differences in relation to the environment by changing the composition of secondary compounds (Jaafar and Rahmat, 2008).

Uniform individuals should be bred to be used in pharmaceutical industries. So to exploit those species resource rationally, a comprehensive research on their phytochemical characteristic seems to be necessary. Therefore, the objective of this study was evaluated the effect different light conditions in production and profile of essential oil constituents of leaves and roots of *P. aduncum* L.

2. Materials and methods

2.1. Plant material

The experiment was conducted in Federal University of Lavras, Brazil. Seedlings of *P. aduncum* L. were produced in the Department of Biology, from seeds. The seeds were pre-germinated in petri dishes, on three filter paper leaves and kept in a Mangesdorf germination chamber, at 25°C and through a 12-hour photoperiod, for 30 days. After this period, the seedlings were transferred to polypropylene trays containing the commercial substrate Tropstrato HA® (Vida Verde®, Brazil) and kept in greenhouses with 50% shading until they reached 2.5 cm height. The plants destined to full-sun cultivation (100% irradiance) were previously acclimatized for 7 days in 70% irradiance and later for 7 days in full sun before they were transplanted to the definitive substrate. Irrigation was performed daily, and the soil was kept under the condition of field capacity. After acclimatization, the seedlings were transplanted to plastic pots, with a capacity of 6 liters, containing a substrate comprised of subsoil, sand and bovine manure, in proportion of 2:1:1, being disposed in the different irradiance treatments. The physicochemical characteristics of the soil were analyzed in the Laboratory Analysis of Soil, and were: pH: 5.4; P: 4.13 mg dm⁻³; K: 73.32 mg.dm⁻³, Ca: 2.30 cmolc.dm⁻³, Mg: 0.30 cmolc.dm⁻³, Al: 0.10 cmolc.dm⁻³, H + Al: 2.90 cmolc.dm⁻³, V: 49.00%; organic matter: 2.10 dag.kg⁻¹, Clay: 70.00 dag.kg⁻¹; Silt: 16.00 dag.kg⁻¹ and Sand: 14.00 dag.kg⁻¹.

The experiment was conducted between April and July of 2012, in total of 150 days of crop, at Gota da Esperança Farm, belonging to the Department of Agriculture, with the following geographical coordinates: 21°14'07"S and 44°58'22"W, at 879 m altitude. The average climatic conditions observed during the experiment were provided by Climatological Station of the Department of Agricultural

Engineering, had maximum temperature of 30.2°C and a minimum of 6.3°C, precipitation of 1.28 mm and a relative humidity of 72.7%.

2.2. Light conditions

The treatments were characterized by cultivation of 25 plants, for each treatment, for 120 days, under four altered ambient light, produced by shading greenhouses with 70% and 50% of natural incident irradiance, two colored shading nets (Chromatinet® Green.tek®, USA) in red (RN) and blue (BN) colors, blocking 50% of the incident radiation and one treatment at full-sun. With the aid of a portable spectroradiometer (USB-650 Red Tide) coupled to a source of electromagnetic radiation DT-MINI (200 to 2000 nm) and a probe reflectance R400-7-VIS-NIR (US BioSolutions Ocean Optics®), the radiation spectrum of different environments was evaluated, with a spectral resolution of 1 nm. The normalized irradiances observed for the treatment at 50% was 6.54 W/m², 13.08 W/m² in the treatment at 70%, 15.42 W/m² at 100%, 8.86 W/m² in RN and 9.07 W/m² in BN. Each environment presented the highest values in terms of amount and size of the spectrum for the atmosphere with 100% irradiance, followed by the RN atmosphere, in which the value found was 70%, and 50% irradiance for the environment with BN. It was also noticed that the blue net provided irradiances, of approximately 450-550 nm, and the red net between 490 and 690 nm.

2.3. Essential oils extraction

To determine the essential oil content, plants were dried in a circulating forced oven, at temperature of 45°C, to constant weight. The hydrodistillation by Clevenger apparatus (Vidrolabor®, Poá, Brazil) was used, with the time of 90 min. For each treatment, three samples were used, i.e, 300g of leaves and roots 250 g, both parts cut into ~ 1 cm with a scissor, and placed in volumetric balloons of 2 liters of capacity. After the hydrodistillation time, essential oil and hydrolate were collected. The condenser and the apparatus collector were washed 3 times, with the aid of a pisete containing dichloromethane, in order to collect oil adhered in glassware. Essential oil, hydrolate and washes of dichloromethane were kept in separation balloon, allowed to stand for 15 min and then separated liquid-liquid partition. The solution was treated with anhydrous magnesium sulfate to remove possible moisture residues. After treatment, the solution was filtered, and the solvent evaporated under gas exhaust hood. The isolated oils were weighed in analytic balance and stored in tightly closed vials at 4 °C until analysis. The content of essential oil was calculated in percent (w/w) in dry mass basis.

2.4. Chromatographic analysis

The essential oil from leaves and roots were analyzed in Phytochemical Laboratory of Agriculture Department, Federal University of Lavras. Three samples of essential oil of each treatment were prepared diluting the essential oil in ethyl acetate (1%, v/v) prior analyses. A GC/MS Agilent 7890A equipment, operated by HP GC ChemStation Ver. A.01.14 software, equipped with automatic injector/sampler CombiPAL Autosampler System (CTC Analytic AG, Switzerland) and flame ionization detector (FID) were used. A HP-5MS capillary column, 30 m × 250 µm; film thickness 0.25 µm and the Helium gas as the carrier gas were used. The conditions of analyses were: injection of 1.0 µL of sample, in split mode, at injection flow 1:20. The selective mass detector operates by impact ionization at 70 eV, in scan mode, at 1.0 scan/s, with mass acquisition interval of 40-400 m/z. In FID analyses, the initial temperature of oven has of 60 °C, maintained for one minute, followed by the ramp of 3 °C/min until 240 °C, followed by one ramp of 10 °C/min until 250 °C, maintaining isothermal for 1 minute. The injector and transference line temperatures for the mass spectrum were maintaining at 250 °C. The gas carrier flow was of 1.0 mL/min.

The constituents were identified for comparison of relative retention index, relative a *n*-alkanes series (C₈-C₂₀) (Sigma-Aldrich®, St. Louis, USA) and comparisons of mass spectrum of literature (NIST, 2008; Adams, 2007). The retention index was calculated by Van den Dool and Krats (1963) equation. The results of analyzes are shows in a table with the calculed retention index, relative percentage of compounds and their classes.

2.5. Statistical analysis

The results of essential oil content and majoritarian compounds were submitted to Tukey`s test (p< 0.05) by SAEG program (SAEG, 2007).

3. Results and discussion

Variation was observed in essential oil content in leaf e roots of *P. aduncum* L. grown in different light conditions (Table 1). The root had the highest essential oil content, independent of the light treatments. This may be due to the high metabolic activity of the leaf, associated with its high plasticity structural and physiological, for the production of secondary metabolites there is inversely related with high metabolic activity tissue (Gobbo-Neto and Lopes, 2007). However, the levels observed in both organs are lower than those found in other studies for the species vary from 1.1 to 3.4% in the leaves (Maia et al., 1998; Potzernheim et al., 2006; Sousa et al., 2008). The variation in the essential oil content

may be due the form of extraction, environmental conditions in which the plants were grown or plant age at the time of collection.

The highest essential oil content in leaves was observed in 50% treatment of light and with longer wavelength in the blue region (BN). While the different light conditions did not promote changes in oil production in the roots (Table 1). Thus, can note that production or accumulation of essential oil of *P. aduncum* L. leaves is influenced by the intensity and quality of light. Moreover, indicates the participation of photoreceptors in the synthesis of secondary metabolites produced by this species. Blue light is absorbed by photoreceptors that stimulate the synthesis of secondary metabolites (Li and Kubota, 2009; Wu and Lin, 2012). The results observed related to essential oil production may be associated with an adaptive strategy for survival in an environment with low light availability. Nascimento et al. (2013) shows that the blue light induces the synthesis of secondary compounds which are antioxidants promoting greater protection in unfavorable situations.

Studies in *Mikania glomerata* Spreng., *Ocimum gratissimum* L. and *Melissa officinalis* L. showed similar results with high essential oil content when grown in low light intensities and enriched environments with blue light (Martins et al., 2009; Brant et al., 2009; Souza et al., 2010). In *Larix gmelinii* Rupr. was also observed an increase in the content of secondary compounds when growing at low light intensities (Yan et al., 2013). However, *Lippia sidoides* Cham. increased the essential oil content when grown at high light intensities, while *Aloysia gratissima* Gillies & Hook had no changes when grown in different light conditions (Souza et al., 2007; Pinto et al., 2007).

A total of 38 and 27 compounds constituting 88.38% and 93.18% of the total oil were identified in the leaves and roots, respectively (Table 2 and 3). Quantitative and qualitative composition of essential oil in the present study is different from those reported in previous studies (Potzernheim et al., 2006; Rali et al., 2007) The existence of variations in oil content and composition may be attributed to factors related plant age and environmental conditions that the plant has been exposed during its development.

The conditions of luminosities showed effect on constitution and composition of the essential oil of *P. aduncum* L. Forty-one to fifty constituents were identified from the essential oils of leaves and twenty-seven to thirty-four at the root. The oil consisted mainly of containing sesquiterpenes in leaves (51.24%) and phenylpropanoids in roots (40.01%), observed in 100% light (Table 3). The blue shade net showed was identified the number of minor constituent (27 constituents). This can is related to participation photoreceptors of blue light in the synthesis of secondary compounds in leaves.

The predominance of sesquiterpenes in leaves has been reported by other authors for the genus *Piper* (Mesquita et al., 2005; Vieira et al., 2011). While the predominance of phenylpropanoids in roots was the first report in the literature for the species *P. aduncum* L. The identification of sesquiterpenes in the leaves and phenylpropanoids in roots may be associated with their role. The monoterpenes and sesquiterpenes have different function but, do not know their action against underground herbivores (Bakkali et al., 2008). Moreover, terpene synthases, key enzymes in the synthesis of terpenoids, shows a pattern of organ-specific transcription correlated with the amount of terpenes produced. In *Matricaria recutita* L., for example, the lowest amount of sesquiterpenes found in the roots was not associated with expression of some genes in terpene synthases (Irmisch et al., 2012).

The principal components in *P. aduncum* L. in the leaves were *E*-nerolidol (14.28-16.65%). Corroborating this study, de Oliveira et al. 2006 also noted this as a major constituent in *P. aduncum* L., Other constituents were linalol (9.33-13.44%); α -humulene (8.45-10.62%); *o* *cis*-cadin-4-en-7-ol (7.48-12.24%) and caryophyllene (7.93-12.24%) (Figure 1). Rali et al. (2007) found as major compound of species grown Papua New Guinea the dilapiolle (43.3%). While, Oliveira et al. (2013) identified a major compound 1,8 cineol. Apparently, the production of the major compound found in leaf *Piper aduncum* L. varies depending on the type and environmental conditions imposed on the development or as the age of the plant.

There was increase of *E*-nerolidol in treatments with 70%, 100% light and under blue shade net. The linalol and α -humulene constituents increased under blue shade net. The contents *cis*-cadin-4-en-7-ol was highest in 100% light (Table 2). Furthermore, the constituents β -elemene, β -copaene, 2-undecanona e *trans*-cadin-1,4-diene was not identified in 50% light, this indicates the need for higher light intensity for synthesis.

The precursor of nerolidol is the farnesyl diphosphate and the reaction is catalyzed by terpene synthase pathways with specific (Dudareva et al., 2013). The terpene synthases are enzymes which have activity and expression regulated by plant development and in response to biotic and abiotic factors, such as the radiation conditions (Tholl, 2006). In *Populus alba* L. the expression of the terpene synthase is enhanced at high temperature and high irradiance (Cinege et al., 2009). Thus, increased levels of its major constituents under high irradiance may be associated with increased expression of the genes of terpene synthases.

The effect of blue light stimulating the synthesis of sesquiterpenes needs further understandings. Studies in flowers of *Arabidopsis thaliana* show that the synthetic terpene blue light has little effect on the expression of genes involved in the synthesis (Hong et al., 2009). However, studies in transformed plants to superexpression of cryptochromes, an increase of terpenoids synthesis when exposed in blue light was observed. In leaves of *Artemisia annua* L., for example, blue light stimulates the synthesis of cytosolic terpenes and promotes increased expression of genes related farnesyl diphosphate synthase, involving the participation of cryptochromes (Hong et al., 2009). Thus, possibly in *P. aduncum* L. the synthesis of terpenes in the leaves can also be activated by blue light. Though, studies involving molecular biology and terpene biosynthesis for the species are needed for more accurate conclusions.

Sesquiterpene indole as *E*-nerolidol and linalol are among the volatile main acting in indirect plant defense against herbivore (Dicke, 1994). These terpenes are released from the leaves of many species after the occurrence of damage caused by herbivores and are used in perfume fragrance (Azuma et al., 1997). Nevertheless, before the attacks these compounds are synthesized in low levels (Degenhardt and Gershenzon, 2000). Thereby, the species cultivation with blue light may be promising not only to obtain terpenes in essential oil and also to increase their defense against herbivores.

The α -humulene and caryophyllene, identified as major compounds, presents important antimicrobial action as already tested for the species (Fidalgo et al., 2004; Duarte et al., 2005). Studies by Pichette et al. (2006), with *Abies balsamea* L., found that 96% of the essential oil were made up by β -caryophyllene and α -humulene who had significant antimicrobial activity against different pathogens. Furthermore, the α -humulene has also been used as anti-inflammatory and as a candidate to be an anti-cancer, to promote the reduction of cellular glutathione content and increased production of reactive oxygen species that promote cancer cell death (Hadri et al., 2010). Future studies with these compounds obtained by *P. aduncum* L. may allow a new medicinal use of this species.

The apiol was the major constituent of roots in all treatments (50%-21.73%; 70%-21.35%; 100%-16.27%; red shade net-22.61% and blue shade net-29.51%) This organ was also observed as the compounds dilapiolle (13.0- 18.36%), α -selinene (14.11- 16.47%) and 2-methylbutanoate-geranila (8.92- 13.6%). The increased levels of the compounds in the treatment under blue light, except dilapiolle which increased in 100% of light (Figure 2).

It has been speculated function dilapiolle as a compound that induces pathways involved in plant defense to stresses (Ferreira et al., 2013). Thus increased levels of these compounds in the roots of plants

grown under 100% light can indicate the presence of an effective defense system in response to adverse environmental conditions.

The increase in phenylpropanoids levels in treatments with 100% light and blue net can be associated with increased expression of the enzyme chalcone synthase, the key enzyme in the synthesis of phenylpropanoids (Bharti and Khurana, 1997). Roots exhibit increased expression of genes related to chalcone synthase, when compared to leaves (França et al., 2001).

The compounds apiol and dilapiol have been observed in *Piper hispidum* Sw. grown in natural environment (Facundo et al., 2008). In addition, the dilapiol and other derivatives as methylenedioxides apiol, safrole and myristicin are often found as major components in essential oils of the genus *Piper* leaves (Guerrini et al., 2009; Ferraz et al., 2010; Parise-Filho et al., 2012). The presence of these compounds in the essential oil of *P. aduncum* L. roots can arouse the interest of the chemical and pharmaceutical industry by this part. The dilapiol and isomers has presented important properties that make it a possible candidate in the development of drugs for the treatment of leishmaniasis and future in the treatment of cancer (Parise-Filho et al., 2012; Titov et al., 2011). Furthermore, it is the first report of the presence of phenylpropanoids in roots and chemical profile observed is different from that reported in leaves suggests the existence of an intraspecific chemodiversity.

The dilapiol and apiol isomer is responsible for much of the biological activities attributed to *P. aduncum* L. (Almeida, 2009; Araújo et al., 2012). Furthermore, the mixture of phenylpropanoids antioxidant plays an important role in helping to combat plant free radicals (Bakkali et al., 2008). Thus increased levels of these compounds in the roots of plants grown under 100% irradiance and blue shading can indicate the presence of an efficient defense system to unfavorable conditions.

4. Conclusion

In this work, we revealed significant differences in the yields and chemical characteristic of leaf essential oils of the *Piper aduncum* L. grown under different light conditions. Most oil production was observed in roots where not occurred influence of light. While the leaves plants grown in low light and in an environment with greater amount of blue light had higher essential oil production. Moreover, the essential oil of leaves was characterized by a high content of sesquiterpenes and roots of phenylpropanoids. The essential oil of leaves is rich in *E*-nerolidol (14.28-16.65%); linalol 9.33-13.44) α -humulene (8.45-10.62%); *cis*-cadin-4-en-7-ol (7.48-12.24%) and caryophyllene (7.93-12.24%). While the root is rich in apiol (16.27-29.51%). Most major compounds increased in the environment rich in blue

light. The variation in the production and essential oil constituents of *P. aduncum* depends on the light conditions and can be related to phenotypic and genetic factors. Therefore control of the light environment in growing conditions make it necessary for obtaining raw material quality.

Acknowledgment

The authors are thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support for and Laboratory of Phytochemistry (Dept. of Agriculture, Federal University of Lavras, Brazil) for the GC–MS analysis.

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Table 1

Essential oil content (% w/w) of leaves and roots of *P. aduncum* grown under different light conditions.

Treatments	Part of plant	
	Leaves	Roots
50%	0.085 aB*	0.139 aA
70%	0.043 cB	0.150 aA
100%	0.040 cB	0.115 aA
RN	0.045 cB	0.128 aA
BN	0.062 bB	0.167 aA
CV%	7.72	11.81

*Means followed by same caps letter in line and minuscule em column no shows difference by Tukey's test $p < 0.05$ %. The percentages indicates the incident light in the treatments. RN: red net; BN: blue net; Coloured nets blocking 50% of incident light.

Table 2

Chemical constituents (%) of essential oil from leaves of *Piper aduncum* L. grown under different light conditions.

Compounds	RI*	100% ^a	70%	50%	RN ^b	BN
<i>Monoterpenes</i>		10.50	9.79	9.33	11.12	13.44
Linalol	1103	10.50	9.79	9.33	11.12	13.44
<i>Oxygenated Monoterpenes</i>		3.33	2.94	2.31	3.59	4.02
1,8-cineol	1032	0.15	—	—	—	0.30
<i>cis</i> -Furanolinalol oxide	1073	0.87	0.51	0.72	1.27	0.94
<i>trans</i> -Furanolinalol oxide	1089	0.12	—	—	1.15	0.11
Terpinen-4-ol	1106	0.30	0.22	0.18	0.15	0.18
α -Terpineol	1166	0.23	0.23	—	0.23	0.29
Neral	1191	0.63	0.77	0.56	0.71	0.86
Piperitone	1255	0.24	0.35	0.35	0.32	0.37
Geranial	1289	0.65	0.68	0.48	0.60	0.77
2-undecanona	1294	0.13	0.18	—	0.13	0.16
<i>Sesquiterpenes</i>		22.05	24.24	23.36	25.94	26.13
α -Copaene	1377	0.67	0.61	0.82	0.74	0.69
β -Cubebene	1386	0.16	0.19	—	0.15	0.19
β -Elemene	1393	0.15	0.16	0.20	0.16	0.24
Caryophyllene	1421	5.29	5.14	5.34	6.70	6.05
β -Copaene	1430	0.12	0.14	—	0.14	0.16
Aromadendrene	1440	0.26	0.39	0.52	0.36	0.38
6,9-Guaiadiene	1445	—	—	0.17	—	0.13
α -Humulene	1456	8.45	8.83	8.75	10.31	10.62
Alloaromadendrene	1462	0.33	0.39	0.47	0.39	0.46
γ -Muurolene	1478	0.69	0.81	0.99	0.73	0.87
α -Selinene	1497	2.24	2.37	2.15	2.46	2.21
α -Muurolene	1502	0.22	0.26	0.30	—	0.30
γ -Cadinene	1516	2.00	2.27	1.83	1.78	2.09
δ -Cadinene	1525	1.17	1.26	1.61	0.28	1.41
<i>trans</i> -Cadina-1,4-diene	1534	0.12	0.19	—	0.14	0.11

1 **Table 2 (Continued)**

Compounds	RI [*]	100%	70%	50%	RN	BN
α -Cadinene	1539	0.17	0.18	0.17	0.15	0.17
<i>Oxygenated Sesquiterpenes</i>		<i>51.24</i>	<i>49.19</i>	<i>49.62</i>	<i>45.48</i>	<i>45.83</i>
<i>E</i> -Nerolidol	1569	16.02	16.65	15.53	14.28	16.10
Eudesma-4(14),11-diene	1487	0.85	—	0.88	0.83	0.86
Caryophyllenyl alcohol	1571	0.16	0.15	0.11	0.14	0.15
Spathulenol	1571	2.82	4.28	5.62	2.88	—
Caryophyllene oxide	1580	—	—	—	—	2.81
Cubebane-11-ol	1596	—	—	—	—	0.31
Guaiol	1601	2.13	2.23	1.80	1.71	1.81
Ledol	1606	0.70	1.05	1.64	1.63	1.68
<i>epi</i> -Globulol	1612	1.61	1.61	1.77	1.81	1.58
6,7-Humulene epoxide	1617	0.17	0.22	0.23	0.19	0.28
<i>epi</i> -Cubenol	1625	0.38	0.42	0.40	0.42	0.42
<i>cis</i> -Cadin-4-en-7-ol	1638	12.24	9.60	7.93	7.98	7.48
<i>epi</i> - α -Cadinol	1640	1.21	1.06	0.71	0.97	0.64
α -Muurolol	1645	1.26	4.26	4.55	1.33	1.28
τ -Muurolol	1649	—	1.35	1.47	—	—
α -Cadinol	1658	2.83	2.76	3.17	2.86	2.81
10- <i>epi</i> - α -Eudesmol	1660	—	2.56	2.23	—	—
(3 <i>Z</i>)-Caryophylla- 3,8(13)-diene-5 α -ol	1661	3.80	—	—	2.39	1.83
Caryophylla-4(12),8 (3)-dien-5- β -ol	1662	3.74	—	—	4.36	4.81
<i>n</i> -hexadecanoic Acid	1963	1.17	0.92	1.25	1.42	0.89
<i>Phenylpropanoids</i>		<i>2.08</i>	<i>2.62</i>	<i>1.87</i>	<i>2.02</i>	<i>2.60</i>
Estragole	1075	0.19	0.41	0.19	0.20	0.30
Elemicin	1560	0.37	0.80	0.35	0.62	0.60
Dilapiolle	1631	1.53	1.40	1.33	1.20	1.54
Safrole	1229	—	—	—	—	0.17
<i>Not identified</i>		<i>5.16</i>	<i>5.15</i>	<i>7.60</i>	<i>6.00</i>	<i>3.65</i>
<i>m/z</i> =220	1554	0.53	0.28	0.15	0.23	0.24
<i>m/z</i> =220	1587	1.16	0.92	2.93	2.65	0.87
<i>m/z</i> =218	1674	3.46	3.93	4.50	3.14	2.53
Total identification (%)		89.20	88.78	86.49	88.15	92.02
Number of compounds		47	43	43	45	41

^{*}RI: retention indices relative to C₈–C₂₀ *n*-alkanes on the HP-5MS column in order of elution.

^a100%; 70% and 50% of total incident irradiance; RN: red net; BN: blue net. Coloured nets blocking 50% of incident light; — Not identified.

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Table 3
Chemical constituents (%) of essential oil from roots of *Piper aduncum* grown under different light conditions.

Compounds	RI*	100%	70%	50%	RN	BN
<i>Monoterpenes</i>		—	—	—	—	—
<i>Oxygenated Monoterpenes</i>		2.40	1.92	1.95	1.66	0.91
1,8-Cineol	1032	0.26	0.35	0.34	0.32	0.23
Myrcenol	1101	0.33	0.33	0.39	0.32	0.27
Camphor	1145	0.66	0.52	0.50	0.40	0.30
Isoborneol	1157	0.39	0.25	0.25	0.19	—
Borneol	1166	0.21	0.15	0.16	0.13	—
α -Terpineol	1191	0.12	—	—	—	—
Piperitone	1255	0.24	0.15	0.15	0.13	—
Geranial	1298	0.16	0.15	0.13	0.14	0.11
<i>Sesquiterpenes</i>		20.99	21.63	21.27	20.69	22.39
Caryophyllene	1421	0.33	0.30	0.35	0.32	0.26
α -Humulene	1455	0.52	0.45	0.49	0.49	0.35
Alloaromadendrene	1461	0.23	0.22	0.21	0.20	0.21
γ -Amorphene	1498	3.17	3.37	3.20	3.05	3.37
β -Selinene	1527	0.20	0.17	0.48	0.30	0.36
α -Selinene	1515	14.11	15.64	15.26	15.03	16.47
α -Calacorene	1543	2.39	1.45	1.26	1.31	1.08
<i>Oxygenated sesquiterpenes</i>		32.22	27.70	27.80	26.91	22.11
Elemol	1551	0.36	0.31	0.39	0.34	0.29
<i>E</i> - Nerolidol	1566	0.74	0.61	0.55	0.59	0.48
Spathulenol	1571	0.13	—	—	0.19	—
Geranyl-2-metil- butyrate	1603	13.60	10.69	10.24	10.25	8.92
<i>epi</i> - Globulol	1611	0.25	0.23	0.22	0.22	0.23
1- <i>epi</i> -Cubenol	1628	2.66	3.09	3.81	3.27	3.85
γ - Eudesmol	1632	0.65	0.49	0.38	0.39	0.28
<i>cis</i> -Cadin-4-en-7-ol	1635	1.32	0.96	0.84	1.01	0.76
α -Muurolol	1645	0.60	0.49	0.48	0.49	0.43
τ -Muurolol	1649	0.39	0.41	0.40	0.42	0.38
α -Eudesmol	1653	2.12	1.79	1.67	1.74	1.50
β -Eudesmol	1657	2.60	2.07	1.91	1.99	1.65
α - Cadinol	1660	0.13	0.15	—	—	—
(3 <i>Z</i>)-Caryophylla-3,8(13)-diene-5- α -ol	1662	0.13	—	0.10	0.11	—
Bulnesol	1671	6.06	5.24	5.09	5.11	4.58
<i>n</i> - hexadecanoic Acid	1692	0.42	1.17	1.72	0.79	0.99
<i>Phenylpropanoids</i>		40.01	42.76	42.16	44.35	48.45
Elimicin	1561	5.37	4.61	5.16	4.78	5.93
Dillapiole	1679	18.36	16.79	15.25	16.96	13.00
Apiole	1690	16.27	21.35	21.73	22.61	29.51
Total identification (%)		95.62	94.01	93.18	93.61	93.86
Number of compounds		34	31	31	32	27

*RI: retention indices relative to C₈–C₂₀ *n*-alkanes on the HP-5MS column in order of elution. 100%; 70% and 50% of total incident irradiance; RN: red net; BN: blue net. Coloured nets blocking 50% of incident light; — Not identified.

Figure 1-Majoritarian compounds of leaves of *Piper aduncum* L. cultivated in different ambient light. Bars-standard error ($n=3$); RN: red net; BN: blue net; Coloured nets blocking 50% of incident light.

Figure 2- Majoritarian compounds of roots of *Piper aduncum* L. cultivated in different ambient light. Bars-standard error ($n=3$); RN: red net; BN:blue net; Coloured nets blocking 50% of incident light.