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**Research Article** 

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# Diurnal variation of the chemical composition and its repercussion on the biological activity polyphenolics of *Salvia officinalis* aerial parts

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**Abstract:** This study reported the diurnal variation of the chemical composition and biological activities of phenolic compounds of *Salvia officinalis* cultivated in Tunisia. The HPLC determination of different phenolic compounds showed that the amounts of total polyphenols reach its maximum at 7 am. Estimation of the antioxidant activity of different methanolic extracts of *Salvia officinalis* showed that the extract of 5 pm had the most effective antiradical power against DPPH and the best iron reducing capacity. The strongest total antioxidant capacity was recorded for extracts of 12 and 5 pm. The methanol extracts of sage also have a capacity to reduce the appetence of *Spodoptera littoralis* larvae. This activity did not vary with the harvesting time.

Keywords: Salvia officinalis; Phenolic compounds; Antioxidant activity; Insecticidal activity

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# Introduction

Medicinal plants are universally considered as an important source of chemical substances with potential therapeutic and biological effects [1,2]. Among these species, Salvia officinalis (S. officinalis), a member of the Lamiaceae family, is cultivated as a culinary herb and as a plant of great medicinal importance. Curative properties of S. officinalis (sage) are particularly recognized since earliest times, and its uses as a tonic, stimulant, carminative, antiseptic, and antihydrotic [3]. Also, this plant has been used since ancient times as a flavor and food condiment, and in cosmetics and perfumes [4]. Nowadays, many Mediterranean countries where sage grows have substantial gains from its production and export [5,6]. In fact, many recent studies have demonstrated that this plant has antidiabetic [7], anticancer [8], anti-inflammatory [9], anti-mutagenic [10], antimicrobial [11] and antiviral [12] properties. Salvia officinalis has been shown to have the strongest antioxidant activity among herbs [13] which is mainly due to its essential oil and phenolic fraction. The major constituents of sage oil include  $\alpha$ -thujone,  $\beta$ -thujone, camphor, and 1,8-cineole, determine also their chemotypes [14-16]. As for phenolic fraction, it is mainly composed of rosmarinic acid, carnosic acid, and carnosol followed by cafeic acid, rosmanol, rosmadial, genkwanin, and cirsimaritin [17].

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental factors of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing [18,19]. Many authors reported that the concentration of plant secondary metabolites such as saponins in *Phytolacca dodecandra* [20], alkaloids in *Papaver somnifemm* [21], essential oils in *Laurus nobilis* [22] and hypericins in some *Hypericum* species [23] can fluctuate diurnally. According to Ramezani et al. [24], the environmental factors such as light (quality,

intensity and duration), temperature, irrigation, elevation, soil type and nutrition elements alone or in combination have great influence on the quantity and quality of secondary metabolites in plants. These authors also reported that temperature variations during the day have more effect on essential oil accumulation in medicinal plants. In Origanum onites [25,26] and other *Origanum* species [27], the reports showed that the variations depending on different times of the day and development period can influence the active substance and the herbal productivity of the plant. DeVasconcelos et al. [28], reported the variation during the daytime in the chemical constituents of the essential oil of Ocimum leaves. They showed gratissimum а considerable variation in the yield of eugenol, i.e. 98 % at 12.00 to 11% at 17.00 am.

According to bibliographic data, it seems that there were few works that have been carried out on the variations of the secondary metabolites accumulation in medicinal plants during the day. These works proved that the accumulation of these metabolites varied significantly during the day which, according to us, would surely affect the biological activities of these extracts. Based on this hypothesis, the present study reports for the the first-time diurnal variation of the essential oil and polyphenolics content and composition of Salvia officinalis aerial parts. The main objective of this work is to evaluate the impact of the the diurnal variation of the studied extracts on their antioxidant and insecticidal potentials. The results of such study may be very interesting in order to optimize the best harvesting hour to obtain extracts characterized by the best yield of active compounds and by the more effective biological activity.



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# **Materials and Methods**

#### **Chemicals**

Solvents used in the experiments were purchased from Merck (Darmstadt, Germany). Anhydrous sodium sulphate (Na<sub>2</sub>SO<sub>4</sub>), 6methyl-5-hepten-2-one used as internal standard, homologous series of C8-C22 nalkanes used for identification of aroma compounds (by calculation of their retention indexes), and commercially pure standards of aroma compounds were purchased from Ridelde Haën (Riedel-de Haën AG, Seelze, Germany). Pure methanol of high-performance liquid chromatography (HPLC)-grade was purchased from Merck (Darmstadt, Germany). Sulfuric acid, acetic acid, and trichloroacetic acid were purchased from Sigma-Aldrich Germany). Butylated (Steinheim, hydroxytoluene (BHT), β-carotene, linoleic acid, ethylenediaminetetraacetic acid, 3-(2pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4triazine (ferrozine), iron(II) chloride (FeCl<sub>2</sub>), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and polyvinyl polypyrolidone were purchased from Sigma. Folin-Ciocalteu reagent, aluminum chloride, sodium nitrite, and sodium carbonate were purchased from Aldrich. Authentic standards of phenolic compounds were purchased from Fluka (Fluka AG, Buchs, Switzerland) and Riedel-de Haën (Riedel-de Haën AG, Seelze, Germany). Stock solutions of pure compounds were prepared in HPLC-grade methanol. All other chemicals used were of analytical grade.

#### Plant material

Sage plants have already been grown in a public garden. Samples were carried out in a day characterized by a non-rainy climate and a temperature of  $25^{\circ}$ C. Aerial parts of *S. officinalis* were collected during the flowering phase of the plants (May, 2011) from a house garden in the region of Hammam-Chatt in the southern suburbs of Tunis (Tunisia)  $36^{\circ}43'49''$ N and  $10^{\circ}22'98''$ E, 2 m above sea

level (average annual minimum temperature=13.2°C; average annual maximum temperature=23.5°C; average annual precipitations=38.5 mm). About forty plants were harvested during 3 harvesting times (7 am, 12 pm and 17 pm) during the same day. Samples were taken in a homogeneous and random way and during the vegetative stage of the plant. Aerial parts were cut at ten centimeters above the ground. About 3 kg of fresh vegetable material was obtained for each harvesting time and then preserved in black plastic bags and directly tacked to the laboratory of Aromatic and Medicinal Plants where they are dried for about thirty days at ambient temperature (24°C). To obtain a constant weight, drayed aerial parts were putted at 60°C. The results were expressed in relation to the weight of the dry matter in the following results. The identity of the plant was confirmed by Prof. Abderrazzak Smaoui, taxonomist, and a voucher specimen was deposited in the herbarium at the Biotechnological Centre of Borj-Cedria under the reference number LN 08002.

# Extraction of polyphenols

Phenolic compounds were extracted by maceration into pure methanol according to the method of Mau et al. [29], One g of dried and powdered sample was immersed into 10 ml of pure methanol and the suspension was stirred for 24 h in darkness at 4°C. The mixture was then filtered through a Whatman filter paper (No. 4) then vanillin acid was added as an internal standard and the methanol macerate was evaporated to dryness under vacuum and stored at 4°C for further analyses.

# Spectrophotometric determination of polyphenols

Total phenolic content was assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto et al. [30]. A calibration curve of gallic acid (ranging from 50 to 400  $\mu$ g/mL) was prepared and the



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results, determined by the regression equation of the calibration curve (y=62.94x-0.67,  $R^2=0.99$ ), were expressed as microgram gallic acid equivalents (GAE) per gram dry weight of raw material (DW). In this method, an aliquot (0.125 ml) of a suitable acetone sample was added to 0.5 mL of deionized water and 0.125 ml of concentrated Folin-Ciocalteu reagent. Solutions were mixed and incubated at room temperature. After 1 min, 1.5 mL of 7 % sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution was added. The final mixture was shaken thoroughly and then incubated for 90 min in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a HACH UV-Vis spectrophotometer.

Total flavonoid contents (TFC) were measured according to Dewanto et al. [30]. An aliquot of diluted sample or standard solution of (+)catechin was added to a 75 µL of NaNO<sub>2</sub> solution and mixed for 6 min, before adding 0.15 mL AlCl3 (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as milligram catechin equivalents (CE) per gram DW, through the calibration curve of (+)-catechin. The calibration curve range was 50-400  $\mu$ g/mL (R2=0.99).

Total tannin contents were measured using the modified vanillin assay described by Sun et al. [31]. To 50  $\mu$ L of properly diluted sample, 3 mL of methanol vanillin solution (4 %) and 1.5 mL of H<sub>2</sub>SO<sub>4</sub> were added. The absorption was measured at 500 nm against extract solvent as a blank. The amount of total condensed tannins is expressed as mg (+)-catechin/g DW. The calibration curve range was 50-400  $\mu$ g/mL (R<sup>2</sup>=0.99).

# **RP-HPLC** analysis of phenolic compounds

Phenolic compounds were analysed using an Agilent Technologies 1100 series liquid chromatograph coupled with an UV-vis multiwavelength detector. Separation of phenolics was carried out on a 250mm×4.6mm, 4µm Hypersil ODS C18 column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml/min. The gradient programme was as follows: 15 % A/85 % B 0-12min, 40 % A/60 % B 12-14min, 60 % A/40 % B 14-18 min, 80 % A/20 % B 18-20 min, 90 % A/10 % B 20-24 min, 100 % A 24-28 min. The injection volume was 20 µL and peaks were monitored at 280 nm. Samples were filtered through a 0.45µm membrane filter before injection. Peaks were identified by comparison of their relative retention times with those of authentic standards analysed in the same conditions.

# Preparation of Phenolic Standards for RP-HPLC

Pure phenol standards including 20 phenolic acids (gallic, 3,4-dihydroxybenzoic, 3.4dihydroxyphenylacetic, chlorogenic, cafeic, 4hydroxybenzoic, 3,5-dimethoxy-4hydroxybenzoic, vanilic, syringic, 2.5dihydroxybenzoic, *p*-coumaric, trans-4hydroxy-3-methoxycinnamic, ferulic, 0coumaric. trans-hydroxycinnamic, protocatechuic, salycilic, rosmarinic, transcinnamic, and carnosic acids) and 15 flavonoids (catechin hydrate, resorcinol, rutine trihydrate, naringin, quercetine-3-rhamnoside, luteolin, dihvdrate. quercetine kaempferol. naphtoresorcinol, 4,5,7-trihydroxyflavone, apigenin, flavone, coumarine, carnosol, and butylated hydroxytoluene) were dissolved in mobile phase (methanol) as 1 mg ml<sup>-1</sup> concentrations. The solutions were prepared freshly, filtered through a 0.45 µm membrane filter and immediately injected to HPLC column. Evaluation of each standard was repeated three times.



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# DPPH Radical Scavenging Assay

The donation capacity of the obtained extracts and EOs was measured by bleaching of the purple-colored solution of the DPPH radical according to the method of Hanato et al. [32]. A total of 1 mL of different concentrations of extracts and EOs prepared in methanol was added to 0.5 mL of a 0.2 mmol/L DPPH methanolic solution. The mixture was shaken vigorously and kept at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity was expressed as IC50  $(\mu g/mL)$ , the concentration required to cause 50 % DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect  $\% = [(A_o - A_1)/A_o] \times 100$ 

Where  $A_0$  is the absorbance of the control at 30 min and  $A_1$  is the absorbance of the sample at 30 min. BHT was used as a positive control.

# Ferric-Reducing Antioxidant Power

The method of Oyaizu [33], was used to assess the reducing power of different organ extracts and essential oils. A total of 1 mL of different concentrations of organ extracts and essential oils in methanol was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide  $[K_3Fe(CN)_6]$  and incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10 % trichloroacetic acid was added to the mixture that was centrifuged at 650g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride solution. The intensity of the bluegreen color was measured at 700 nm. Results were expressed as  $EC_{50}$  (mg/mL) which is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as positive control.

# Total antioxidant capacity

The total antioxidant capacity of the extract was evaluated by the method of the phosphomolybdenum according to Prieto et al. [34]. The test is based on the reduction of ions Mo (VI) to Mo (V) by the antioxidant molecules present in the plant extract. Following this reduction reaction, a green complex phosphate/Mo (V) is formed in acidic medium. The absorbance measured at 695 nm is then evaluated to measure the total antioxidant capacity of the extract and results were expressed in gallic acid equivalents (GAE). 100 µL of the diluted plant extract is mixed with 1000 µL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM molybdate ammonium). After stirring, the mixture is kept in a water bath at a temperature of 90°C for 90 minutes. The absorbance is measured at 695 nm with a UV/visible spectrophotometer, referring to a control without extract. The total antioxidant capacity of each sample is expressed as mg of GAE per gram of dry matter.

# Insecticidal activity of extracts

The insecticidal potential of sage extracts was tested against *Spodoptera littoralis* species.

# Antifeedant assay with leaf discs

The antifeedant effect of sage methanolic extracts against larvae of *Spodoptera littoralis* (stage 4) was determined. For this, 10 mg of the dry residue of sage methanolic extract was diluted in 10 ml of distilled water. The diluted extract was then subjected to vigorous mixing until completely dissolved in water. After this step, leaves of castor bean (*Ricinus* communis L.) were cut into discs of 30 mm diameter and were well soaked in the diluted extract for about 10 minutes and then dried on Whatman paper. Every two-disc leaves were placed in a petri dish in the presence of only one larva stage 4 of *Spodoptera littoralis*. Ten replicates were made and control leaves were soaked in pure distilled



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water. After 24 h, the leaf area consumed was measured for treated samples and control.

The regression of insect appetence was estimated by antifeeding index, which was determined by the following formula:

AFI = (C-T) \* 100 / (C + T)

C = leaf area consumed by controls larvae of Spodoptera littoralis expressed in mm<sup>2</sup>. T = leaf area of the treated samples consumed by the larvae of Spodoptera littoralis expressed

by the larvae of *Spodoptera littoralis* express in  $mm^2$ .

# Statistical analysis

All extractions and determinations were conducted in triplicates. Data are expressed as means $\pm$ SD. The means were compared by using the one-way and multivariate analysis of variance followed by Duncan's multiple range test. The differences between individual means were deemed to be significant at *p* <0.05. All analyses were performed by using the "Statistica v 5.1" software.

Concerning the results of the antifungal activity, the trial is conducted in a completely randomized design in which various treatments tested are the only fixed factor. The averages of three replicates were compared by SNK test at  $p \le 0.05$  using the SPSS program.

Regarding the insecticidal activity data were subjected to a comparison of the average according to the Duncan test using SPSS version 11 program. Differences between means were considered significant at  $p \le 0.05$ . Five repetitions were carried out for the fumigant and repulsive test, ten for antifeeding.

# **Results and Discussion**

# Variation of total polyphenols, flavonoids and condensed tannins

Contents of total polyphenols, flavonoids and condensed tannins were presented in table 1. Results showed that the levels of total polyphenols and condensed tannins remained unchanged from one time of harvest to another. On the other hand, levels of total flavonoids varied significantly (p<0.05) with a maximum detected at 5 pm (673.68 µg CE/g DMW) followed by 7 am and 12 pm, with amounts of 505.26 and 463.15 µg CE/g DMW respectively.

According to bibliographic data, it seemed that diurnal variation of polyphenolic contents in plants was limited to only one study carried out on the phenolics of whole plants of *Hypericum* species and which reported by Ayan et al. [35]. According to these authors, the greatest accumulation of phenolics was found in the species *H. pruinatum* harvested at 24 h during the stage of full flowering.



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Table 1: Changes in the levels of total polyphenols (µg GAE/g DW), total flavonoïds (µg CE/g						
DW) and condensed tannins (µg CE/g DW) of Salvia officinalis, depending on the time of harvest.						
Harvests hours	7 am	12 pm	5 pm			
Total polyphenols (µg	1042.28±220.27 <sup>a</sup>	1107.04±160.66 <sup>a</sup>	1110.85±197.97 <sup>a</sup>			
GAE /g DMW)						
Total flavonoids (µg CE/g	505.26±47.64 <sup>b</sup>	463.15±32.70 <sup>b</sup>	673.68±73.00 <sup>a</sup>			
Condensed tannins (µg	58.94±11.68 <sup>a</sup>	49.47±49.47 <sup>a</sup>	21.04±22.04 <sup>a</sup>			
CE/g DMW)						
Values in the same row with different superscripts (a-b) are significantly different at P<0.05.						

# Variation of individual phenolic compounds

The RP-HPLC analysis of chromatographic profiles obtained from methanol extracts of the aerial parts of *S. officinalis* collected at three different times of the day allowed the identification of 11 compounds with the predominance of rosmarinic acid and *p*-coumaric (Figure 1; Table 2).

**Figure 1**: Chromatographic profil obtained by HPLC of methanolic extracts of *S. officinalis* collected at different times of the day.





**Figure 2:** Variation of leaf area consumed by *Spodoptera littoralis* under the effect of methanolic extracts of *S. officinalis* collected at different times of the day.

Values in the same line with different superscripts (a-b) are significantly different at P < 0.05.



Obtained results showed that all the compounds identified varied significantly with the harvesting time during the day (p<0.05) with the exception of *trans*-cinnamic acid, carnosol and coumarin which were stable during the day.

The major phenolic acids identified were rosmarinic ranging from 9.3 % (296.29 g/g DMW) at 7 am to 6.8 % (180.71 g / g DMW) at 12 pm followed by *p*-coumaric ranging from 6.2 % (197.03 g/g DMW) at 7 am to 6 % (159.4 g/g DMW) at 12 pm and cafeic acid ranging from 5.1 % (161.09 g / g DMW) at 7 am to 2.9 % (78.45 g / g DMW) at 12 pm (Table 3). Rosmarinic acid has been also reported by Gomes et al. [36], to be the major phenolic acid present in the shoots of sage grown *in vitro*. In all, the percentage of total phenolic acids was stable during the day (p < 0.05), whereas the quantities of these compounds varied significantly from one hour of harvest to another with a maximum at 7 am (1093.16 g/g DMW) and a minimum at 12 pm (804.61 g / g DMW). The major flavonoïd compound was coumarin which the percentage and amounts were stable regardless of the time of collect followed by flavone which peaked at 7 am harvest (5.4 %, 171.06 g / g)DMW). The flavonoic compounds that undergo a significative daily variation (p < 0.05) were flavone, kampferol and epicatechin. Flavone showed the highest percentages and amounts at 7am while the maximum rates of epicatechin were the same in both the extract from 7 am and pm. this compound 5 However, was quantitatively more concentrated in the extracts of 17 pm (93.37 g / g DMW). In all, the extract from 7 am was the richest in flavonoïds (19.5 %, 616.33 g/g DMW) followed by that of 5 pm (18.1 %, 528.42 g/g DMW) and finally 12 pm (17.4 %, 469.23 mg/g DMW). The quantification of total polyphenols of S. officinalis aerial parts of by HPLC revealed that these compounds varied significantly (p < 0.05)during the day. According to our results, it seemed that the extracts of 7 am were the richest in these metabolites (3.15 mg / g DMW) followed by those of 5 pm (2.92 mg/g DMW)and finally those of 12 pm (2.67 mg/g DMW). Finally, it has been demonstrated that the biosynthesis and accumulation of phenolic compounds could be enhanced based on biotic and abiotic conditions. Temporal or permanent environmental conditions influenced polyphenol concentration in plants [37,38].



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Table 2: Changes in percentages and amounts ( $\mu g / g DW$ ) of phenolic compounds in methanol extracts of of S. officinalis								
aerial parts depending on the time of harvest.								
	Harvesting time							
	7 am		12 pm		5 pm			
	% (w/w)	µg/g DW	% (w/w)	µg/g DW	% (w/w)	µg/g DW		
Phenolic acids	31.97±2.98b	1093.16±9.12 A	31.58±3.02b	804.61±9.25C	32.32±3.54a	974.3±8.45B		
Cafeic acid	4.71±0.51b	161.09±12.20A	3.08±0.33c	78.45±6.45C	4.80±0.42a	144.69±9.78B		
Ferulic acid	2.77±0.25a	94.54±8.15A	2.41±0.21c	61.44±5.45C	2.74±0.22b	82.64±7.49B		
P-coumaric acid	5.76±0.44b	197.03±9.87A	6.26±0.52a	159.40±9.56B	4.68±0.33c	141.01±8.94C		
Rosmarinic acid	8.67±0.74b	296.29±8.69A	7.09±0.65c	180.71±9.84C	9.02±0.77a	271.81±8.95B		
Carnosic acid	4.33±0.32	148.03±8.67B	6.25±0.57	159.15±9.89A	4.84±0.45	145.99±9.89C		
Salicylique acid	3.12±0.24b	106.70±9.86A	3.34±0.28a	85.15±7.85C	3.33±0.41a	100.40±8.98B		
Trans-cinnamic acid	2.62±0.22c	89.45±7.85A	3.15±0.28a	80.27±6.59C	2.84±0.26b	85.73±7.65B		
Flavonoids	18.03±1.54b	616.33±9.67A	18.42±1.74a	469.23±9.76C	17.53±1.66c	528.42±9.46B		
Carnosol	2.42±0.21b	82.80±8.64A	2.68±0.25a	68.22±5.12C	2.43±0.21b	73.19±6.86B		
Flavone	5.00±0.45a	171.06±9.57A	5.08±0.47a	129.44±8.61B	3.94±0.27b	118.92±8.64C		
Kampferol	3.13±0.25b	106.85±9.54A	3.12±0.32b	79.50±8.43C	3.32±0.33a	100.23±8.94B		
Epicatechin	2.28±0.20b	77.82±6.58B	1.79±0.15c	45.65±3.49C	3.10±0.32a	93.37±8.61A		
Coumarin	5.20±0.44b	177.78±9.54A	5.75±0.51a	146.41±8.36C	5.10±0.49c	153.88±8.69B		
Total	100.00±8.23a	3418.93±12.58A	100.00±7.85a	2547.63±20.58C	100.00±9.51a	3014.58±34.29B		
The values of the quantities and percentages of phenolic compounds represent the average of three repetitions±SD, letters (a-								

c) between percentages) and (A-C)

between quantities indicate significant differences at P < 0.05.

# Variation of antioxidant activity

In this study, antioxidant radical scavenging capacity using the DPPH, reducing power and total antioxidant capacity were tested for methanolic extracts of *S. officinalis* and results obtained were resumed in table 3.

Methanol extracts of *S. officinalis* showed a radical scavenging activity measured by DPPH test. This activity varied significantly (p<0.05) with harvesting time (Table 3). According to our results, it seemed that the highest radical scavenging activity (IC50=13.03 mg/mL) characterized the extracts of 5 pm. This activity was stronger than that of the synthetic antioxidant BHT (IC50=16 µg/mL). Extracts from 7 am and 12 pm had the same radical

scavenging activity (IC50=29.34 and 29.11 mg/mL respectively).

These results were in agreement with those of Hamrouni Sellami et al. [39], who demonstrated that IC50 corresponding to radical scavenging activity against DPPH of S. officinalis aerial parts fresh or dried with different drying methods were ranged from 21.52 to 58.48  $\mu$ g/mL. Nevertheless, our results were different from those of Termes and schwarz [40], who reported that the IC50 of the methanol extract of S. officinalis was in the order of 41 µg/mL. According to Lu and Foo [41], the antioxidant activity of the methanol extract of S. officinalis was attributed to the



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presence of rosmarinic and caffeic acids and also to its high flavonoïd contents.

A study reported on the chemical structure of various phenolic acids of sage showed that derivatives of cinnamic acid had a strong antiradical activity. This activity may be due to the presence of conjugated instauration facilitating the re-allocation of the resulting free radicals. The cinnamic and caffeic acids were more effective in scavenging radicals than ferulic and *p*-coumaric acids [41]. Cuvelier et al. [17], also attributed the antioxidant activity of sage to the presence of abietanes diterpenoids (carnosic acid and carnosol). According to Gomes et al. [36], the antioxidant effectiveness of phenolic compounds varied from one compound to another. The antioxidant activity of sage could also be attributed to the synergistic effect of phenolic compounds (salicylic acid and flavonoïds) or total phenolic content of extracts [42].

Lu and Foo [41], showed that orthodihydroxybenzene (catechin or epicatechin) was essential for improving the antioxidant activity of extracts. According to these authors, this compound was the best flavonoid that enhanced antiradical activity of sage extracts. Finally, it should be noted that the difference in the antioxidant properties of different extracts could be attributed to the presence of different substrates as well as the variability of the products generated by the reaction system [42]. On the other hand, the estimation of reducing power of S. officinalis methanolic extracts presented in Table 3 and results were expressed as EC50 values, which were concentrations of the extracts corresponding to an absorbance of 0.5. The reference used as positive control was ascorbic acid. Results showed that all the tested extracts presented a more effective reducing activity as compared with that of ascorbic acid  $(EC50 = 34.96 \ \mu g/ \ ml).$ 

The reductive capacity of iron was significantly affected by harvesting time. Indeed, the extract

of 5 pm had the most important activity (EC50 equal to 375.45 g/ml) followed by that of 12 pm and 7 am (625 and 578.7  $\mu$ g / mL, respectively). The high reducing power of methanolic extracts of sage has been also reported by Hamrouni Sellami et al. [39].

Finally, the screening of the total antioxidant capacity of *S. officinalis* methanolic extracts was evaluated by reduction of molybdate method. Results of this test (table 3) showed that the studied extracts had different antioxidant capacity. In this way, the extracts of 5 pm and 12 pm had the highest total antioxidant capacity equivalent to 792 and 722 mg GAE /g DMW respectively. Whereas the extract of 7 am showed the lowest total antioxidant capacity corresponding to 553 GAE mg/g DMW.

Grzegorczyk et al. [42], showed that acetonic and methanolic extracts of *S. officinalis* cultivated *in vitro* had different degrees of molybdate reduction and methanol also was the most effective solvent for this activity. The ability of methanolic extracts of *S. officinalis* to reduce molybdate was mainly due to their high rosmarinic acid content whereas acetone extracts were able to reduce molybdate by their wealth carnosol and carnosic acid contents [41,42] showed that antioxidant capacities of all sage polyphenols (with the exception of apigenin) were better than that of synthetic antioxidant trolox.



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Table 3: Variation of antioxidant activity of methanol extracts according to the time of harvest.						
	DPPH (IC <sub>50</sub> µg/mL)	Reducing power(EC <sub>50</sub> µg/mL)	Total antioxidant capacity (mg GAE/g DW)			
7 am	29.34±2.43 ª	625 <sup>a</sup> ±42.12a	553±19.30 <sup>b</sup>			
12 pm	29.11±0.47 °	625 <sup>a</sup> ±51.28a	722±64.50 ª			
5 pm	13.03±2.78 <sup>b</sup>	375.45 <sup>b</sup> ±17.94b	792±81.64 <sup>a</sup>			
BHT	16±1.89 <sup>b</sup>	-	-			
Ascorbic acid	-	34.96±1.30	-			
The values IC <sub>50</sub> , EC <sub>50</sub> and total antioxidant capacity represent the average of three repetitions $\pm$ SD, letters (a-c) indicate significant differences at <i>P</i> <0.05.						

#### Insecticidal activity

#### Antifeeding test

As presented in figure 2, results of this test showed that control leaf area was 282 mm<sup>2</sup>. In cases where the leaves are processed by extracts of S. officinalis, leaf area consumed decreased considerably and achieved 183.2 mm<sup>2</sup> for extract of 7 am, 87.2 mm<sup>2</sup> for extract of 12 pm and 157.6 mm<sup>2</sup> for 5 pm extract. These results indicated that different diluted methanol extracts of S. officinalis reduced appetence of Spodoptera littoralis larvae. Pavela [43], announced that the methanol extract of S. officinalis was one of the most toxic extracts and could affect various parameters in insects such as growth index. On the other hand, insecticidal activity of plant extracts was treated by different authors. Acetonic extract of Ajuga pseudoiva leaves also had an antifeeding activity against Spodoptera *littoralis* [44]. The  $\beta$ -caryophyllene and its oxide compound had a strong antifeeding activity against Leptinotarsa decemlineata and Spodoptera littoralis [45]. Methanolic extract of Othanthus maritimus had a larvicidal activity against Culex quinquefasciatus with LC50

equal to 7 ppm [46]. The regression of appetence insects is estimated by the antifeeding index (AFI) and results showed that antifeeding index was about 38 for the extract of 7 am, 67.04 for the extract of 12 pm and 39.43 for the 5pm.

The statistical analysis of our results showed no significant differences between the different extracts antifeeding index. This stability of antifeeding activity probably may be due to the synergy between the different phenolic acids or flavonoides.

# Conclusion

Methanolic extract of *S. officinalis* collected at 7 am seemed to be effective to replace chemical drugs used for the treatment of diseases caused by oxidative stress. In addition, methanolic extract of this plant can be also used as an alternative to the chemical's insecticide especially in organic agriculture.

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# **Graphical Abstract**



# Highlights

- Chemical composition of Salvia officinalis methanolic extract varied significantly during the day.
- Methanolic extract of 7 am had the best antiradical activity.
- > Methanolic extract of 12pm and 5pm had the best total antioxidant activity.
- Methanolic extract of sage had a capacity to reduce appetence of the larvae of Spodoptera littoralis.

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