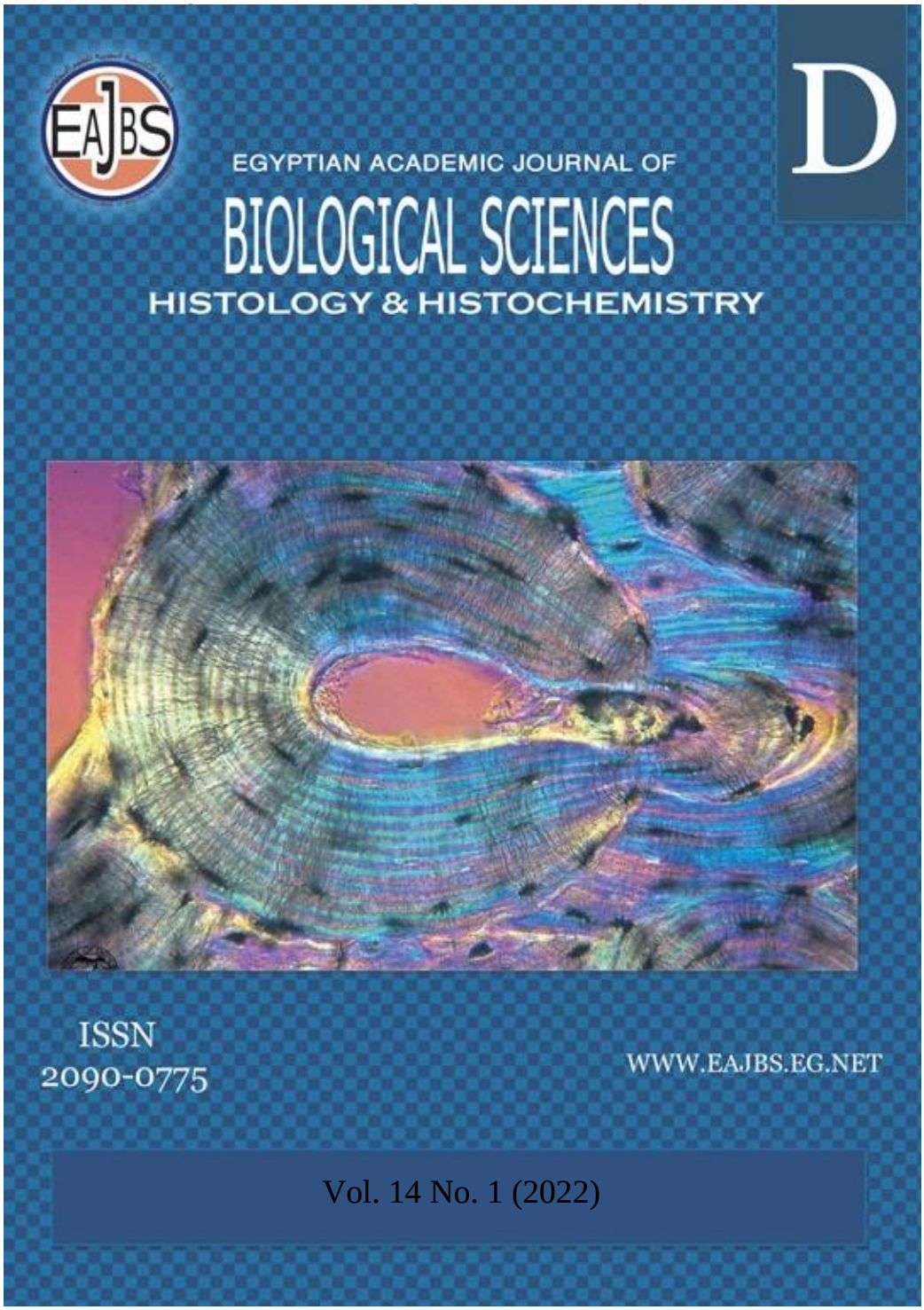

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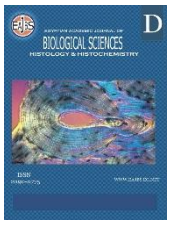

Effect of Ultraviolet on Molecular Structure and Photochemistry Compounds for *Salvia hispanica* Medical Seeds

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ABSTRACT

Background: In some developing countries and due to economic conditions, plant seeds are used as a medicinal source to treat infectious diseases. The seeds are the main medicinal part of the plant. Phenolic compounds are secondary metabolites that have diverse structures containing one or more hydroxyl groups produced by plants mainly for protection against biotic or abiotic stress. A study of its exposure is essential in order to obtain a higher yield of these valuable compounds. The aim of this research is to study the influence of UVA on molecular structure, chemical composition and photochemistry compounds of *Salvia hispanica*. **Materials and methods:** *Salvia hispanica* seeds were received from the Egyptian Ministry of Agriculture. The total phenolic content was determined using the Florin-Ciocalteu method. The total flavonoids content was determined using the aluminum chloride method. **Results:** UVA caused a great effect on molecule arrangement, size and orientation, chemical composition, enzymatic and non-enzymatic antioxidants in *Salvia hispanica*. Total phenolic content in *Salvia hispanica* varied after exposure to UVA for 1, 2, 3 and 4 hours at 5 cm and 20 cm from UV source. Total flavonoids content in *Salvia hispanica* varied after exposure to UVA. Also, DPPH scavenging activity, phenolic and flavonoids in *Salvia hispanica* varied after exposure to UVA. A significant change in carbohydrates, protein and fats with little variation in total fibers in *Salvia hispanica* after exposure to UVA.

INTRODUCTION

Secondary metabolites of medicinal plants are the material basis of their clinically curative effects, but the synthesis and accumulation of its very complex and are affected by many factors including internal as enzymes and external environmental factors such as radiation. Ultraviolet radiation is produced more total phenolic, flavonoids and antioxidants against adverse conditions which caused intracellular redox homeostasis. UVB radiation is serious environmental stress that enhances the generation of reactive oxygen species, including superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals (Mackerness *et al.*, 1998 & He and Hader, 2002). Plants respond to oxidative damage by activating the enzymes superoxide dismutase and peroxidase, which scavenge ROS and offer protection to nucleic acids, lipids and proteins (Jain *et al.*, 2004 & Zu *et al.*, 2010).

Plants can produce UVB absorbing compounds such as phenolic and flavonoids in order to protect from UVB radiation damage (Tegelberg *et al.*, 2001). The abiotic stresses, such as drought soil nutrient deficiency and heavy metals, in turn, the ROS may act as a mediator in initiating biosynthesis of certain secondary metabolites (Shohael *et al.*, 2006 & Pu *et al.*, 2009). Also, the enzymatic and non-enzymatic antioxidants in plants can provide adequate protection against ROS and free radicals induced by photooxidative damage under UVB radiation (Rai *et al.*, 2011 & Takshak and Agrawal, 2015). Growth behavior, structure, antioxidants and vitamins of *Nigella Sativa* and *garden cress* changed after exposure to UVC for different period's time and at dissimilar distances (El-Bediwi *et al.*, 2018 & El-Bediwi *et al.*, 2018). *Prunella vulgaris* exposed to abiotic factors, such as drought (Chen *et al.*, 2011), heavy metals (Wu *et al.*, 2010) and different soil nutrient concentrations (Chen *et al.*, 2013), exhibited significantly increased major bioactive components. The aim of this research is to study and analyze the effect of UVA on the structure and photochemistry compounds of *Salvia hispanica*.

MATERIALS AND METHODS

Total Phenolic:

The total phenolic content was determined using the Florin–Ciocalteu method as described by (Attard, 2013). The procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1M Na_2CO_3 was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at 630 nm. Data are represented as means ± SD.

Total Flavonoids:

The total flavonoids content was determined using the aluminum chloride method as described by (Kiranmai *et al.*, 2011), with minor modifications to be carried out in microplates. 15 µL of sample/standard was placed in a 96-well microplate, then, 175 µL of methanol was added followed by 30 µL of 1.25 % $AlCl_3$. Finally, 30 µL of 0.125 M $C_2H_3NaO_2$ was added and incubated for 5 min. At the end of incubation time, the resulting yellow color was measured at 420 nm. Data are represented as means ± SD.

DPPH Radical-Scavenging Activity:

Concentrations ranging from 0.4g/100g to 2g/100g are prepared with methanol from each sample (100 µl) extract and DPPH radical (100 µl, 2Mm) dissolved in methanol. The mixture is stirred and left to stand for 15 min in dark. Then the absorbance is measured at 517 nm against a blank. The percentage scavenging effect is calculated as $[(A_o - A_1)/A_0]\times100$ where A_o is the absorbance of the control (without sample) and A_1 is the absorbance in the presence of the sample.

Structure:

The structure of *Salvia hispanica* is studied by scanning electron microscope (JEOL JSM-6510LV, Japan). The molecular structure of *Salvia hispanica* is studied by Nicolet™ iS™ 10 FT-IR Spectrometer from USA.

RESULTS AND DISCUSSION

Phenolic:

Total phenolic content in *Salvia hispanica* varied after being exposed to UVA as presented in Figure 1. It increased by 18.53% and 76.2% after exposure for 1 hour at both used distances but decreased by 33.5% and 22.1% after exposure for 4 hours at 5 cm and 20 cm distances from the UVA source. Phenolic content varied after exposure to UVA due to the change in hydroxyl group, O-H, carbohydrate, protein, total fibers, and fat in *Salvia hispanica* bio-content matrix. Moreover, phenolic may also be associated with other components where protein work as a base for phenolic components and quantity change of it caused variation phenolic content.

Phenolic compounds protect seeds from UV damage that were affected by their power change also (Hideg *et al.*, 2013 & Kohler *et al.*, 2017).

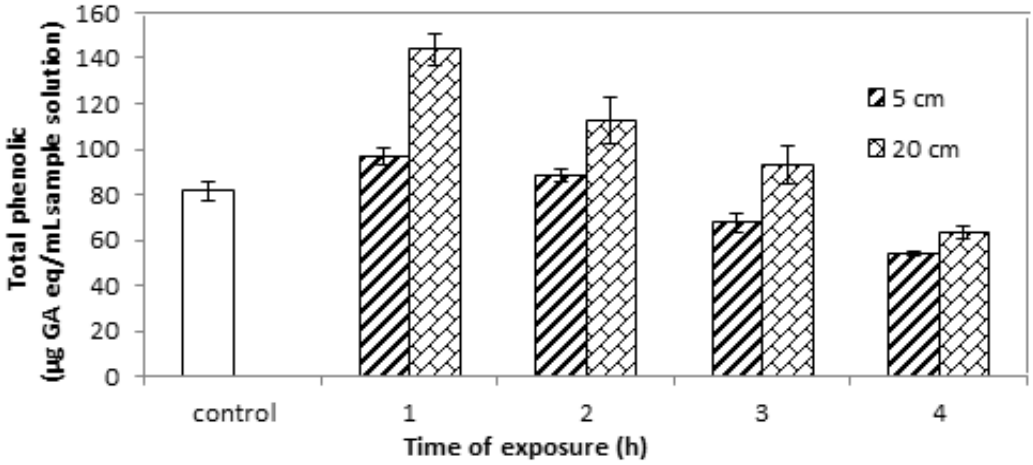

Fig. 1. Total phenolic content in *Salvia hispanica* after exposure to UVA

Flavonoids:

Total flavonoids content in *Salvia hispanica* varied after exposure to UVA as shown in Figure 2. Total flavonoids increased by 23.5% and 27.3% after being exposed for 1 hour at 5 cm and 20 cm distances but decreased by 18.8% and 7.5% after exposure for 4 hours at the same distances. The change in flavonoids content in *Salvia hispanica* is caused due to the use of it as a protection wall against UV radiation damage and this agrees with previous studies (Zu *et al.*, 2010 & Sun *et al.*, 2010). Also, the flavonoid is a type of phenolic compound which are affected by exposure to UVA depending on exposure time and distances from the source.

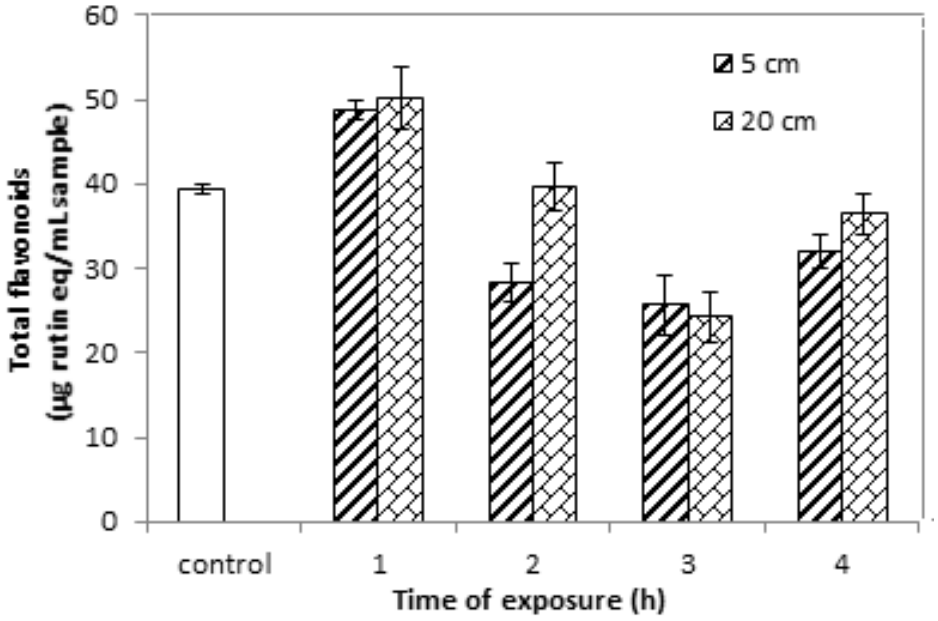

Fig. 2. Total flavonoids in *Salvia hispanica* after exposure to UVA

DPPH Scavenging Activity:

Figure 3 shows that DPPH scavenging activity for Salvia hispanica varied after being exposed to UVA and increased after being exposed for 1 and 4 hours at 5 and 20 cm distances from the source. Similar results were observed in other studies (Dang, 2015 & Liu *et al.*, 2012).

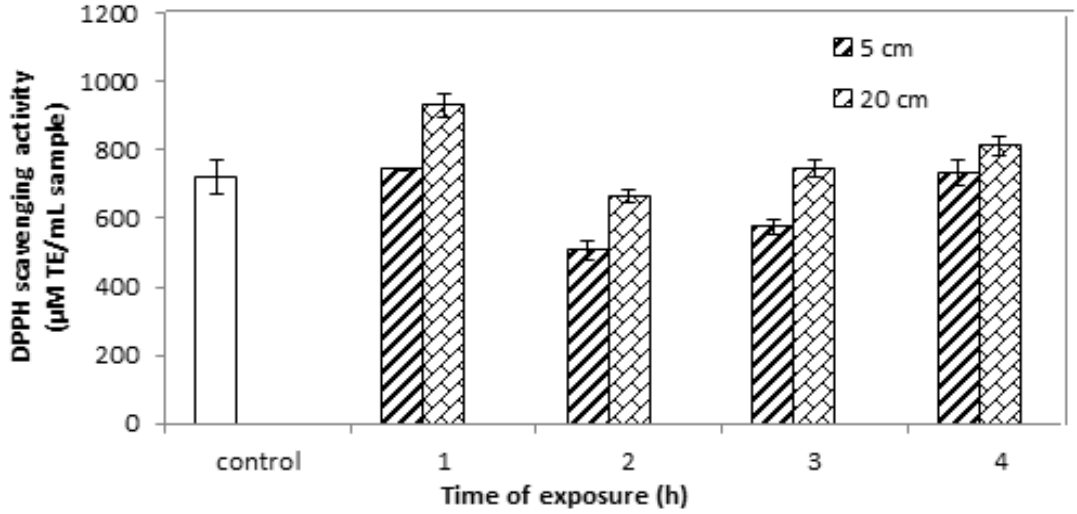

Fig. 3. DPPH scavenging activity for *Salvia hispanica* after exposed to UVA

Molecular Structure (IR Analysis):

IR spectrum for Salvia hispanica, (Fig. 4), is a plot of wavenumber (X-axis) vs. present transmittance (Y-axis). There is a significant change in the O-H bond position, broadness and intensity after being exposed to UVA, and a little change was caused on some bonds as C-O ~ 1745 cm^{-1} and C- H~ 2926 cm^{-1} after being exposed to UVA, because each interatomic bond vibrates in several different motions, (stretching or bending), and individual bonds absorbed at more than one IR frequency.

Structure and Chemical:

Composition:

Figure 5 shows the scanning electron micrographs, SEM, for Salvia hispanica after being exposed to UVA for different periods of time and dissimilar distances. The micrographs show a change in *Salvia hispanica* molecules shape, size and orientation. These changes in structural and cell disruptions are due to oxidative stress caused by UVA radiation interaction with molecules.

Chemical composition results for *Salvia hispanica* after exposure to UVA, listed in (Table 1), show a significant change in carbohydrates, protein and fats with little variation in total fibers. Carbohydrates increased after 1 hour exposed and decreased after 4 hours exposed at 5 and 20 cm distances from the UVA source. Protein increased after being exposed to UVA for 1 and 4 hours at a 5 cm distance. Fats decreased after exposure for 1 hour then increased after exposure to 4 hours at 5 and 20 cm distances.

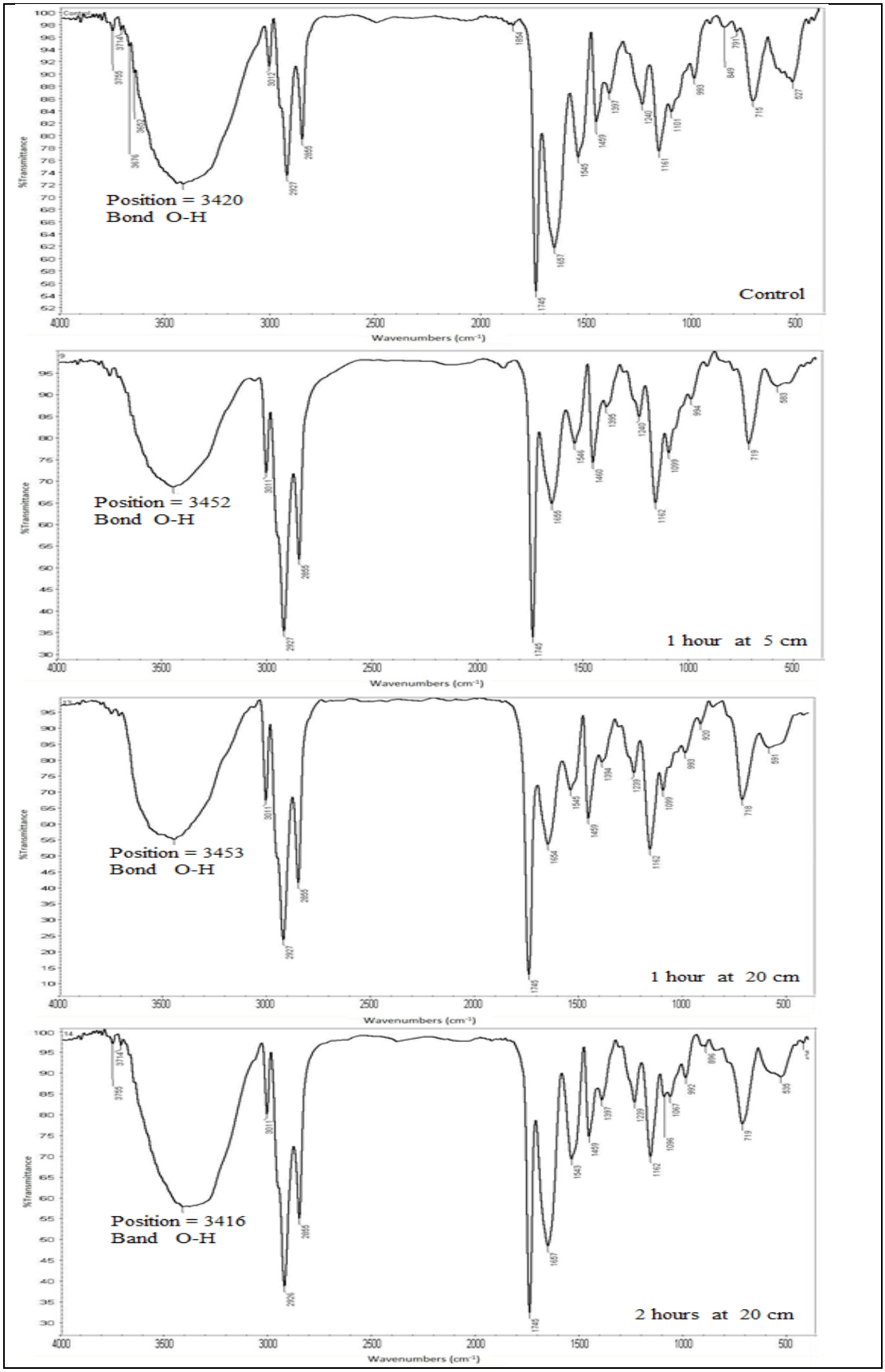

Fig. 4. IR spectrum for *Salvia hispanica* after exposure to UVA

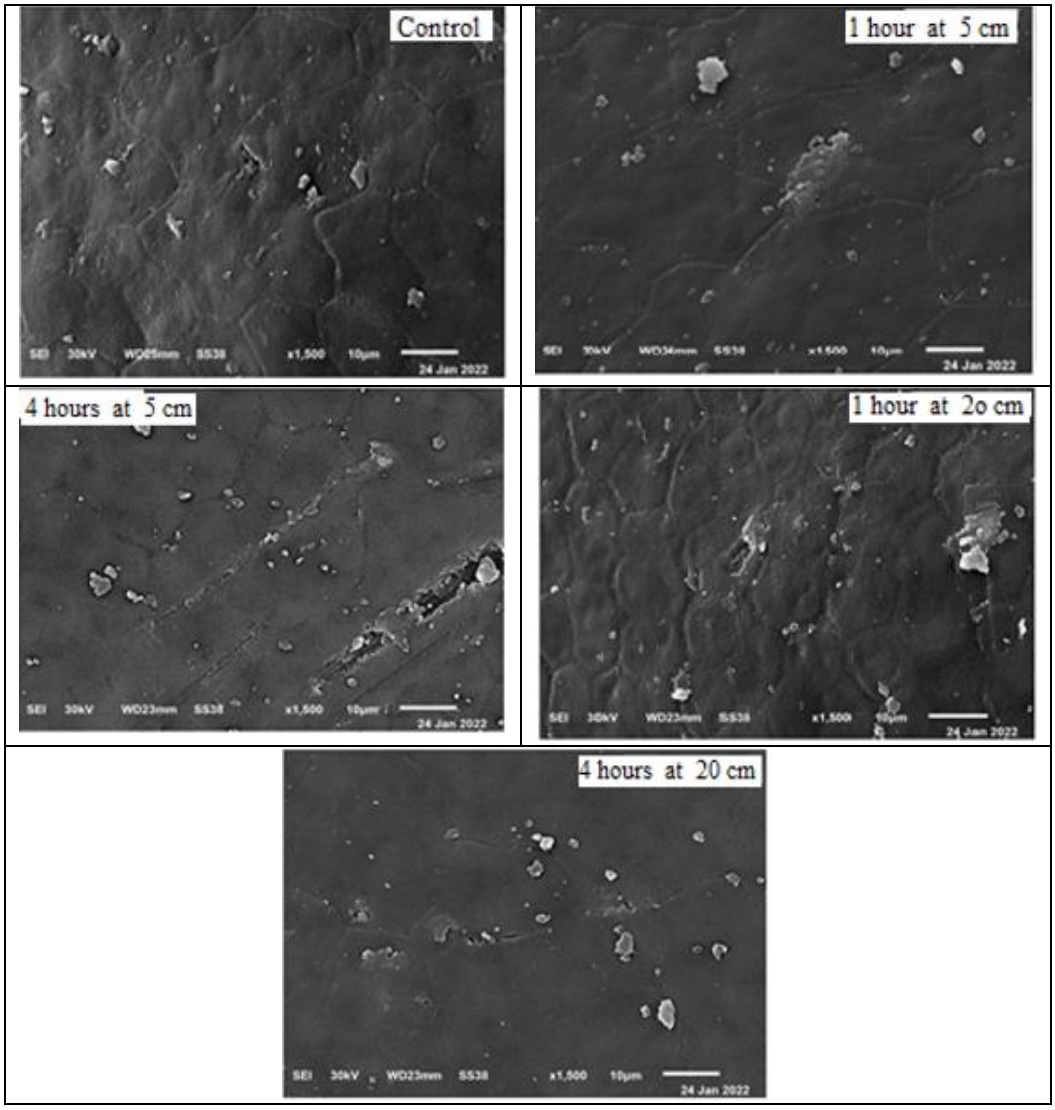

Fig. 5. SEM of *Salvia hispanica* after exposure to UVA

Table 1. Chemical composition of *Salvia hispanica* after exposure to UVA at 5 and 20 cm for different period times

Time (h)	(Exposure at 5 cm)					
	Carbohydrates	Protein	Fats	Moisture	Fibers	ASH
control	11.04%	15.56%	12.4%	7.18%	57.4%	3.6%
1	22.46%	16.31%	10.3%	8.52%	46.5%	4.43%
4	1.54%	16.44%	23.2%	8.01%	54.9%	3.92%

Time (h)	(Exposure at 20 cm)					
	Carbohydrates	Protein	Fats	Moisture	Fibers	ASH
control	11.04%	15.56%	12.4%	7.18%	57.4%	3.6%
1	14.95%	11.69%	10.7%	7.05%	58.5%	4.16%
4	9.32%	16.94%	16.1%	7.65%	53.7%	3.94%

Conclusion:

Molecular structure such as molecules arrangement, size, orientation and bonding strength or position, for *Salvia hispanica* changed after being exposed to UVA for 1, 2, 3 and 4 hours at 5 and 20 cm distances from the source. Also, a chemical composition such as

protein, carbohydrate, fat and total fiber and bio-content (phenol, flavonoid) of *Salvia hispanica* were greatly affected after exposure to UVA.

Declarations

Conflict of Interest: The authors declare that there are no conflicts of interest.

Ethical Approval and Consent to Participate: I would like to tell us, that all authors are accepted ethical approval and consent to participate Issued by the journal.

Consent for Publication:I would like to tell us, that all authors agreed for publishing this paper in your journal.

Availability of Data and Material: I would like to show, that all materials, experimental tools and data are available.

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors Contribution: Reham Ebrahim did the experiments such as (irradiated samples and preparing the used samples for other measurements) in physics and biology laboratories. Also collecting data, discussing and analyzing data.Aya Abdelrazek contributed to collecting data, discussing, and analyzing data.

Hamed El-Shra shared to put the title of the paper, doing some biological experimental and interpretation data.Abu Bakr El-Bediwi put the title of the paper (under a big research plan), and contributed with other authors in interpreting the results and writing the paper.

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