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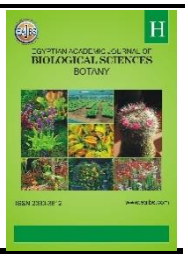

Impact of Ultraviolet C on Silymarin Content and Structure of Milk Thistle Medical Seeds

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Milk thistle, UVC, morphological structure, silymarin, yield.

ABSTRACT

Background: The use of ultraviolet C radiation is a low-cost technique that is easy to apply to plants, and is widely used for disinfection in many industries and food processing. The milk thistle seeds have medicinal properties and antioxidant and anti-inflammatory effects. The aim of this work is to study the effect of ultraviolet C on silymarin content, germination growth and internal structure of milk thistle medical seeds. **Methods:** The seeds of milk thistle were received from the Egyptian Ministry of Agriculture, and used as research samples. The ultraviolet irradiation system consists of one fluorescent lamp (type- C) which is covered totally with aluminum foil to illuminate the sample from all sides. The structure of the used samples was studied by x-ray and IR analysis. **Results:** The concentration and yield values of silymarin in various extracts for milk-thistle seeds increased after being exposed to UVC at different times at dissimilar distances. The weight and germination height values of irradiated milk thistle seeds by UVC are much less than untreated seeds. From x-ray and IR analysis, it is obvious that the arrangements and position of milk-thistle molecules changed after being exposed to UVC for different times and distances.

INTRODUCTION

Ultraviolet (UV) light is the range of electromagnetic radiation that is more energetic than the visible range, UV wavelength lies from 100 to 400 nm, including vacuum ultraviolet, ultraviolet C, ultraviolet B, and ultraviolet A. UVC light (2000 Å–2800 Å) is useful for disinfection and inactivation of microorganisms, is a function of the radiation dose, which is determined by the intensity of radiation and time. The milk thistle seeds contain silymarin, a group of compounds said to have antioxidant and anti-inflammatory effects and are used as a home remedy to treat liver problems. UVC light is absorbed by both nucleic acids and proteins and has been found useful for air disinfection, water and wastewater treatment, laboratory disinfection, especially inside biosecurity cabinets, food and beverage preservation, and medical applications such as wound care (Cutler & Zimmerman, 2011; Gupta *et al.*, 2013). There are various environmentally friendly methods used for disinfection processes, where UV-C radiation is considered one of the emerging trends in food processing (Otto *et al.*, 2011; Shah *et al.*, 2016; Zhao *et al.*, 2019; Jermann *et al.*, 2015), where considered an environmentally friendly non-thermal technology for decontamination (Zhang *et al.*, 2011), and it is necessary to know related to its effects on secondary metabolites. More research was done on the effect of UVC on growth, internal structure and antioxidant activity for

different seeds such as *Foeniculum vulgare*, *yellow mustard*, Ammimajus, *Nigella Sativa* and *garden cress* is studied and the results showed there is a change occurred in gross, structure and antioxidant after exposed by UVC (Ebrahim *et al.*, 2022; El-Bediwi *et al.*,, 2021; El-Bediwi *et al.*,, 2020; El-Bediwi *et al.*,, 2018; El-Bediwi *et al.*,, 2018). This work aimed to study the influence of UV- C on silymarin content, morphological structure and antioxidants of milk thistle seeds.

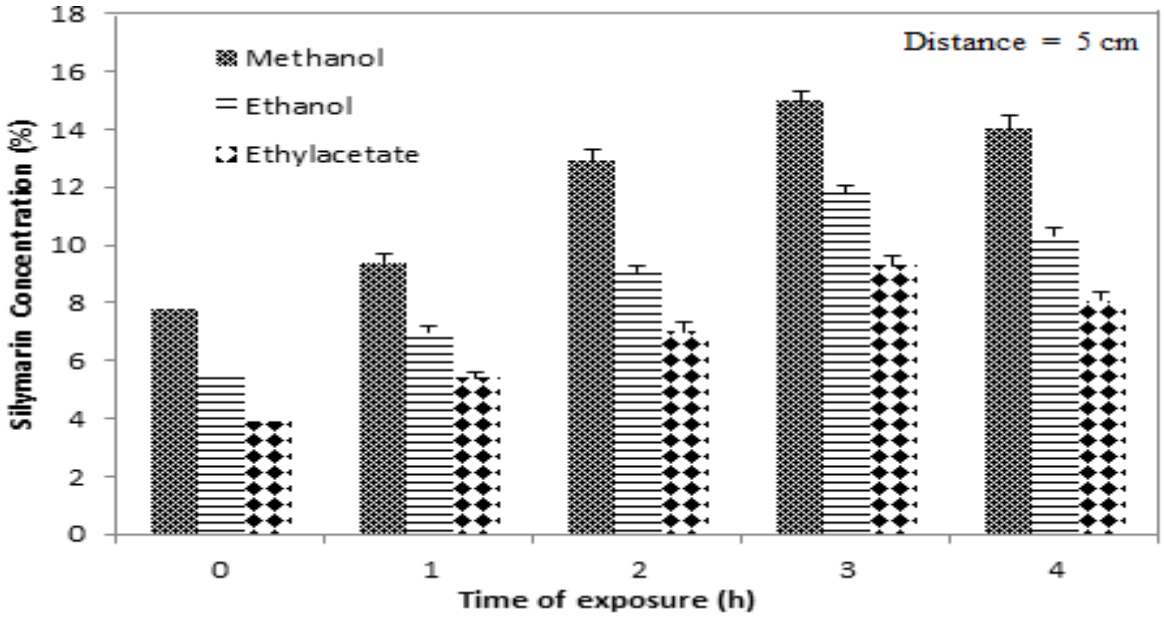
MATERIALS AND METHODS

The seeds of milk thistle were received from the Egyptian Ministry of Agriculture and used as research samples. The ultraviolet irradiation system consists of one fluorescent lamp (type- C) which is covered totally with aluminum foil to illuminate the sample from all sides. The structure of the used samples was studied by Shimadzu X-ray diffractometer, (Dx-30, Japan) while the molecular structure was studied by Nicolet™ iS™ 10 FT-IR Spectrometer from USA.

RESULTS AND DISCUSSION

1-**Silymarin Content:**

Silymarin concentration was tested in the three extracts such as methanol, ethanol and ethylacetate. The results in Figure 1 indicated that the methanol extract was the best among the three extracts and the concentration increased gradually from the first hour to the 4th hour exposed to UVC at 5 cm. After the 4th hour, the concentration was 14 mgg^{-1}. Ethanol was the second solvent (10.3 mgg $^{-1}$) and ethylacetate was the third 8.1 mgg^{-1}. After exposure to UVC at 15 cm for 1, 2, 3 and 4 hours, the concentration of silymarin was highest in methanol extract followed by ethanol and ethylacetate extracts. In methanol after the 4th hour, it was 20.7 mgg^{-1} followed by ethanol (16 mgg^{-1}) and ethylacetate (12.8 mgg^{-1}). After exposure to UVC at 20 cm for 1, 2, 3 and 4 hours, silymarin concentration was highest (16-9%) in methanol compared to ethanol (13%) and ethylacetate (10.3%) respectively.



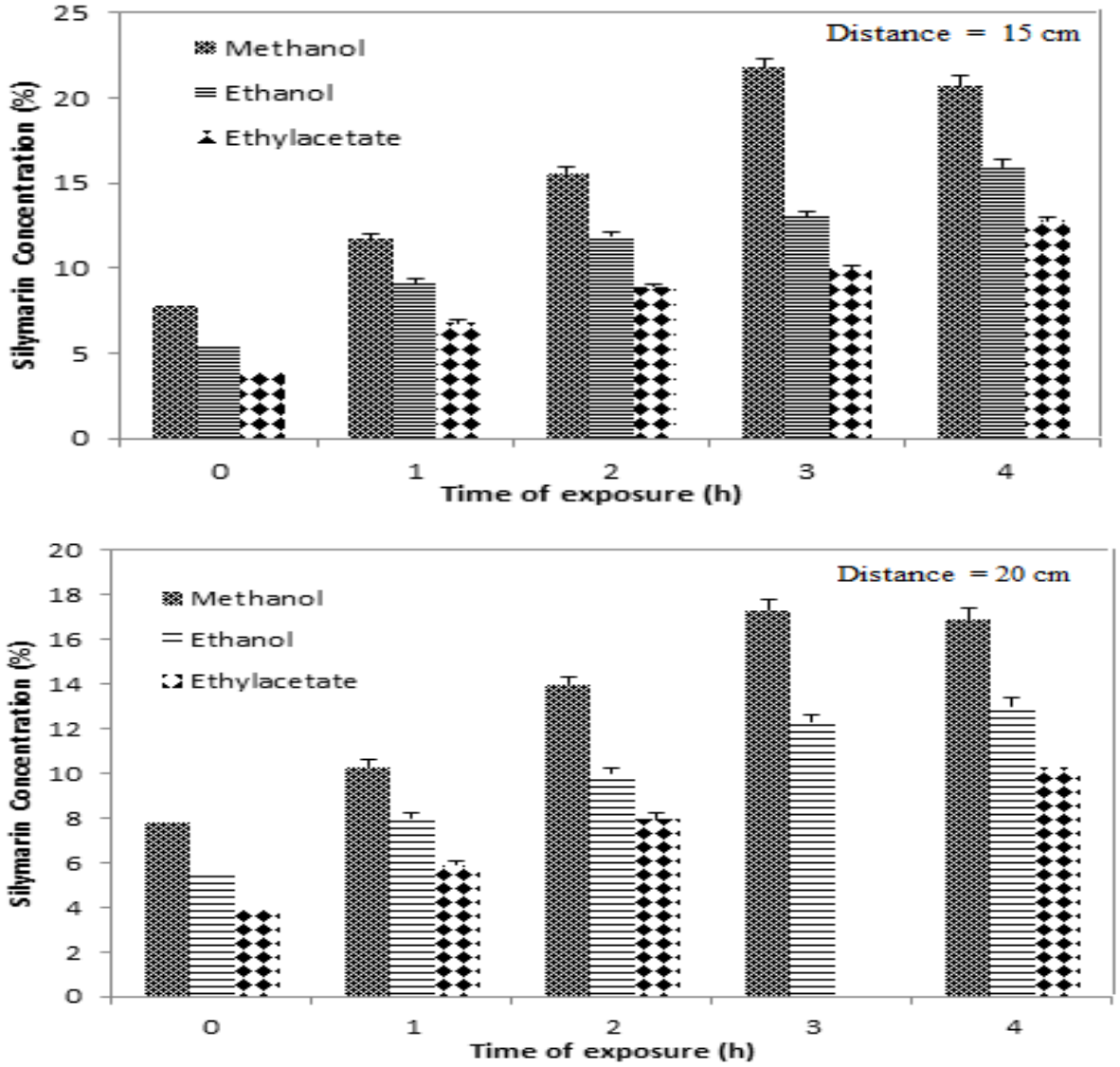


Fig. 1: concentration of silymarin in milk- thistle seeds in various extracts after exposure to UVC.

Silymarin was prepared in three different solvents namely methanol, ethanol and ethylacetate. The results in Figure 2 revealed that methanol was the best solvent for silymarin extraction. It was observed that the yield of silymarin increased gradually from the time of exposure to UVC throughout from hours and it reached 6.8% with methanol. However, it was 5.9% and 3.8% with ethanol and ethylacetate respectively. It was observed that the yield from 10 cm was higher than from 5 cm and continuous increase in the yield from one hour to the 4th hour. Also, ethanol was the best solvent compared to methanol and ethylacetate. The yield of silymarin was determined after exposure to UVC at 15 cm in methanol, ethanol and ethylacetate for 1, 2, 3 and 4th hours. Methanol was the best solvent compared to ethanol and ethylacetate. In ethanol extract, the yield of silymarin increased gradually and reached 11% after 4th hour. However, methanol, ethanol and ethylacetate were 10% and 8.5% respectively.

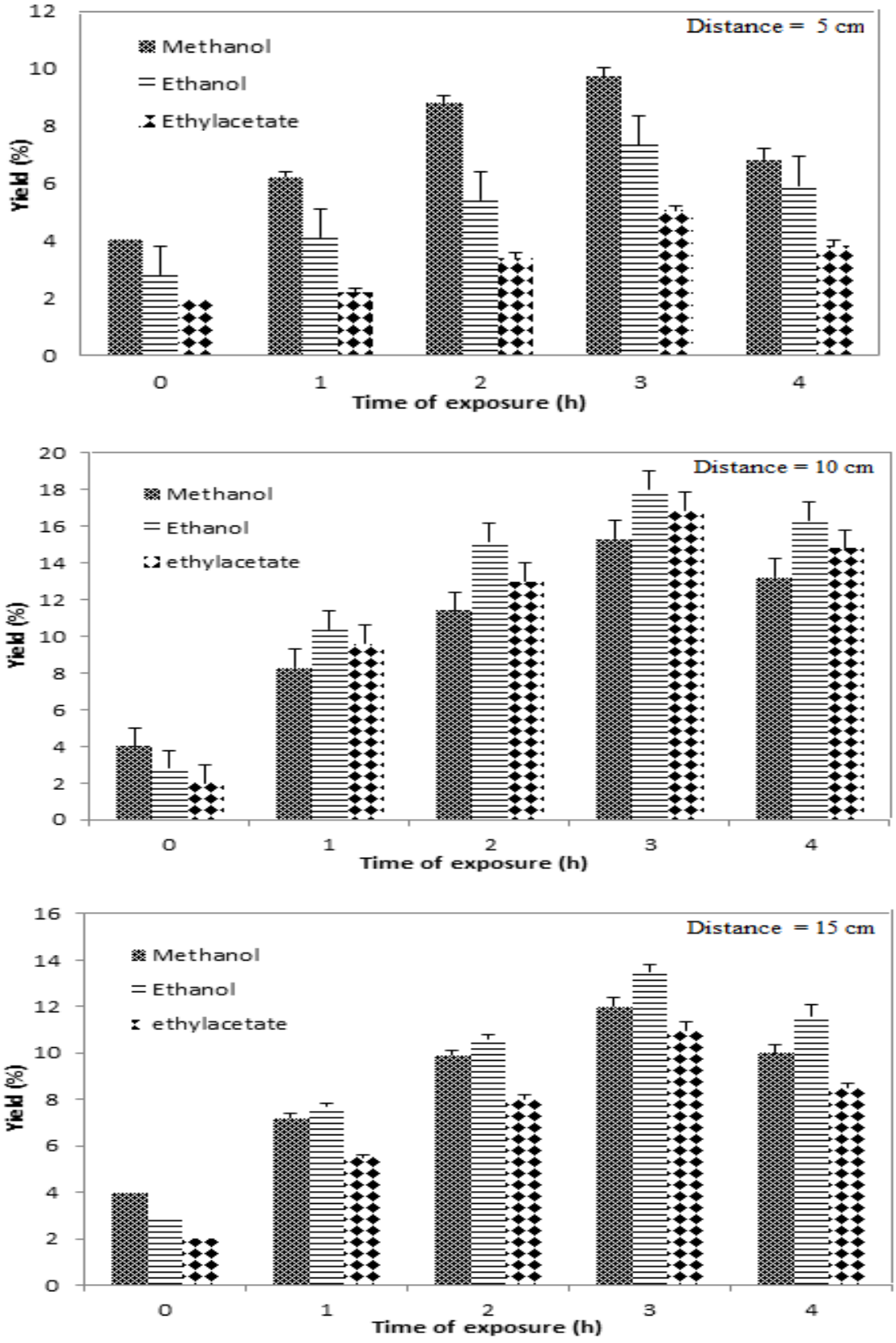


Fig. 2: yield of silymarin in milk-thistle seeds in various extracts after exposure to UVC

3.2. Morphological structure

The weight loss in milk thistle seeds after exposure to UVC for different times at dissimilar distances was listed in Table 1. Weight loss from seeds powder increased by increasing exposure time but it decreased with increasing the distance from the UV source. That may be due to changes in the geometric shape of seeds or bonds.

The germination height value of milk thistle seeds was decreased after exposure by UVC at 5, 15 and 20 cm from UV source for 1, 2, 3 and 4 hours as listed in Table 2, where UVC due to a change in bio-components for growth or generation cells.

Table 1: weight loss from milk thistle after being exposed to UVC

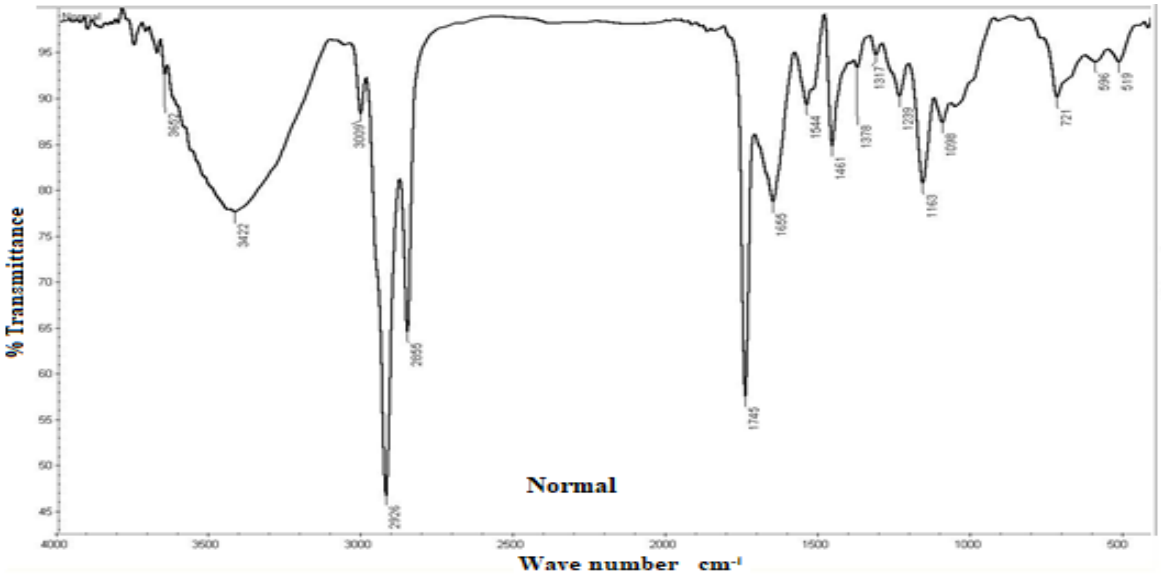
Exposure time/ h	Weight difference/ gm.		
	5 cm	15 cm	20 cm
	Δg	Δg	Δg
1	0.19	0.105	0.095
2	0.36	0.19	0.18
3	0.41	0.315	0.25
4	0.52	0.37	0.225

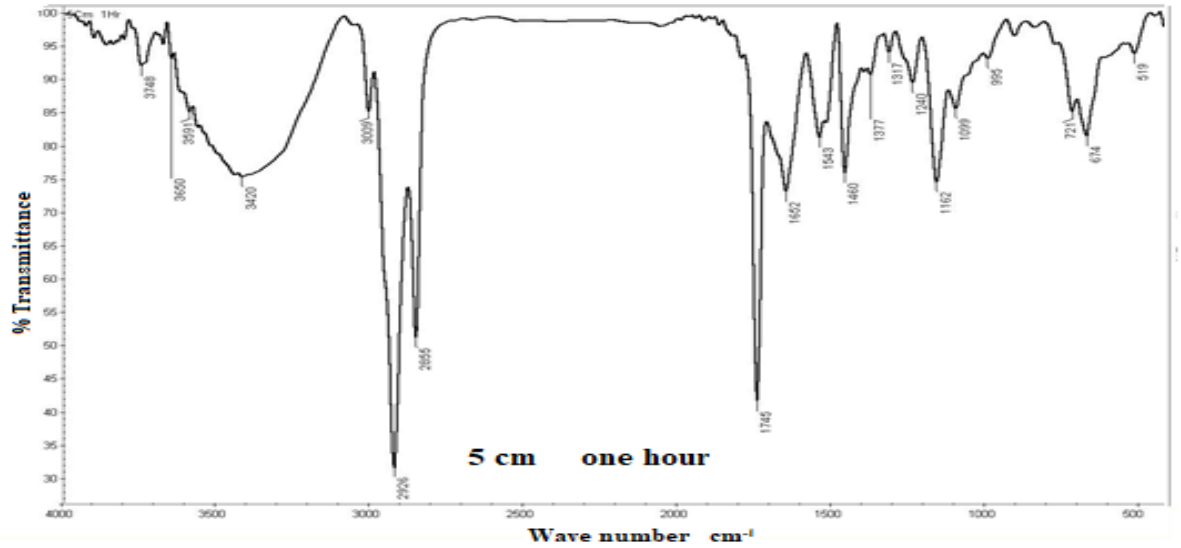
Table 2: germination height from milk thistle after exposure to UVC

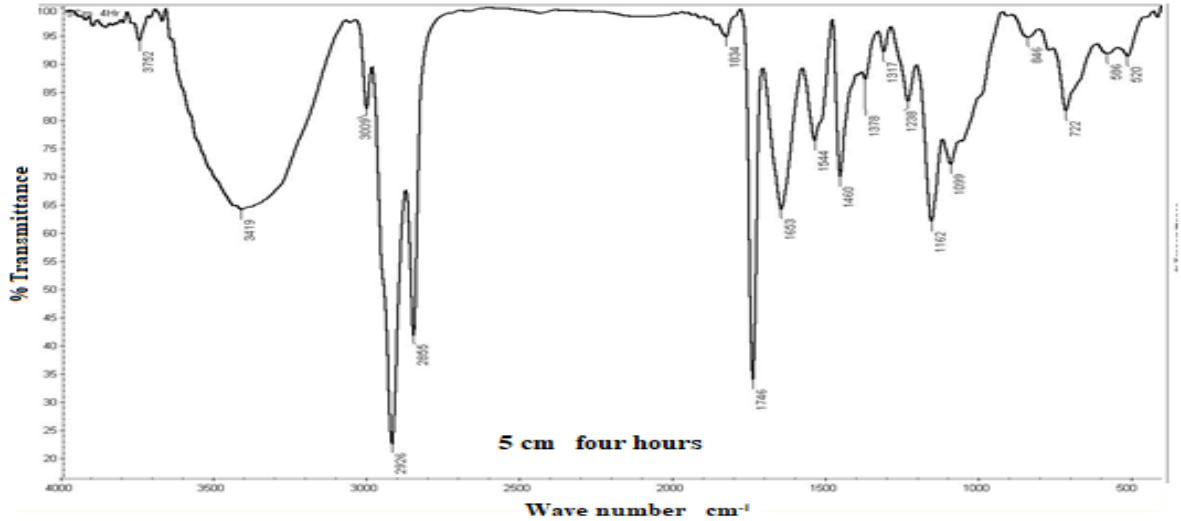
Exposure time/ h	Germination height / cm		
	5 cm	15 cm	20 cm
Untreated	H = 8.03		
1	3.9	3.67	4.13
2	4.32	4.72	4
3	4.18	4.53	4.75
4	4.23	4.48	4.18

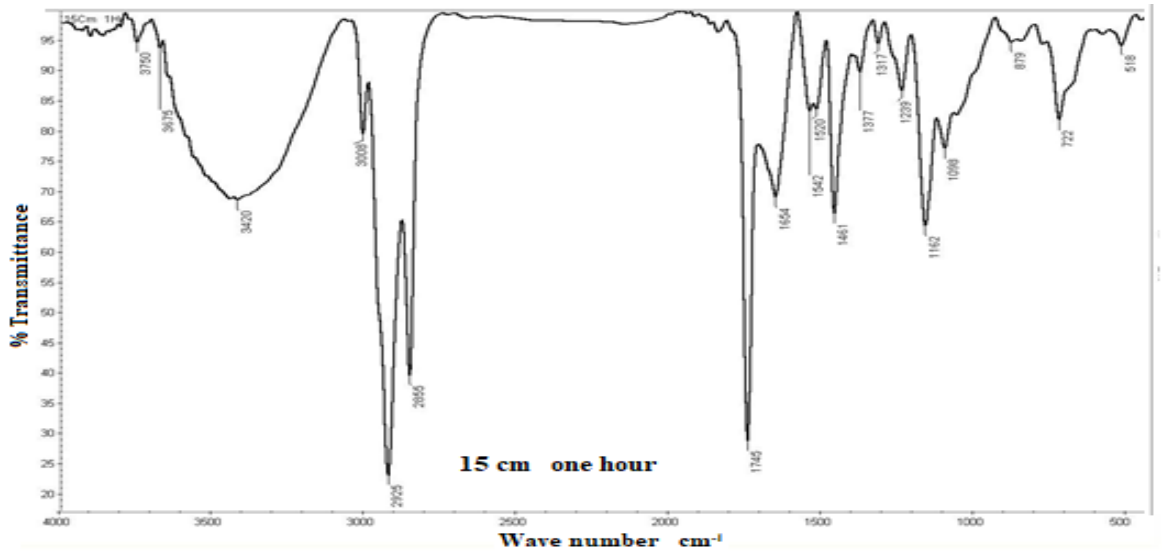
IR Analysis:

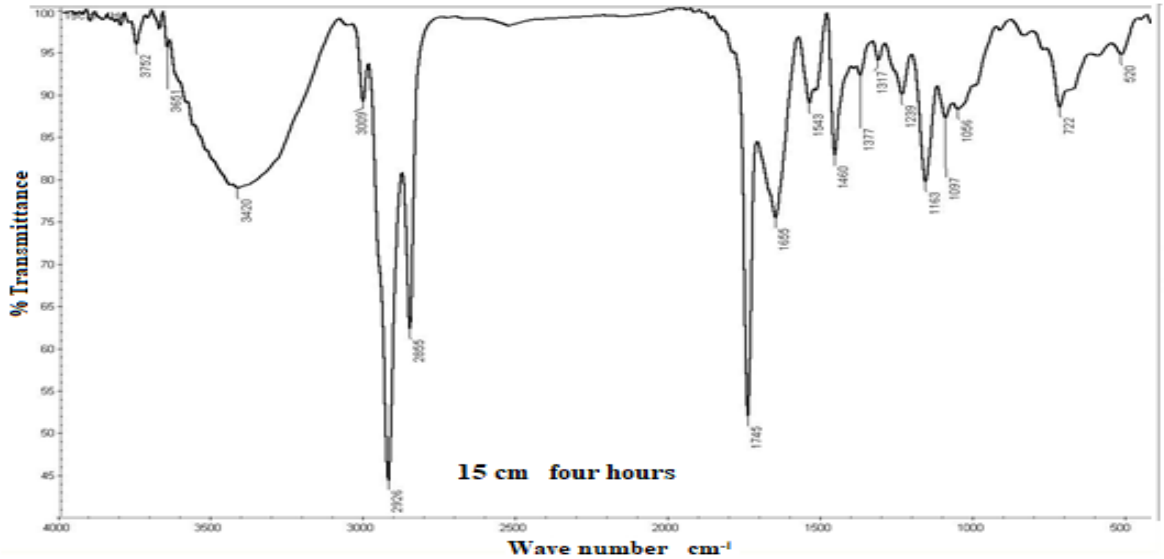
The relation between wavenumber (X-axis) and % transmittance (Y- axis) of normal milk-thistle seeds and after exposure to ultraviolet C for one, two, three and four hours at dissimilar distances are shown in Figure 3. Infrared (IR) spectrum analysis of milk-thistle seeds shows that there is a change in {C- O bond at (1745 cm^{-1}), C-H bond at (2925 cm^{-1}) and O- H bond at (~3420 cm^{-1}) bonded} for milk-thistle-seeds after exposure to UVC because each interatomic bond vibrates in several different motions (stretching or bending) and individual bonds absorbed at more than one IR frequency. These effects may be due to deriving from chemical changes induced by the absorption of UV light by various biological molecules. Or UVC irradiation can induce photochemical reactions of pi systems (multiple bonds) in many organic molecules. Or The UVC photons have enough energy to destroy chemical bonds causing a photochemical reaction and the biological effect is due to these processes.

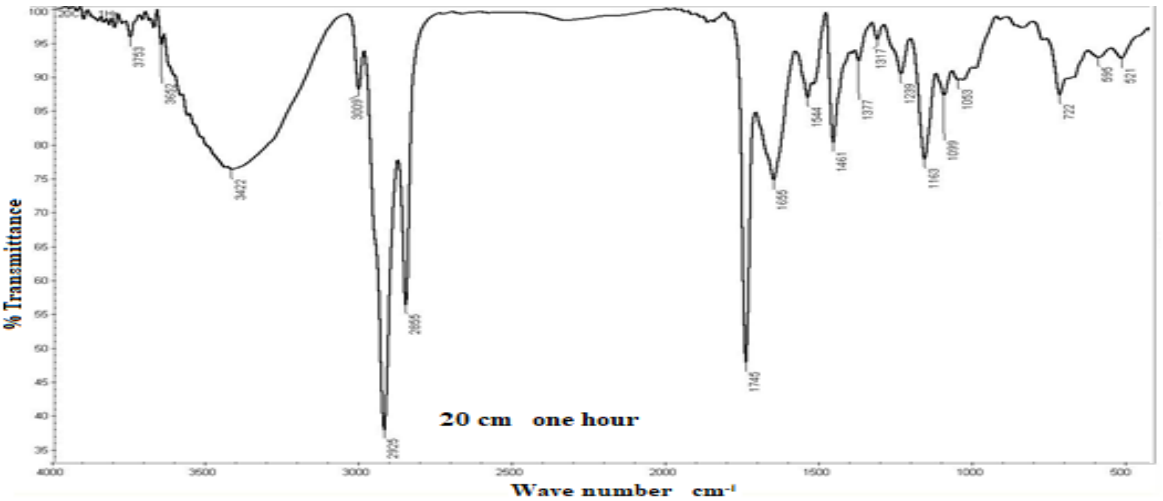

% Transmittance
Normal
Wave number cm-1


% Transmittance
5 cm one hour
Wave number cm-1


% Transmittance
5 cm four hours
Wave number cm-1


% Transmittance
Wave number cm-1
15 cm one hour
3760
3675
3420
3009
2925
2855
1745
1654
1542
1520
1461
1377
1317
1239
1162
1098
879
722
518


% Transmittance
Wave number cm-1
15 cm four hours
3762
3651
3420
3009
2926
2855
1745
1655
1543
1460
1377
1317
1239
1163
1097
1056
722
520


% Transmittance
Wave number cm-1
20 cm one hour
3755
3650
3422
3009
2925
2855
1745
1655
1544
1461
1377
1317
1239
1163
1099
1063
722
695
521

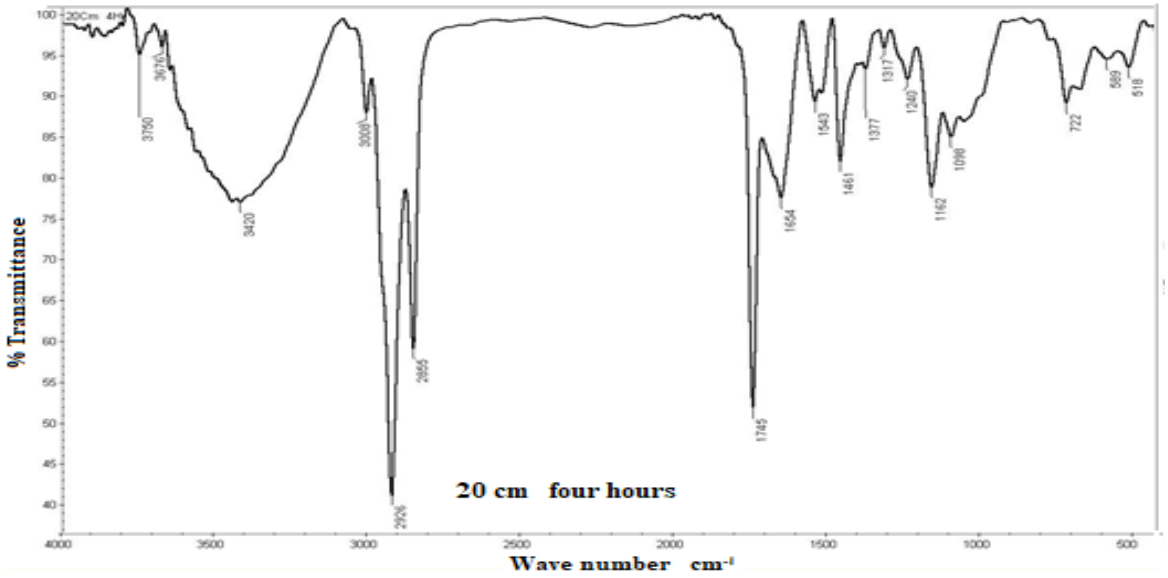
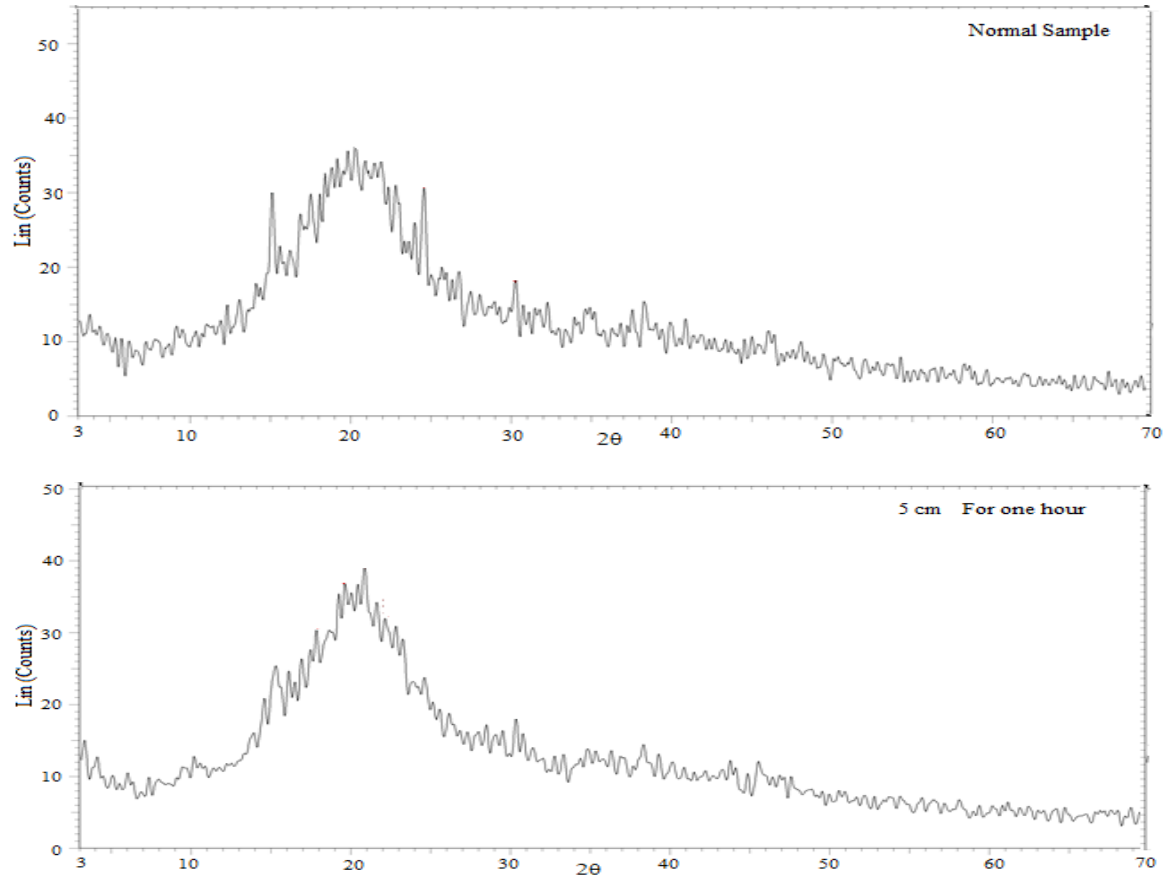


Fig. 3: IR spectrum of milk-thistle seeds after exposure to UVC

X-ray analysis

Figure 4 shows x-ray diffraction patterns of milk-thistle seeds after being exposed to UVC for 1 and 4 hours at 5 cm and 20 cm distances from the source, where there is a change in the peak (intensity, broadness, started baseline and area under the peak) after exposed by UVC. That may be due to the colliding of UVC rays with matrix molecules causing breaking or modifying bonds or forming free radicals, which changed the molecular structure of it.



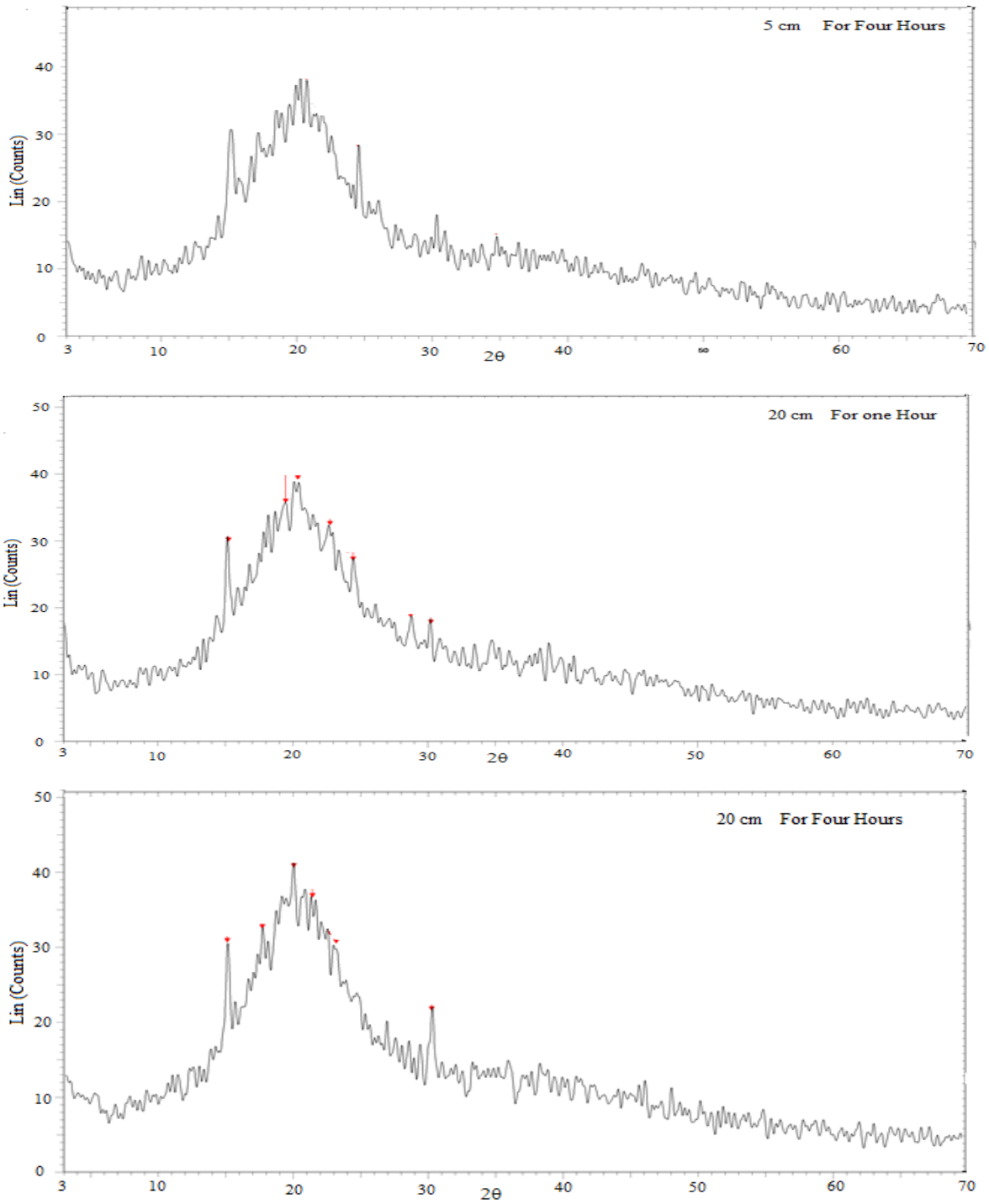


Fig. 4: x-ray diffraction patterns of milk-thistle seeds after exposure to UVC

Conclusion

The research results show that it can improve the quantity of silymarin in milk-thistle seeds using UVC, which is useful for the medical industry. Also don't expose milk-thistle seeds to UVC before cultivated.

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