Phytochemical compositions and in vitro anticancer effects of ethanolic extracts from Syrian hawthorn fruit on the human breast cell line MDA-MB-231

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Abstract

Hawthorn is an important medicinal plant among other wide spread herbal species. Only few studies have been performed to exhibit therapeutic effects of hawthorn extracts on cancer cell lines. Herein, three types of hawthorn fruits were collected from different places in Syria, samples were air dried until reaching a constant dry weight and then powdered. Hawthorn bioactive compounds were extracted from the powder with absolute ethanol. Qualitative phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, saponins and glycosides in the three types of hawthorn fruits. The red hawthorn contained anthracene derivatives, unlike both yellow and green hawthorns. FT-IR analysis revealed the presence of functional groups such as alcohols, alkanes, alkyl halides, aromatic groups and alkenes. UV—Visible analysis represents the absorption spectrum and shows a broad absorption band at 350–400 nm and a peak at approximately 300 nm. Chromatography–mass spectrometry (GC/MS) analysis showed 13, 5 and 22 chemical constituents representing an average of 100% in the EOs of yellow, green and red hawthorn respectively. Cell proliferation assessment results showed that hawthorn inhibits cell viability of MDA-MB-231 cancer cells lines, which was strongly related to increasing concentration of hawthorn.

Introduction

The traditional treatments for cancer diseases are based on surgical treatment, radiotherapy and chemotherapy or even all together. Despite the benefits of these treatments, they have side effects that negatively impact the patient's health, especially chemical and radiological treatments that are toxic to normal tissues in the body, cause genetic mutations in cells, or weaken the immune system. Chemotherapy causes harmful effects in normal cells in terms of genetic mutations, DNA methylation and histone modification, which also leads to resistance to some chemotherapy treatments. In addition, most of the drugs that are used as chemotherapy can lose their effectiveness due to the development of drug resistance factors. Botanical medicines are the main source of chemotherapy drugs, and some of the chemotherapy agents currently in use have been derived from plants, such as paclitaxel[1]. It is used to treat breast cancer, vinca alkaloids for leukemia[2], and flavopiridol for colorectal cancer[3]. The ongoing search for new anticancer compounds in plant medicines and traditional foods is both realistic and promising. However, the properties of many plants, especially their toxicity and antioxidant activity, have not yet been fully studied. Syria has a very rich flora with approximately 3100 registered plant species that belong to more than 600 genera and 130 families as well as approximately 112 gymnosperms[4–7]. The Hawthorn herb (Crataegus spp.) is one of the most important medicinal plants in syria. There are five types of hawthorn (Crataegus) in Syria: Crataegus aronia L. (Aaron'shawthorn), Crataegus azarolus L (Azarole hawthorn.), Crataegus monogyna L. (monogynous hawthorn), Crataegus sinaica (Sinai's hawthorn.) and Crataegus Sp. (cultivated hawthorn) (Fig. 1). The therapeutic properties of hawthorn are because its flowers, fruits and leaves contain a number of active ingredients, which are collectively responsible for the preventive and therapeutic effects of its preparations. Hawthorn species have been widely used in medicine and in all regions in which it grows[8–10] to lower blood pressure and cholesterol[11]

and to treat congestive heart failure[12]. It contains many effective compounds, the most important of which are phenolic compounds and flavonoids, which are antioxidants[13–16], such as epicatechin, rutin, vitexin, and chlorogenic acid. Experiments have shown[8,17–21] that hawthorn extracts, especially fruits, lead to a significant reduction in the negative effects caused by radiotherapy, especially in lymphocytes. Abd El-Azime [22] showed that eating hawthorn fruit extract before and after radiation exposure leads to a significant reduction in the negative effects caused by radiation, especially in the liver. A study by Mustapha and associates[23] using the ethyl acetate extract of hawthorn (crataegusazarolus) showed remarkable results in the apoptosis of colorectal cancer cell lines HT-29 and HCT-116. The study conducted by M T Sáenz and associates in 1997 using the combination of hawthorn monospices and mistletoe on HEp-2 laryngeal cancer cells showed that all samples showed significant cytotoxic activity against cultured HEp-2 cells [24 –26].

Based on these facts and due to the scarcity of studies that dealt with the importance of the toxicity of hawthorn extracts on cancerous cell lines, this study aims to determine preliminary chemical detection of the active constituents of three types of hawthorn fruit, which are wild species distributed in mountainous and coastal regions, and study the cytotoxic effect of these extracts on the breast cancer cell line MDA-MB-231.

Results and Discussions

Fourier transform infrared (FT-IR) spectroscopy

The IR spectra (Fig. 2) of yellow, red and green hawthorn exhibit vibrational bands in the usual region of 3000–3382 cm^{-1} for the hydroxyl function (alcohol) and N-H stretching. One absorption band, located at 2936 cm^{-1}, can be assigned to –CH2- aliphatic stretching, whereas bands at 1419 and 1636 cm-1 can be assigned to –C = C- olefinic and aromatic ring stretching. Sharp bands at 1080 and 1045 cm-1 can be assigned to C = O and (C-O-C) stretching. These vibrational bands generally express the presence of flavonoids, tannins, glycosides, saponins, alkaloids and antricines in hawthorn fruits.

UV–Vis spectrophotometer

The UV–vis spectrum of the hawthorn fruit extracts (Fig. 3) shows a broad absorption band at 350–400 nm (bond I) and a peak at approximately 300 nm (bond II) with absorptions of 0.58 and 0.02–0.25, respectively.

Energy-dispersive X-ray analysis (EDX)

samples indicated high atomic percentages of carbon 52.45–75.92%, and oxygen 22.48–46.99%, and small atomic percentages for Na, Mg, Si, P, Cl, K, S, Cu and Fe elements (see Supplementary Information). This results confirms the presence of these elements in the compounds that we obtained in the Gc-MS results.

Determination of total phenolic content

The concentration of total phenolic compounds in the tested extracts was determined spectrophotometrically (the method is based on the measurement of the reducing capacity of phenolic compounds), and the values were calculated based on the equation obtained from the calibration curve. The total phenolic contents of red, green and yellow hawthorn fruit extracts were 33.46 ± 0.18, 30.5 ± 0.23 and 29.2 ± 0.11 mg, respectively (Table 1).

Table 1
content of total phenolics of red, green and yellow hawthorn fruits extracts

Extract	total phenolics content
Red hawthorn	33.46 ± 0.18
Green hawthorn	30.5 ± 0.23
Yellow hawthorn	29.2 ± 0.11

DPPH assay

All tested extracts showed strong and significant antioxidant activity. Yellow fruit hawthorn extract showed stronger antioxidant activity (98.22%) than red (84.22%) and green (88.15%) extracts (Fig. 4).

Preliminary phytochemical screening

In the present study, qualitative phytochemical analysis revealed the presence of flavonoids, glycosides, saponins, tannins, alkaloids, and phenols in the three types of hawthorn. The red hawthorn also contained anthracine derivatives, unlike both the yellow and green hawthorns (Table 2).

Table 2
Preliminary phyto analysis of red, green and yellow hawthorn fruits

	constituents	Tests	hawthorn		
			Red	Green	Yellow
1	**Flavonoids**	Wilson-Tauback	-	-	+
		Shinoda test	+	-	-
		Pew	+	+	+
2	**Glycosides**	Kedde	+	+	+
		Baljet	+	+	+
		Keller-kiliani	+	+	+
3	**Saponins**	Foaming experiment	+	+	+
		Reaction with aromatic aldehyde	-	+	+
		Reaction with H2SO4 & anhydrous acetic acid	-	+	-
4	**Tannins**	Reaction with Fecl3			
		Reaction with lead acetate			
5	**Alkaloids**	Dragendrof	+	+	+
		Mayer	-	-	-
		Wagner	-	-	-
		Bicric acid	+	+	+
6	**Anthracine derivatives**	Borntrager	+	-	-
		shouteten	+	-	-
7	**Phenols**	Phenol (aglycon)	+	+	+
		sugar	+	+	+
(+) indicate Positive; (-) indicate Negative					

Gas chromatography–mass spectrometry (GC–MS) technique

A comparative study among three hawthorn species based on their EO chemical composition was performed using GC–MS analysis. Essential oil (EO) composition analysed by GC–MS analysis was presented for yellow hawthorn (Table 3), green hawthorn (Table 4) and red hawthorn (Table 5) grown in Syria. The data revealed that 13, 5 and 22 chemical constituents representing an average of 100% were

identified in the EOs of yellow, green and red hawthorn, respectively. These compounds were identified by comparing the fragmentation patterns of the resulting mass spectra with those published in the literature and using the National Institute of Standards and Technology Mass Spectral Database of a gas chromatograph-mass spectrometer (Agilent Technologies Co., Ltd.). However, alkanes, fatty acids, esters, alcohols, amines, glucose, ether, ketones and aldehydes are important components in hawthorn. The data were partially identical to those in previous studies by Chen et al. (1997) and Xie et al. (1997), who reported that 32 volatile components from hawthorn contained alcohols, aldehydes, esters, alkanes and ketones. Moreover, the major constituents recorded varied according to each studied hawthorn. In this regard, for *green hawthorn*, they were n-hexadecanoic acid (83.2%), perfluorotributylamine (20.13%) and pyrimidin-2-one, 4-[N methylureido]-1-[4-methylaminocarbonyloxymethyl (15.7%). Sorbitol (85.50%), β-D-glucopyranose, and 4-O-β-D-galactopyranosyl (2.18%) were present in red hawthorn. For yellow hawthorn, they were heptacosane (27.5%), octacosane (21.7%) and oleic acid (5.9%).

Table 3
GC/MS spectrum of Yellow hawthorn .

Peak number	Retention time	Area %	P, %	Compound name
1	18,26	2,0	11.72	Tetradecane
2	19,83	5,9	19.61	Oleic Acid
3	20,98	2,5	14.16	Nonadecane
4	21,78	1,8	10.20	trans-(2-Chlorovinyl)dimethylethoxysilane
5	22,95	3,3	16.05	1,2-Benzenedicarboxylic acid, butyl octyl ester
6	23,47	2,5	12.93	Nonadecane
7	24,62	3,3	12.06	Pyrimidin-2-one, 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl Dodecane, 5,8-diethyl-
8	25,72	2,9	13.32	Heptadecane
9	26,78	8,8	14.21	Heptacosane
10	27,79	8,0	58.52	Pyrimidin-2-one, 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl
11	28,80	**21,7**	10.28	Octacosane
12	29,94	9,7	44.10	Docosane, 1,22-dibromo-
13	31,26	**27,5**	10.85	Heptacosane

Table 4
GC/MS spectrum of Green hawthorn.

Peak number	Retention time	Area %	P, %	Compound name
1	8,64	5,6	84.65	Cyclotetrasiloxane, octamethyl-
2	11,01	4,5	81.45	Cyclopentasiloxane, decamethyl-
3	13,57	2,4	10.36	Phenol, 2-ethyl-4,5-dimethyl-
4	21,77	4,2	19.89	Diamyl phthalate
5	22,96	3.5	16.00	Dibutyl phthalate
6	23,02	83,2	41.74	n-Hexadecanoic acid
7	31,23	15.70		Pyrimidin-2-one, 4-[N methylureido]-1-[4-methylaminocarbonyloxymethyl
8	28,78	16.12	53.06	Perfluorotributylamine
9	34,76	20.13	64.57	Perfluorotributylamine

Table 5
GC/MS spectrum of Red hawthorn.

Peak number	Retention time	Area %	P, %	Compound name
1	5.690	0.277	15.74	Tetraacetyl-d-xylonic nitrile
2	6,540	1.396	52.67	3-Ethoxy-1,2-propanediol
3	6.730	0.819	54.27	1,2-Butanediol
4	8.450	0.635	31.53	Dihydroxyacetone
5	9.250	0.122	12.22	dl-α-Methylglutamic acid
6	11.120	0.820	12.37	Methanol, TMS derivative
7	12.220	0.384	21.93	3,4Dehydro-dl-proline
8	13.050	0.135	21.91	5-Aminosalicylic acid, N,O,O'-tris(trimethylsilyl)-
9	13.287	0.225	27.99	5-Aminosalicylic acid, N,O,O'-tris(trimethylsilyl)-
10	13.701	1.515	10.51	N-[3,5-Dinitropyridin-2 yl]proline
11	15.870	1.219	17.66	[(trimethylsilyl)amino]-5H-chromeno[2,3-b]
12	17.860	0.793	10.64	2-Formyl-9-[β-d-ribofuranosyl]hypoxanthine
13	18.140	1.875	10.04	Carbamazepine-10,11-dihydrodiol,2TMS derivative
14	20.120	0.627	21.10	Spiro[isoquinoline-1,2'-indene],1,2,3,4,2',3'-tetrahydro-6'-hydroxy-6,7,3',7'-tetramethoxy-2
15	20.330	2.294	11.08	β-D-Glucopyranose, 4-O-β-Dgalactopyranosyl-
16	21.837	0.108	10.11	Trimethylsilyl 7-(1-methylethyl)-5-oxo-2-[(trimethylsilyl)amino]-5H-chromeno[2,3-b]
17	23.195	**85.50**	11.95	Sorbitol
18	25.920	0.140	19.52	Amodiaquine, 2TMS derivative
19	27.040	0.120	13.51	Amodiaquine, 2TMS derivative
20	28.100	0.130	11.60	Amodiaquine, 2TMS derivative
21	28.330	0.176	10.45	2-Oxo-4,6-diphenyl-3-(4-tolyl)-1,2,3,4-tetrahydropyrimidine
22	30.590	0.694	14.41	Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)-

Microscopic tests:

The results of the microscopic study indicated that all types of hawthorn contain sclerotic cells and an outer epithelium, endosperm, and whorls. The green hawthorn contained calcium acids, pollen grains, sclerotic fibres, sclerotic cells and tentacles, while the red hawthorn contained parenchyma tissue, elastic tissue, and intestinal cells (Figs. 5, 6, 7).

Cell proliferation assessment

As shown in Figs. 8 and 9, hawthorn inhibits the viability of MDA-MB-231 cancer cells, and this decrease in cell viability percentage is strongly related to increasing the concentration of hawthorn. yellow hawthorn had a greater effect on MDA-MB-231 cells than red and green hawthorn under the same treatment conditions. The IC_{50} values for MDA-MB-231 cells were 3.85, 4.21 and 4.67 mg/mL for yellow, green and red hawthorn, respectively, after 48 h of treatment (Fig. 10). The results showed that upon treatment with hawthorn for 48 h, a significant decrease in cell viability was observed from 6.37 to 7.37 µg/ml in MDA-MB-231 cells ($P < 0.05$). It is worth noting that treating MDA-MB-231 cells with yellow, green and red hawthorn at concentrations ranging from 3.37–8.37 µg/ml decreased cell viability by nearly 70%. Thus, the results of the XTT assay clearly demonstrated that hawthorn exerts cytotoxic effects on MDA-MB-231 cancer cells in a dose-dependent manner.

Statistical analysis

One-way ANOVA showed that the difference between control and treated cells using red, yellow and green hawthorn was statistically significant, with a p value < 0.0001.

METHODS

Chemicals and reagents

DMEM (Dulbecco's modified Eagle's medium), fetal calf serum, L-glutamine, and penicillin/streptomycin were obtained from EuroLoan. Sodium 3´-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate [XTT], dimethyl sulfoxide (DMSO), and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All the other chemicals were of the highest grade available. The MDA-MB-231 breast cancer cell line was obtained from the Cell Bank of the Department of Biology, Atomic Energy Commission of Syria. The Scanning electron microscopy SEM and Energy dispersive X-ray (EDX) were obtained using OnTescan Vega II XMU SEM.

Plant materials and bioactive compound extraction.

Plant materials

In this study, three types of Syrian hawthorn fruits were collected in autumn 2022 from different places of Syria in compliance with appropriate International Union for Conservation of Nature IUCN (1989). Samples were identified by Prof M. Alodat, from the Atomic energy commission of Syria (ref. 2009810).

Green hawthorn (*Crataegus aronia L*) and red hawthorn (*Crataegus monogyna L*) were collected from AL Tawaheen in Tartous City, and yellow hawthorn (*Crataegus Sp*) were collected from Sarabuon in Latakia City. Samples were washed with clean tap water and air dried for nearly 60 days in the dark until reaching a constant dry weight and then powdered by a special electric mill, and the powder was stored at 4°C until use.

Extraction

Hawthorn bioactive compounds were extracted from the powder with absolute ethanol at a 1:5 (w/v) ratio. After incubating for 2 hours in the dark at room temperature, the samples were centrifuged at 3500 rpm for 30 minutes. The ethanol extract was collected and concentrated by a vacuum rotary evaporator (Buchi, SwitZerland) at 40°C. The residue was weighed and dissolved in 10% dimethyl sulfoxide (DMSO) in water at a 20 mg/ml concentration before the experiment and then filtered through a 0.22 µm syringe filter.

Essential oil (EO) extraction

Essential oils (EOs) were extracted from the fine powder (100 × g for each sample) by the hydrodistillation method using 400 ml of distilled water. The process was obtained by a Clevenger-type apparatus for 4 hours, and the produced oil was saved in sealed glass vials at 4–5°C.

Fourier transform infrared (FT-IR) spectroscopic analysis

Infrared spectra were recorded in the range 400–4000 cm^{-1} using an FTIR-JASCO 300E. Ten milligrams of the dried extract powder was encapsulated in 100 mg of KBr pellet to prepare translucent sample discs (Fig. 2).

UV–Visible (UV–Vis) spectroscopic analysis

The aqueous plant extract was examined by UV–visible spectral analysis. The sample was diluted to 1:10 with methanol. The extract was scanned at wavelengths ranging from 200–900 nm using a Thermo Scientific Multiskan GO spectrophotometer, and the characteristic peaks were detected (Fig. 3).

Energy-dispersive X-ray analysis (EDX)

The **EDX** analysis were employed to determine the elemental composition of hawthorn and subsequently estimated the weight and atomic ratios of carbon, oxygen, nitrogen and any other element present in the samples based on the EDX data (Fig. …..).

Determination of total phenolic content

The total phenolic content of the tested hawthorn fruit extracts was determined spectrophotometrically with Folin Ciocalteu reagent according to a previously described method [25], with slight modifications. The dry extracts were dissolved in methanol (1000 µg/ml) and used during the analysis. Briefly, 250 µL of Folin-Ciocalteu reagent was added to a test tube with 50 µL of extract and then shaken. After 2 minutes,

1.25 mL of 7.5% Na_2CO_3 solution and 450 µL of water for injection were added. The test tubes were slightly shaken and then incubated at room temperature for 90 minutes in the dark. The same procedure was repeated for gallic acid, which was used to construct the calibration curve, and it was constructed based on different concentrations of gallic acid solution (10, 15, 20, 25 and 30 µg/ml). The absorbance was measured at 760 nm relative to the blank (250 µL of Folin-Ciocalteu reagent, 1.25 mL of 7.5% Na2CO3 and 450 µL of water for injection were added to 0.5 ml of methanol) on an LLG UniSPEC 2 spectrophotometer. The total phenolic content of the samples was calculated from the calibration curve equation, and the results are presented as mg of gallic acid equivalents per g of dried weight extracts (mg GAE/g dw) (Table 1).

DPPH assay

The antioxidant activity of the extracts obtained from hawthorn fruits was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, according to a previously described method [26], with small modifications. The procedure was as follows: 0.8 ml of ethanolic solution of extracts (0.93–960 µg/ml) was placed in a test tube and mixed with 0.5 ml of (0.1 mM) DPPH methanolic solution. The test tubes were allowed to stand for 30 minutes in the dark and at room temperature. The blank contained 0.5 ml of methanol instead of extracts. The same procedure was repeated for the standard solution of ascorbic acid (0.937-480 µg/ml), which in our case was used as a measure of comparison of efficacy with the tested extracts (positive control). The absorbance was measured at 517 nm on a spectrophotometer. The inhibition of DPPH radicals in the presence of tested samples was calculated by the formula and expressed as a percentage of inhibition (%) (Fig. 4).

I% = 100 ×(A blank- A sample/A blank)

Preliminary phytochemical screening

Preliminary analysis of extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols [27]. The results are summarised in Table 2 after conducting the following chemical tests: **Tests for Flavonoids** (Wilson-Tauback- Shinoda test- Pew interaction), **Glycosides (**Kedde-Baljet- Keller-kiliani), **Saponins (**Foaming experiment- Reaction with aromatic aldehyde- Reaction with H2SO4 & anhydrous acetic acid), **Tannins (**Reaction with Fecl3 **-** Reaction with lead acetate), **Alkaloids (**Dragendrof **–** Mayer**-** Wagner- Bicric acid**), Anthracine derivatives (**Borntrager**-** shouteten**)** and **Phenols** (Phenol (aglycon)-sugar).

Gas chromatography–mass spectrometry (GC–MS) technique

The chemical composition of the extracts was identified by GC–MS analysis using a GC-Agilent 7890A (indicator: inert-MS) apparatus equipped with an HP-5MS capillary column DB-35 fused silica with dimensions of 30 m×0.25 mm (id×0.25 µm). Analytical conditions were as follows: injector and transfer line temperatures 250°C for each; oven temperature programmed from 50 to 250°C at 5°C/min; carrier

gas helium at 1 mL/min; and triplicate injections of 0.2 mL (10% hexane solution). Identification of each chemical compound was carried out by comparing its retention index with the spectral library of Wiley 99, NIST69 and NIST10.

Microscopic tests:

Samples were treated with drops of distilled water, placed on a microscope slide and examined. Using a 10x weak light microscope lens to ensure the clarity of the sample, the best 40X magnification area was selected to identify the components and compounds incorporated within the cells and to identify the shape of the ovaries and successive tissues. The following anatomical features were observed (Figs. 5, 6, 7).

Cell cultures

The MDA-MB-231 breast cancer cell line was used as the model chosen to test the combined anticancer effects of hawthorn. MDA-MB-231 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 lg/mL) in a humidified incubator with an atmosphere containing 5% CO2 at 37°C. All experiments were performed independently in triplicate per experimental point.

Cell proliferation assessment

We investigated the anticancer effect of hawthorn on human cancer cells using the XTT assay in a dose-dependent manner at different time points. MDA-MB-231 cancer cells were seeded in 96-well plates. After 4 h (the cells were grown to 70% confluence), the cells were treated with serial concentrations of hawthorn (3.37, 4.37, 5.37, 6.73, 7.37, 8.37, and 9.37 µg/mL) for 48 h. After the exposure time, 100 µL of XTT solution (0.3 mg/mL) was added, and the plate was further incubated for 4 h. The absorbance was measured at 450 and 640 nm using a Thermo Scientific Multiskan GO spectrophotometer. Cell viability was expressed as a percentage of absorbance values in the treated group to that in the control group, which was considered 100%.

Conclusion

In this study, three types of Syrian hawthorn fruits were collected in autumn from different places in Syria. Samples were washed with clean tap water and air dried until reaching a constant dry weight and then powdered by a special electric mill. Qualitative phytochemical analysis revealed the presence of flavonoids, glycosides, saponins, tannins, alkaloids, and phenols in the three types of hawthorn. The red hawthorn also contained anthracine derivatives, unlike both the yellow and green hawthorns. The concentration of total phenolic compounds in the tested extracts was determined spectrophotometrically, and the results were 33.46 ± 0.18, 30.5 ± 0.23 and 29.2 ± 0.11 mg for red, green and yellow hawthorn fruit, respectively. The DPPH assay showed strong and significant antioxidant activity for all tested extracts. The IR spectra of yellow, red and green hawthorn exhibit vibration bands in the usual regions, and these vibrational bands generally express the presence of flavonoids, tannins, glycosides, saponins, alkaloids

and antricines in hawthorn fruits. Using GC–MS analysis, 13, 5 and 22 chemical constituents representing an average of 100% were identified in the EOs of yellow, green and red hawthorn, respectively. The results of the microscopic study indicated the presence of sclerotic cells, an outer epithelium, endosperm, and whorls in the three types of hawthorn. The green hawthorn contained calcium acids, pollen grains, sclerotic fibres, sclerotic cells and tentacles, while the red hawthorn contained parenchyma tissue, elastic tissue, and intestinal cells. Cell proliferation assessment showed that hawthorn inhibits cell viability of MDA-MB-231 cancer cells lines, and this decrease in cell viability percentage is strongly related to increasing the concentration of the hawthorn. Red hawthorn had a greater effect on MDA-MB-231 cells. The IC_{50} values for MDA-MB-231 cells were 3.85, 4.21 and 4.67 mg/mL for yellow, green and red hawthorn, respectively, after 48 h of treatment.

Abbreviations

GC–MS Gas chromatography–mass spectrometry.

FT-IR Fourier transform infrared.

UV–Vis Ultraviolet–visible spectroscopy.

DMEM Dulbecco's modified Eagle's medium.

DMSO Dimethyl sulfoxide.

EDTA Ethylenediaminetetraacetic acid.

DPPH 2,2-diphenyl-1-picrylhydrazyl.

IC_{50} Half maximal inhibitory concentration.

XTT *Cell Proliferation Kit II* (Sodium 3´-[1-(phenylaminocarbonyl)- 3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate).

Declarations

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Data availability

Correspondence and requests for materials should be addressed to T.A.

Author contributions

All authors have contributed to the work and reviewed the manuscript. Specifically, TA contributed to the study design, performed the laboratory experiments, analysis, and interpretation of data and wrote the manuscript. M Al M, MD and MY were involved in the project administration. AY, AN, RM, RZ, LAb and AM performed the laboratory experiments. LAs participated in interpretation of some data, writing and review.

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Competing interests

The authors declare no competing interests.

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Figures

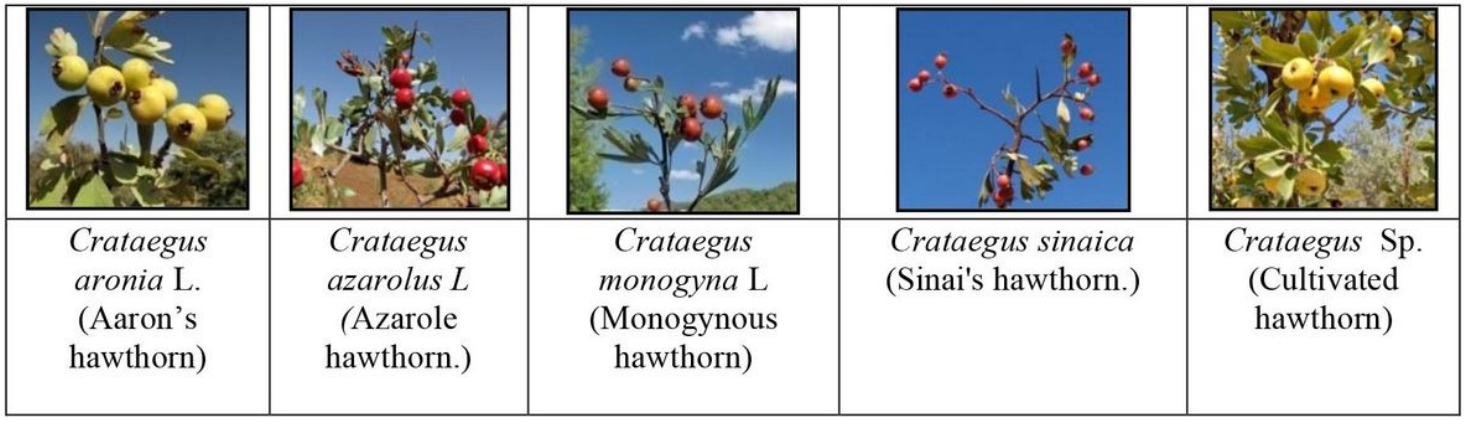

Figure 1

types of hawthorn*(Crataegus*) in Syria

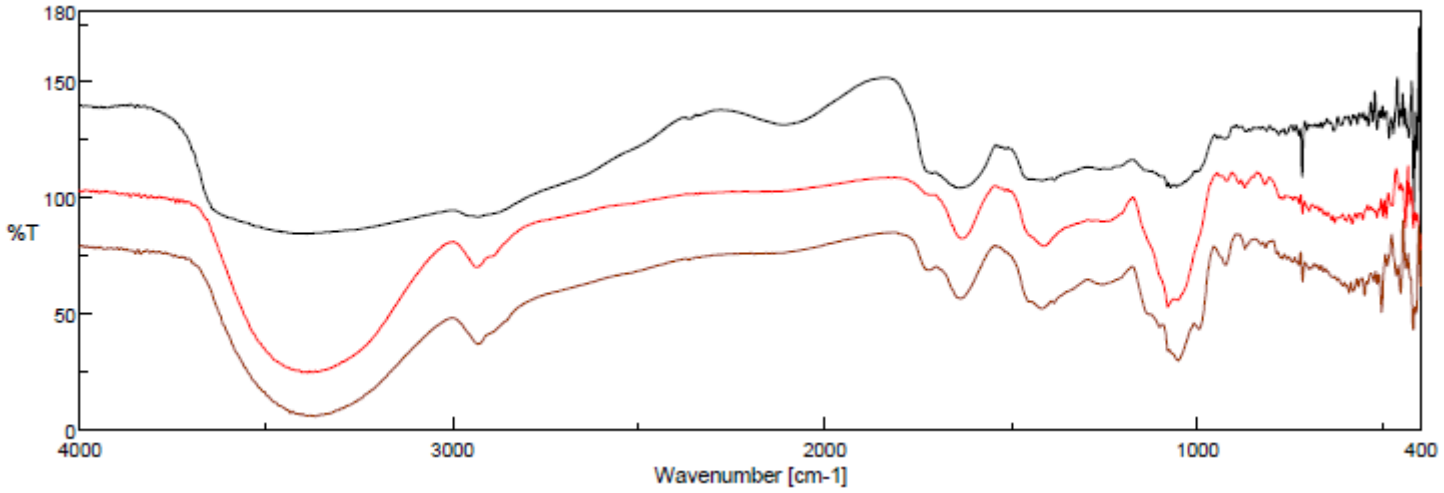

Figure 2

FT- IR spectra of yellow, red and green hawthorn (from down to up)

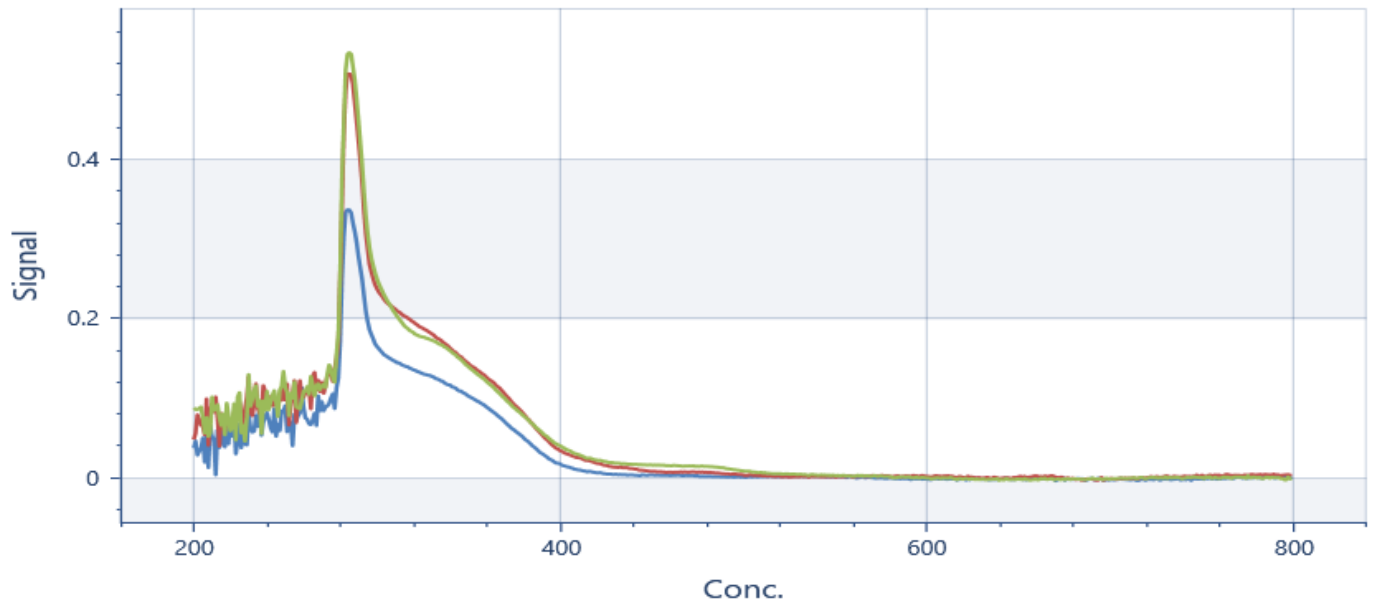

Figure 3

UV–vis spectrum of yellow, red and green hawthorn (from down to up)

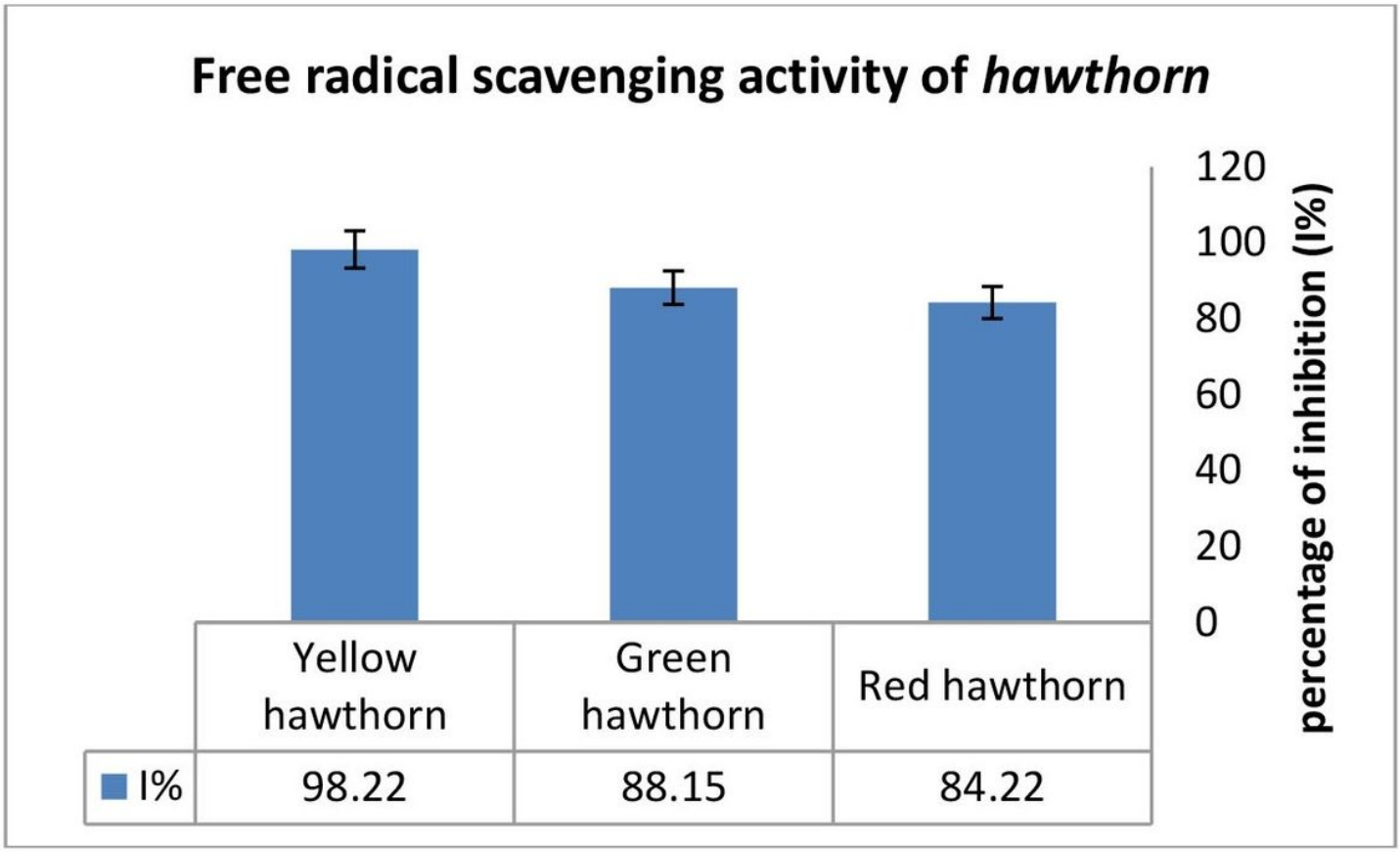

Figure 4

Free radical scavenging activity of *hawthorn*

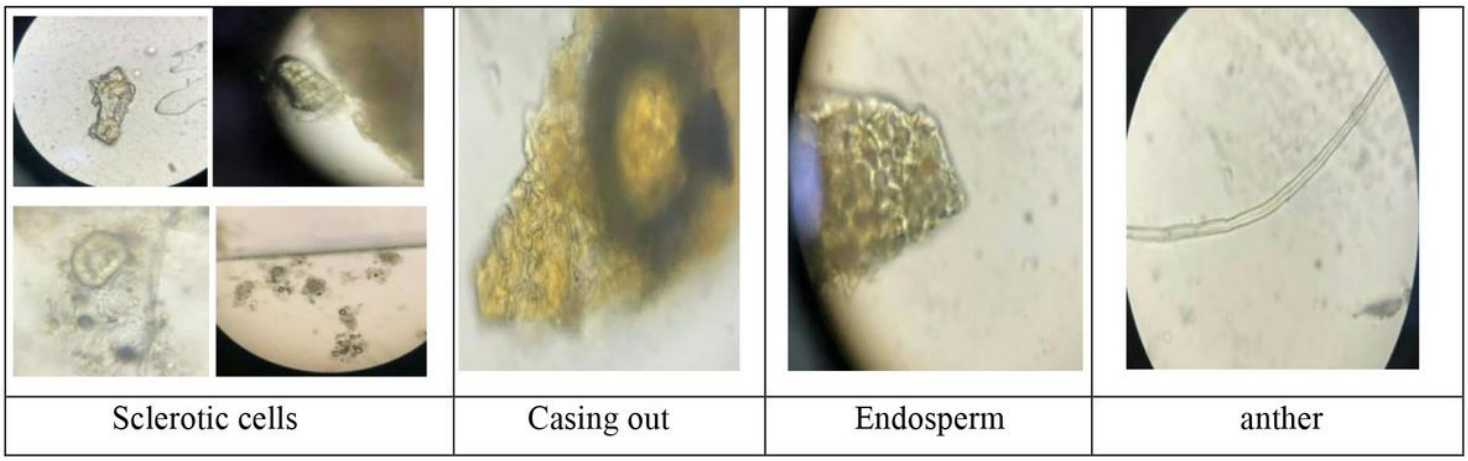

Figure 5

microscopic study Yellow hawthorn

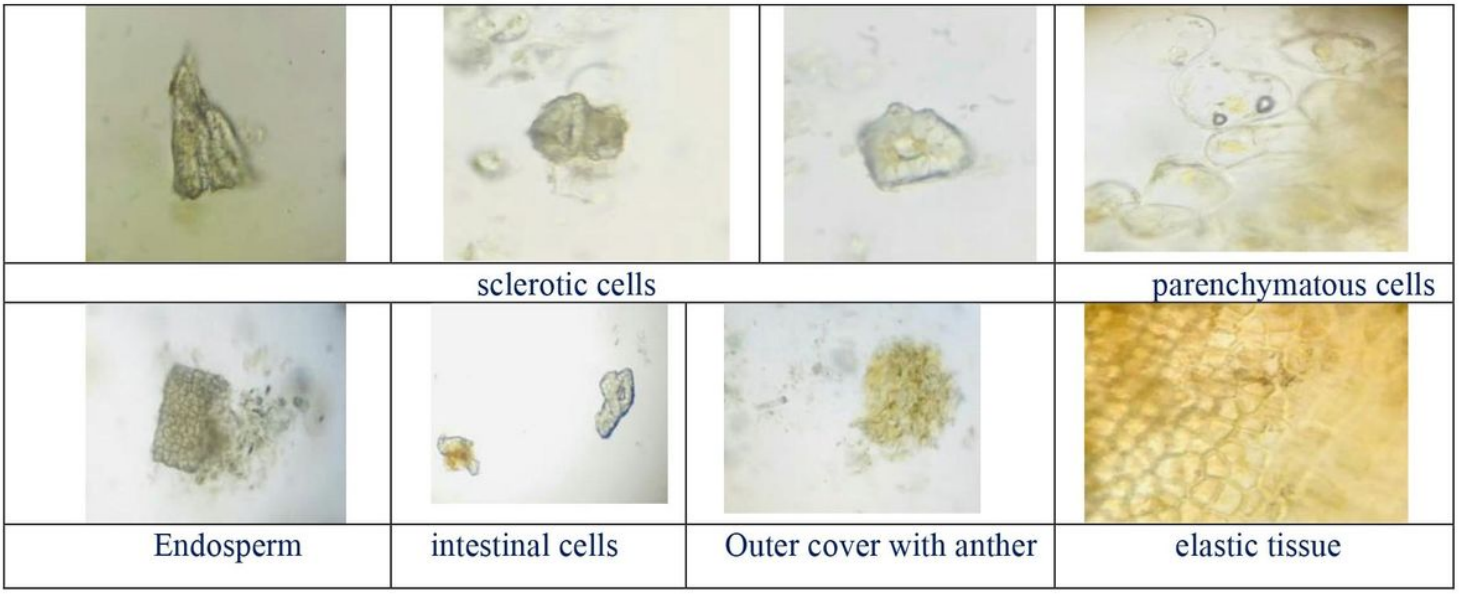

Figure 6

microscopic study of Red hawthorn

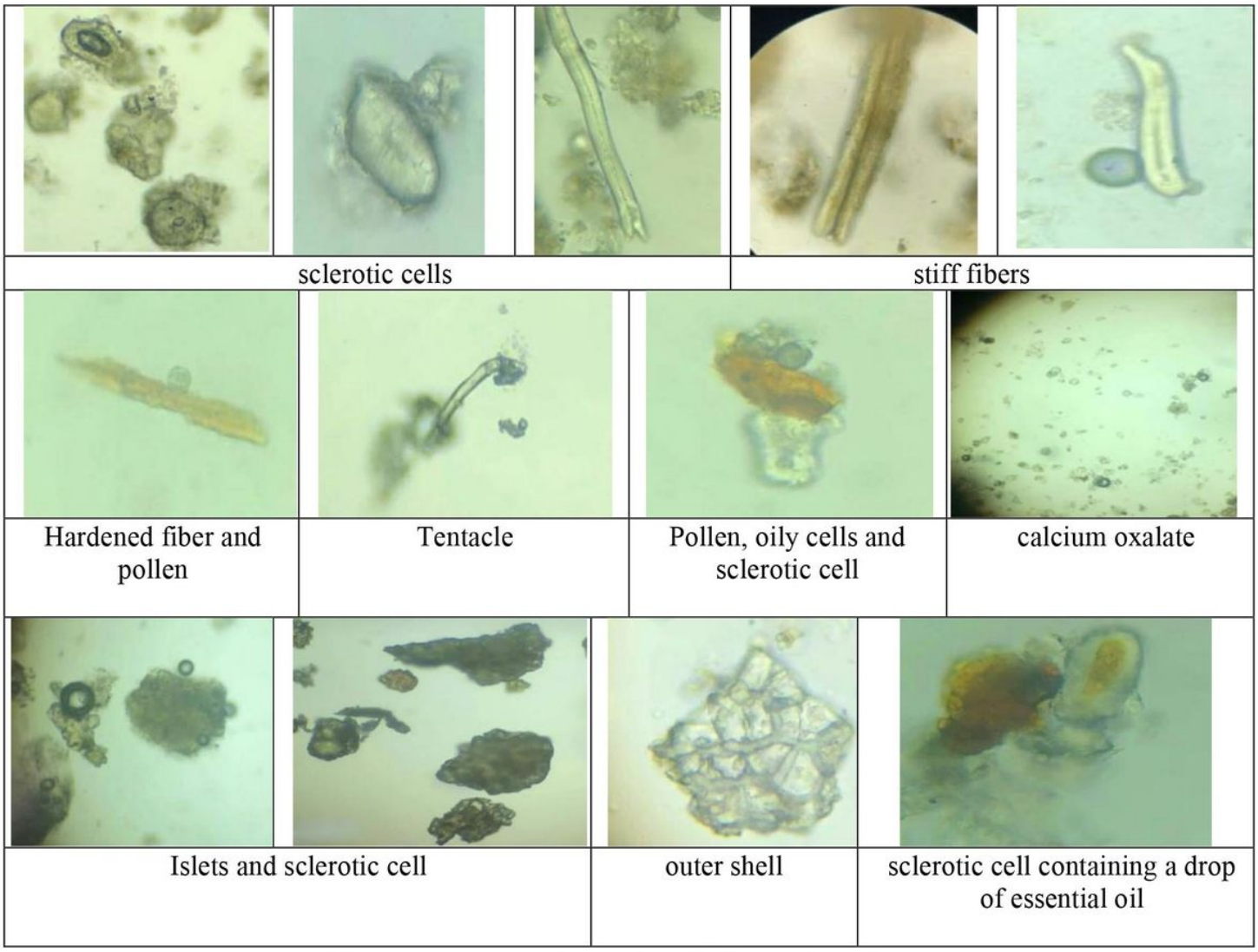

Figure 7

microscopic study of Green hawthorn

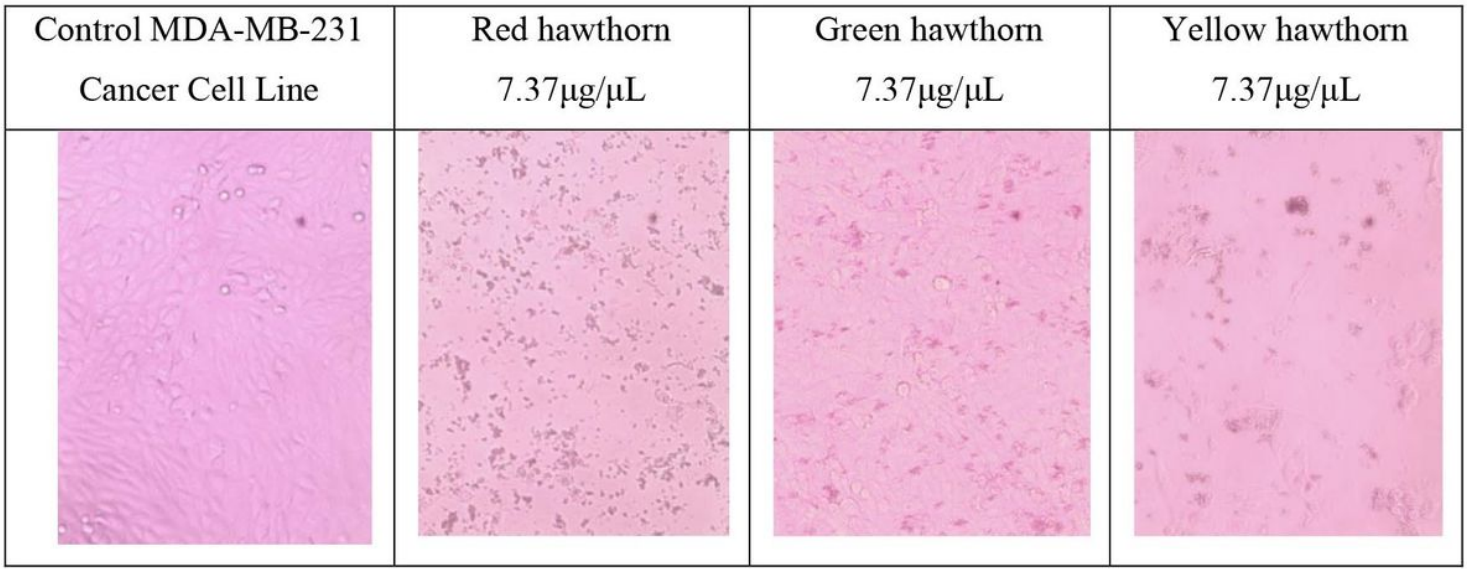

Figure 8

The cytotoxicity effect of hawthorn

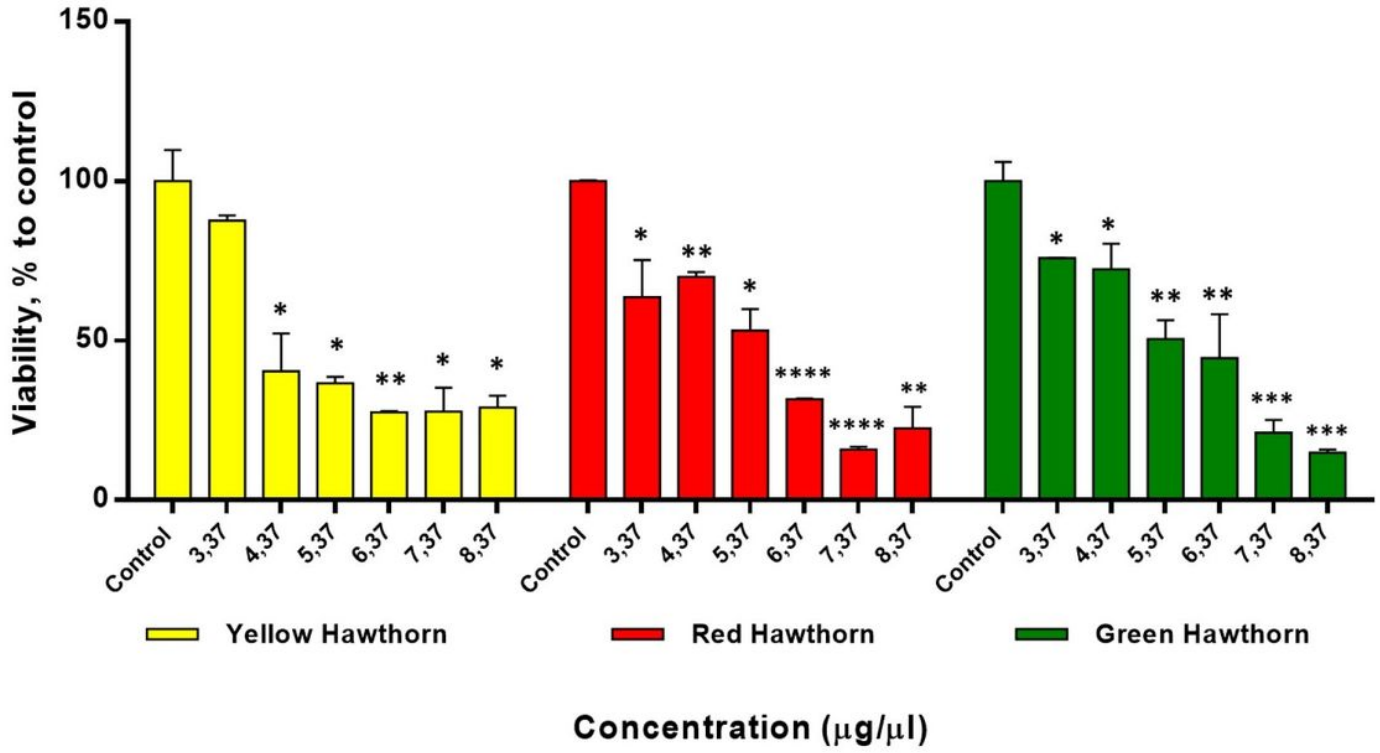

Figure 9

The cytotoxicity effect of hawthorn on MDA-MB-231 cancer cell lines after 48 h. The cells were treated with indicated concentrations of hawthorn and the cell viability was determined by the XTT assay.

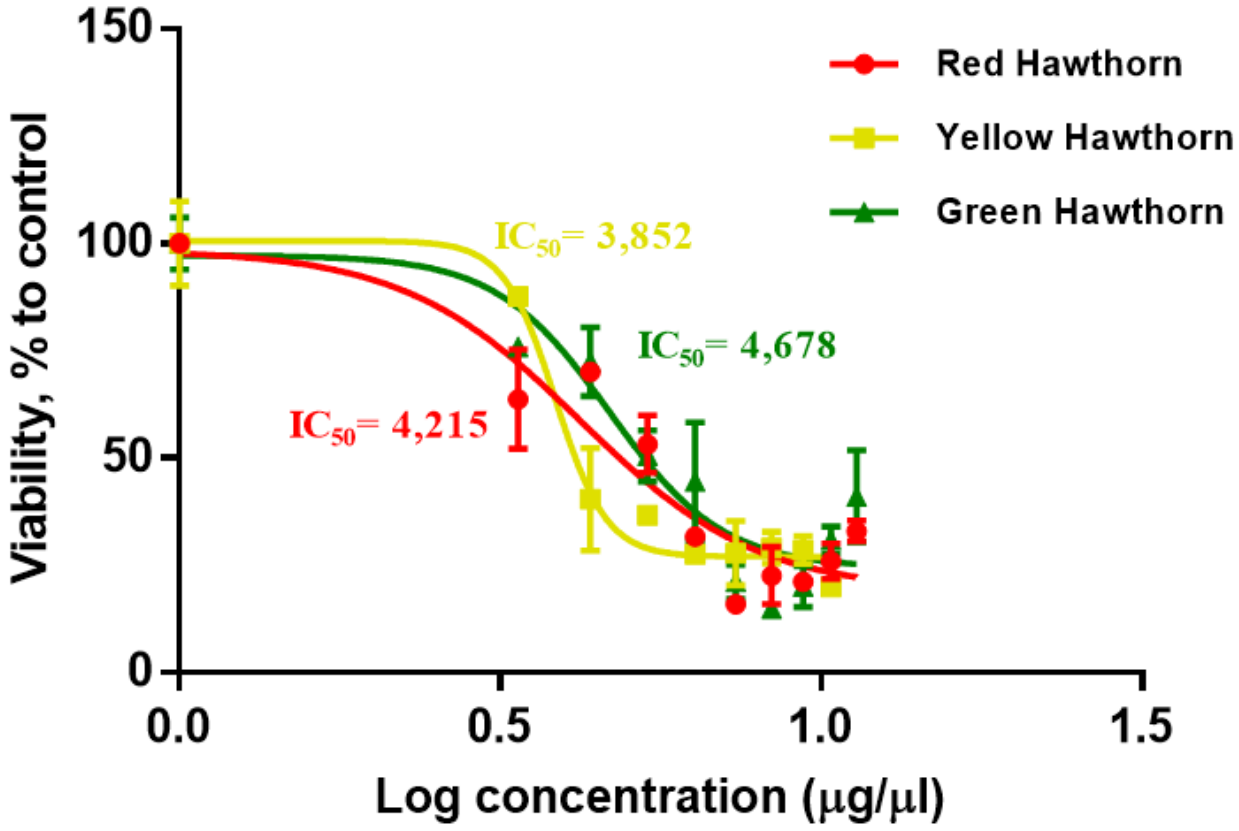

Figure 10

IC_{50} of hawthorn on MDA-MB-231 cancer cell lines after 48 h.

Supplementary Files

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