Research Article

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Citrullus colocynthis (L.) Schrad: Chemical characterization, scavenging and cytotoxic activities

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Abstract: *Citrullus colocynthis* (L.) Schrad (*C. colocynthis*) called colocynth is a wild species that belongs to the family cucurbitaceae. The present research work aimed to study the phytochemical composition, cytotoxic and antioxidant activities of *C. colocynthis* seed extract. The chemical characterization of *C. colocynthis* seeds was effectuated using a gas chromatograph coupled to a mass spectrometer (GC-MS). The cytotoxic activity of *C. colocynthis* seed extract against breast cancer cell lines (MDA-MB-231) and colon cancer cell lines (HT-29) was assessed using the WST-1 bioassay. The antioxidant power was evaluated by the DPPH assay. The phytochemical characterization of *C. colocynthis* seed extract showed the richness of *C. colocynthis* seed extract in several families of bioactive compounds. Regarding the cytotoxic activity, the IC_{50} (the half-maximal inhibitory concentration) of *C. colocynthis* seed extract in inhibiting MDA-MB-231 and HT-29 were 170.34 and 132.31 µg/mL, respectively. The cancerous cell lines MDA-MB-231 seem to be more sensitive to *C. colocynthis* seed extract than HT-29 cell lines. *C. colocynthis* seed extract exhibited a strong antioxidant power with an IC_{50} value of 1.37 mg/mL. Insight into the results obtained, *C. colocynthis* seed extract may be used as a promising weapon to fight against cancer and free radicals' damage.

Keywords: *Citrullus colocynthis*, seeds, phytochemical characterization, cytotoxic activity, antioxidant activity, GC-MS

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1 Introduction

When traditional medicine has not become of great interest for being used in the treatment, doctors and health organizations strongly call for its application in the treatment of diseases because of its promising therapeutic effects. About 80% of the world population and more than 90% of those based in developing countries depend on herbal medicine for primary health care [1–3]. For many decades, medicinal plants have played an essential role in pharmacological research studies and drug realizations. Plants contain many active compounds, the great majority of which are derived from secondary metabolism. These constituents are used as therapeutic agents, as raw materials for drug synthesis, or as models for pharmacologically active compounds [4]. Plants possess very interesting biological properties which are attributed to their content in secondary metabolites like polyphenols, alkaloids, terpenes, and essential oils that are applied in various fields like medicine, pharmacy, cosmetology, and agriculture [5].

The knowledge of medicinal plants goes to be lost since the younger generations have not been interested in keeping the natural heritage [7]. As a result, substances developed in laboratories turn out to be more expensive to be used in the treatment [6]. This type of knowledge has a real cultural value and may ultimately lead to the development of new pharmaceutical drugs. The sustainable exploitation of medicinal plants could contribute not only to the preservation of a significant part of biological diversity but also to the improvement in the living conditions of local communities by developing the trade in medicinal plants or their derivatives [8].

C. colocynthis called colocynth is a wild species that belongs to the family cucurbitaceae and is considered to be one of the most genetically diverse plant groups [9]. *C. colocynthis* fruits have naturally globular aspects, yellow or red color with a potential of poisonous content [10].

The traditional use of *C. colocynthis* has been largely described in the earlier literature. The plant studied in this research work has been used in the treatment of several diseases comprising diabetes, asthma, constipation, toothache, leprosy, bronchitis, jaundice, joint pain, mastitis, and skin infections [11,12].

To the best of our knowledge, no previous literature has investigated the medicinal properties of *C. colocynthis* seeds; therefore, the present study was undertaken to screen the phytochemical composition, the cytotoxic effect, and the antioxidant activity of *C. colocynthis* seeds.

2 Materials and methods

2.1 Plant material and extract preparation

The plant material was collected from the surrounding regions of Tangier city located in the north-west of Morocco. The plant was botanically identified by the taxonomist Pr M. Bakkali (Team of Research in Biotechnology and Biomolecular Genius – Faculty of Sciences and Techniques Tangier – Morocco) and the voucher specimen # LMB 06/04 has been deposited in the local herbarium. After drying the fruits at room temperature in the shade for ten days, seeds were salvaged and ground into a fine powder. One hundred milligrams of powder was extracted by maceration using ethanol for 72 h. Afterward, the mixture was filtered under reduced pressure using a rotary evaporator to obtain 2.4 g of crude extract and then kept at –20°C until further use [13].

2.2 Identification of bioactive constituents by GC-MS analysis

The analysis of *C. colocynthis* seed ethanolic extract was carried out using a GC-MS. The Clarus 580 chromatography apparatus equipped with a capillary column (5% phenyl, 95% methypolysiloxane) (30.0 MX 250 µm) and coupled to a mass spectrometer (Polaris Q) (EI 70 eV) was used in this assay. The carrier gas was helium at 1 mL/min. The split was 1/75 and the injection volume was 1 µL. The injection and detection temperatures were set to 250 and 280°C, respectively. The temperature of the furnace regulating the temperature of the column was programmed as follows: from 50°C to 200°C at a rate of 11°C/min, then from 200°C to 240°C at a rate of 6°C/min. The spectra of the major unknown compounds were compared to the spectrum of the known component in the NIST library [13].

2.3 Cell culture

Breast cancer cell lines (MDA-MB-231) and colon cancer cell lines (HT-29) were used to perform the current research work. The cancer cell lines were kindly provided by Dr H. Morjani, UFR Pharmacy, Reims, France. Cells were cultured in the DMEM (Dulbecco's Modified Eagle Medium) with the following supplements: Glutamine and fetal calf serum, and a mixture of streptomycin/penicillin was added at the rate of 1% to each. The cancerous cells were kept at 37°C with 5% CO_2 and 95% moisture for 24 h (Figure 1).

2.4 Cell viability assay

Cell viability assay was performed using the WST-1 assay, according to the methods described in the earlier data [13]. Briefly, during the exponential growth of cells, MDA-MB-231and colon HT-29 were initially seeded on 96-well microplates. The *C. colocynthis* extract was applied at concentrations that ranged from 7.81 to 250 µg/mL. At the end of the treatment period, 10 µL of the medium was replaced with 10 µL of the WST-1 reagent in each well and then the plates were incubated for further time. Cell viability was evaluated by reading the absorbance at 450 nm using a multiplate reader.

The percentage of cytotoxicity was calculated using the following equation:

$$\text{Cell death (\%)} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100$$

In the present study, the concentration giving 50% cell growth inhibition (IC_{50}) was calculated from the

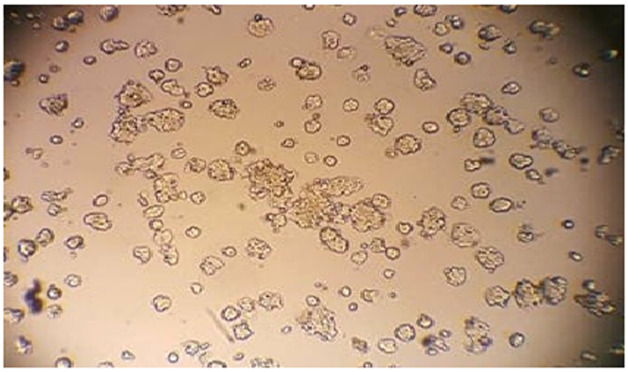

Figure 1: Cancerous cell lines (HT-29).

dose–response curve obtained by plotting the percentage of inhibition vs concentrations (μg/mL).

2.5 Antioxidant activity

The antioxidant activity was conducted, according to the earlier found protocols [14]. *C. colocynthis* extract was tested in different concentrations that ranged from 0.313 to 10 mg/mL. Briefly, each concentration was dropped in a microplate with 96 wells and then 50 μL of 1 mM DPPH was added to each well. The microplates were placed at room temperature for 30 min under dark conditions and then the absorbance was read at 517 nm. BHT (butylated hydroxytoluene) was used as a positive control. The percentage of free radical inhibition was calculated as follows:

$$\%\ \text{inhibition} = \frac{\text{OD sample} - \text{OD control}}{\text{OD control}} \times 100$$

IC_{50} (the required concentration for inducing 50% inhibition of free radical) was calculated from the graph.

2.6 Statistical analysis

Data were expressed as the means of duplicate assays ± SD (standard deviation). The significant difference between the means was calculated using the Student's *t*-test. Statistically, a significant difference was considered, when $P < 0.05$.

Ethical approval: The conducted research work is not related to either human or animal use.

3 Results

3.1 Gas chromatography-mass spectrometry analysis

The results of *C. colocynthis* seed extract showed the presence of interesting bioactive molecules (Figure 2; Table 1).

3.2 Antioxidant effect

C. colocynthis ethanolic extract showed a promising free radical inhibition in dose-dependent concentration. The IC_{50} value (the required concentration for inhibiting 50% of free radicals) generated by *C. colocynthis* ethanolic extract was determined at 4.56 mg/mL by the time the BHT, which

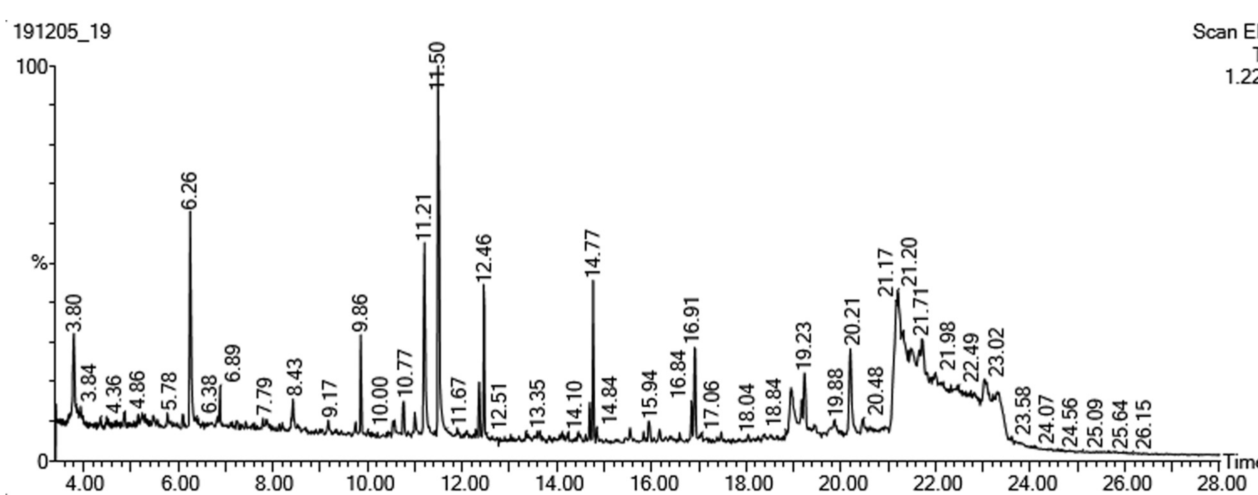

Figure 2: GC-MS chromatogram of *C. colocynthis* seed extract.

Table 1: Bioactive molecules identified in *C. colocynthis* seed extract using a GC-MS

Pk #	RT	Hit	Compound name	Match	R. Match	Prob.	CAS	Library
1	3.420	1	Methylene chloride	665	887	83.6	75-09-2	mainlib
		2	Methane-d, trichloro-	545	747	4.4	865-49-6	mainlib
		3	1,3-Dioxolane, 2-heptyl-4-phenyl-	542	643	3.9	55668-40-1	mainlib
2	3.798	1	Hexanal	825	829	53.6	66-25-1	mainlib
		2	Cyclopentanol, 2-methyl-, trans-	794	812	14.6	25144-04-1	mainlib
		3	2-Hexen-1-ol, (*E*)-	774	776	6.7	928-95-0	mainlib
3	3.944	1	Tetrachloroethylene	763	826	95.1	127-18-4	mainlib
		2	1,4-Dichloro-2-fluorobenzene	533	594	0.9		mainlib
		3	1,2-Dichloro-4-fluorobenzene	525	560	0.7	1435-49-0	mainlib
4	4.130	1	4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloromethylpyrimidine	504	603	28.2		mainlib
		2	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16 S,19*E*)-	474	525	7.8	6393-66-4	mainlib
		3	Cyclopentanemethanol, 1-amino-	471	621	6.9	10316-79-7	mainlib
5	4.235	1	Methylene chloride	537	732	67.9	75-09-2	mainlib
		2	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16*S*,19*E*)-	471	516	10.7	6393-66-4	mainlib
		3	Dichloroacetaldehyde	450	626	4.6	79-02-7	mainlib
6	4.361	1	1-Heptanol, 3-methyl-	634	742	10.2	1070-32-2	mainlib
		2	2,4-Dimethylhept-1-ene	610	724	3.4		mainlib
		3	Octyl chloroformate	610	692	3.4	7452-59-7	mainlib
7	4.487	1	*N*-(2,2-Dichloro-1-hydroxy-ethyl)-2,2-dimethyl-propionamide	509	573	12.0	58956-78-8	mainlib
		2	1,3-Dioxolane, 2-heptyl-4-phenyl-	501	572	8.9	55668-40-1	mainlib
		3	Cyclopentanone, 2-(1-methylpropyl)-	491	676	6.3	6376-92-7	mainlib
8	4.663	1	Methylene chloride	534	688	7.4	75-09-2	mainlib
		2	2-Piperidinecarboxylic acid	531	631	6.5	535-75-1	mainlib
		3	Pyrrolidine, 2-ethyl-1-methyl-	529	765	6.0	26158-82-7	mainlib
9	4.703	1	Methylene chloride	531	694	27.8	75-09-2	mainlib
		2	Methane-d, trichloro-	528	746	24.6	865-49-6	mainlib
		3	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16*S*,19*E*)-	497	534	6.7	6393-66-4	mainlib
10	4.788	1	Butanoic acid, 4,4′-dithiobis[2-amino-,[*S*-(*R**,*R**)]-	390	400	9.2	626-72-2	mainlib
		2	Ethanethiol, 2-(3-(3-chloro-2- pyridyloxy)propyl)amino-, hydrogen sulfate	386	413	7.8	41286-95-7	mainlib
		3	DL-Homocystine	381	387	6.3	870-93-9	mainlib
11	4.884	1	*N*-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic acid, benzyl ester	560	582	22.8		mainlib
		2	Ethylbenzene	542	839	11.8	100-41-4	mainlib
		3	Benzaldehyde, 4-benzyloxy-2-fluoro-5-hydroxy-	528	606	7.4	141523-16-2	mainlib
12	4.964	1	Methylene chloride	415	580	21.4	75-09-2	mainlib
		2	Aethylephedrin propionyl	388	630	6.3		mainlib
		3	2-[4-Chloro-2-nitrophenyl]-1-(2-diethylaminoethyl)-3-formyl-1*H*-indole	376	418	4.2	65287-47-0	mainlib
13	5.072	1	2-Cyclohexylpiperidine	460	570	5.2	56528-77-9	mainlib
		2	2-[*p*-Chlorobenzoyl]piperidine	456	615	4.4	63587-52-0	mainlib
		3	Pyrrolidin-2-one, 5-pentyl-	455	582	4.2	3817-18-3	mainlib
14	5.157	1	Nitrous acid, cyclohexyl ester	554	695	12.6	5156-40-1	mainlib
		2	Cyclohexanol	548	620	9.9	108-93-0	mainlib
		3	2-Hexen-1-ol, (*E*)-	545	627	8.7	928-95-0	mainlib
15	5.242	1	Isonicotinic acid, 2-phenylethyl ester	579	730	6.4		mainlib
		2	Nicotinic acid, 2-phenylethyl ester	578	731	6.2		mainlib
		3	Benzene, (nitromethyl)-	572	634	4.8	622-42-4	mainlib
16	5.307	1	3-Piperidinecarboxamide, 6-oxo-	504	672	30.8	189763-34-6	mainlib
		2	Carbonic acid, (ethyl)(1,2,4-triazol-1-ylmethyl) diester	465	506	7.3		mainlib

Table 1: Continued

Pk #	RT	Hit	Compound name	Match	R. Match	Prob.	CAS	Library
		3	2(3*H*)-Furanone, 3-(2-bromoethyl)-4,5-dihydro-	455	555	5.1	54815-24-6	mainlib
17	5.477	1	*meso*-3,4-Hexanediol	528	969	51.4	22520-39-4	mainlib
		2	Oxirane, 3-ethyl-2,2-dimethyl-	491	830	12.5	1192-22-9	mainlib
		3	2-Methoxyethoxymethyl chloride	458	654	3.3	3970-21-6	mainlib
18	5.567	1	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16*S*,19*E*)-	449	479	26.9	6393-66-4	mainlib
		2	4-Chlorobuten-3-yne	401	760	5.6	40589-38-6	mainlib
		3	*N*-Morpholinomethylidene-3-morpholino-2-(2-thienyl) thioacrylamide	398	402	4.9		mainlib
19	5.776	1	Ethanethioic acid, *S*-(1-ethylbutyl)ester	527	622	6.7	55590-84-6	mainlib
		2	2-Hexanol, 3,4-dimethyl-	509	645	3.5	19550-05-1	mainlib
		3	Methoxyacetic acid, 4-methylpentyl ester	508	612	3.3		mainlib
20	6.097	1	2-Heptenal, (*E*)-	570	736	9.9	18829-55-5	mainlib
		2	2-Pentenal, (*E*)-	560	634	7.0	1576-87-0	mainlib
		3	2-Butenal, 3-methyl-	559	744	6.7	107-86-8	mainlib
21	6.258	1	2-Heptenal, (*Z*)-	940	961	77.7	57266-86-1	mainlib
		2	2-Heptenal, (*E*)-	850	869	6.6	18829-55-5	mainlib
		3	1-Hexene, 3,5-dimethyl-	843	874	5.1	7423-69-0	mainlib
22	6.379	1	Benzoic acid 3-methyl-4-(1,3,3,3-tetrafluoro-2-methoxycarbonyl-propenylsulfanyl)-phenyl ester	471	520	9.6		mainlib
		2	3-Fluoro-3-(2-oxo-2-phenyl-ethylsulfanyl)-2-trifluoromethyl-acrylic acid methyl ester	460	520	6.6	329735-34-4	mainlib
		3	Benzamide, *N*-(2-chloro-4-nitrophenyl)-	447	707	4.3	64160-38-9	mainlib
23	6.888	1	Decane, 2,5,6-trimethyl-	766	863	14.0	62108-23-0	mainlib
		2	Heptane, 2,3,6-trimethyl-	746	841	6.4	4032-93-3	mainlib
		3	Oxalic acid, isobutyl nonyl ester	742	849	5.4		mainlib
24	7.878	1	*N*-Methyl-3-piperidinecarboxamide	535	657	8.3	5115-98-0	mainlib
		2	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester, (16*S*,19*E*)-	533	564	7.7	6393-66-4	mainlib
		3	4-[(5-Oxopyrrolidin-2-yl)carbonyl]-morpholine	531	637	7.1	76284-13-4	mainlib
25	8.431	1	2,4,6,8-Tetramethyl-1-undecene	760	878	8.5	59920-26-2	mainlib
		2	Oxalic acid, allyl nonyl ester	746	825	5.3		mainlib
		3	1-Octanol, 2-butyl-	742	792	4.5	3913-02-8	mainlib
26	9.858	1	Dodecane	846	849	10.2	112-40-3	mainlib
		2	Undecane, 2,6-dimethyl-	824	857	4.1	17301-23-4	mainlib
		3	Undecane	822	833	3.7	1120-21-4	mainlib
27	10.577	1	1-Oxaspiro[2.2]pentane, 5-isopropylidene-2,2,4,4- tetramethyl-	548	640	7.7	15448-69-8	mainlib
		2	6,6-Dimethyl-2-vinylidenebicyclo[3.1.1]heptane	539	597	5.6	39021-75-5	mainlib
		3	1-Propene, 2-nitro-3-(1-cyclooctenyl)	534	579	4.5	80255-21-6	mainlib
28	10.769	1	2-Decenal, (*Z*)-	818	882	39.6	2497-25-8	mainlib
		2	2-Decenal, (*E*)-	799	879	19.2	3913-81-3	mainlib
		3	*cis*-7-Decen-1-al	733	792	3.0	21661-97-2	mainlib
29	10.998	1	(*Z*)-3-Phenylacrylaldehyde	791	862	47.9	57194-69-1	mainlib
		2	2-Propenal, 3-phenyl-	761	826	13.3	104-55-2	mainlib
		3	Benzylidenemalonaldehyde	751	822	9.4	82700-43-4	mainlib
30	11.209	1	2,4-Decadienal	894	923	53.7	2363-88-4	mainlib
		2	2,4-Decadienal, (*E*,*E*)-	874	878	24.5	25152-84-5	mainlib
		3	2,4-Nonadienal	798	848	3.1	6750-03-4	mainlib
31	11.496	1	2,4-Decadienal	911	939	53.9	2363-88-4	mainlib
		2	2,4-Decadienal, (*E*,*E*)-	893	902	27.8	25152-84-5	mainlib
		3	2,4-Nonadienal	828	880	4.5	6750-03-4	mainlib
32	12.361	1	Germacrene D	752	823	8.0	23986-74-5	mainlib
		2	alfa-Copaene	751	774	7.7		mainlib
		3	Copaene	751	771	7.7	3856-25-5	mainlib
33	12.458	1	Heptadecane, 2,6,10,14-tetramethyl-	850	855	4.0	18344-37-1	mainlib

Table 1: Continued

Pk #	RT	Hit	Compound name	Match	R. Match	Prob.	CAS	Library
		2	Heptadecane, 2,6-dimethyl-	850	854	4.0	54105-67-8	mainlib
		3	Tetradecane	849	850	3.9	629-59-4	mainlib
34	14.697	1	7-Hexadecene, (*Z*)-	827	856	5.3	35507-09-6	mainlib
		2	9-Octadecene, (*E*)-	825	852	4.9	7206-25-9	mainlib
		3	Cetene	820	853	4.0	629-73-2	mainlib
35	14.769	1	Hexadecane	872	874	10.7	544-76-3	mainlib
		2	Pentadecane, 7-methyl-	848	849	3.6	6165-40-8	mainlib
		3	Tridecane	846	866	3.3	629-50-5	mainlib
36	15.942	1	Ethyl *trans*-α-cyanocinnamate	748	830	63.4	2169-69-9	mainlib
		2	2-Propenoic acid, 2-cyano-3-phenyl-, ethyl ester	727	803	27.0	2025-40-3	mainlib
		3	2-Propenoic acid, 3-(3-cyanophenyl)-, ethyl ester	662	736	4.3	87087-44-3	mainlib
37	16.843	1	2-Dodecanol	838	888	4.7	10203-28-8	mainlib
		2	Cetene	831	878	3.6	629-73-2	mainlib
		3	7-Tetradecene, (*Z*)-	831	871	3.6	41446-60-0	mainlib
38	16.908	1	Pentadecane	858	870	6.6	629-62-9	mainlib
		2	Hexadecane	847	859	4.5	544-76-3	mainlib
		3	Dodecane, 2-methyl-	846	883	4.3	1560-97-0	mainlib
39	18.951	1	*n*-Hexadecanoic acid	787	809	55.3	57-10-3	mainlib
		2	Tridecanoic acid	723	755	9.1	638-53-9	mainlib
		3	Tetradecanoic acid	720	749	8.0	544-63-8	mainlib
40	19.168	1	9-Eicosene, (*E*)-	735	808	3.2	74685-29-3	mainlib
		2	*E*-14-Hexadecenal	734	785	3.0	330207-53-9	mainlib
		3	Trichloroacetic acid, tetradecyl ester	733	810	2.9	74339-52-9	mainlib
41	19.228	1	Dodecane, 2,6,10-trimethyl-	820	871	13.7	3891-98-3	mainlib
		2	Sulfurous acid, 2-ethylhexyl hexyl ester	819	922	13.2		mainlib
		3	Tridecane	797	850	5.2	629-50-5	mainlib
42	19.439	1	Ethanol, 2-(9-octadecenyloxy)-, (*Z*)-	552	556	6.0	5353-25-3	mainlib
		2	9-Hexadecenoic acid	544	571	4.5	2091-29-4	mainlib
		3	Dodecanoic acid, 3-hydroxy-	536	577	3.4	1883-13-2	mainlib
43	19.876	1	5-Heptyn-3-ol, 2-benzyloxy-7-methoxy-1-(*t*-butyldimethylsilyl)oxy-	548	647	8.4		mainlib
		2	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	540	717	6.2	13754-10-4	mainlib
		3	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	540	602	6.2	86071-95-6	mainlib
44	20.207	1	1-fluorenecarboxylic acid, 2,2,2-trifluoroethyl ester	676	688	26.6		mainlib
		2	1*H*-Phenalene	671	800	21.4	203-80-5	mainlib
		3	9*H*-Fluorene, 9-bromo-	670	768	20.6	1940-57-4	mainlib
45	20.478	1	Heptadecane, 9-hexyl-	670	670	13.7	55124-79-3	mainlib
		2	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	646	646	4.6	55282-12-7	mainlib
		3	Dodecane, 5,8-diethyl-	643	662	4.1	24251-86-3	mainlib
46	21.200	1	Linoelaidic acid	851	881	10.3	506-21-8	mainlib
		2	11,14-Eicosadienoic acid, methyl ester	839	878	6.8	2463-02-7	mainlib
		3	17-Octadecynoic acid	823	828	3.9	34450-18-5	mainlib
47	21.712	1	Pentadecane, 2,6,10-trimethyl-	707	790	4.4	3892-00-0	mainlib
		2	Tetradecane	705	793	4.0	629-59-4	mainlib
		3	Pentadecane	703	783	3.7	629-62-9	mainlib
48	22.007	1	2,6-Nonadienal, 3,7-dimethyl-	513	579	16.9	41448-29-7	mainlib
		2	7-Heptadecene, 1-chloro-	481	496	4.5	56554-78-0	mainlib
		3	2-(4-Nitrobutyryl)-cyclopentanone	478	567	4.0	79630-91-4	mainlib
49	23.043	1	Disulfide, di-*tert*-dodecyl	683	712	4.8	27458-90-8	mainlib
		2	Tetradecane, 2,6,10-trimethyl-	666	716	2.6	14905-56-7	mainlib
		3	Ethanol, 2-(octadecyloxy)-	666	676	2.6	2136-72-3	mainlib
50	23.304	1	2,4-Heptadiene, 5-diethylboryl-2-methyl-4- trimethylsilyl-	494	553	15.6		mainlib
		2	*t*-Butyl-(2-[3-(2,2-dimethyl-6-methylene-cyclohexyl)-propyl]-[1,3]dithian-2-yl)-dimethyl-silane	474	483	7.1	95472-42-7	mainlib

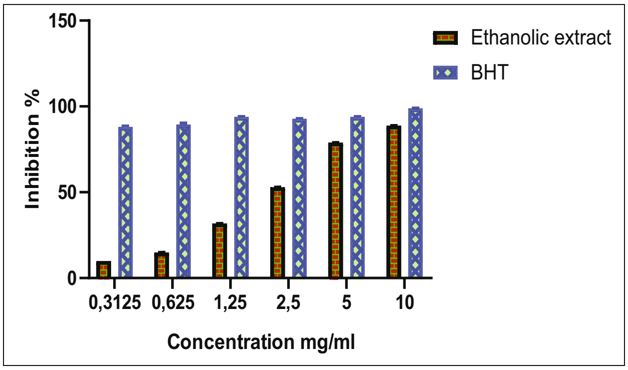

Figure 3: Inhibition percentage of the scavenging activity of *C. colocynthis* ethanolic extract (results are presented as means ± SD).

was used as a reference, exhibited an IC_{50} value of 1.37 mg/mL (Figure 3). A significant difference between the IC_{50} value (4.56 mg/mL) of *C. colocynthis* ethanolic extract and BHT IC_{50} 1.37 mg/mL was recorded ($P < 0.05$).

3.3 Cytotoxic effect of *C. colocynthis* seeds

The finding of the cytotoxic effects investigated in the current research work showed that all cancerous cell lines were sensitive to the plant extract. The ethanolic extract induced toxic effects on both HT-29 and MDA-MB-231 cell lines after 72 h of treatment with IC_{50} values of 170.34 and 132.31 µg/mL, respectively. The MDA-MB-231 cell lines were more sensitive to *C. colocynthis* seed extract, compared to colon HT-29 cell lines (Figure 4).

Figure 4 shows that the extract studied induced pronounced cell toxicity in a dose-dependent manner. The statistical analysis showed a significant difference between the IC_{50} value induced by the mitomycin used as positive control 0. 2 vs the IC_{50} values generated by the extract on both cancerous cell lines HT-29 (170.34 mg/mL) and MDA-MB-231 (132.31 mg/mL). The statistical analysis also showed the presence of a significant difference between the IC_{50} value of HT-29 vs MDA-MB-231 ($P < 0.05$).

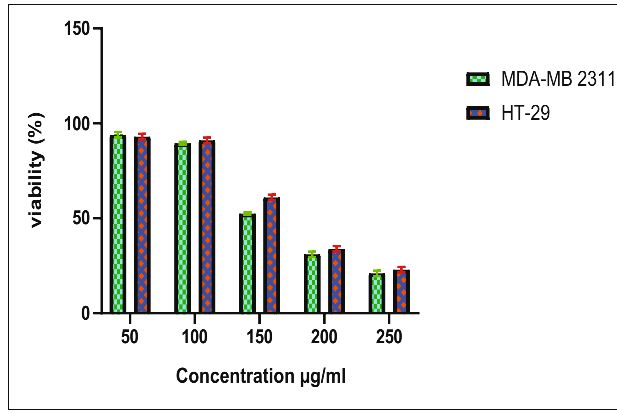

Figure 4: Dose–response curve of cell viability after 72 h of treatment with the *C. colocynthis* ethanolic extract (results are presented as means ± SD).

4 Discussion

Cancer is a major burden worldwide, although the modern medicine has succeeded to limit its impact by involving large therapeutic categories, resistance to classical modern therapeutic agents continues to be a major challenge in cancer therapies. It is worth searching for new effective agents with no or few secondary effects for cancer treatment which is highly appreciated. In this context, natural products have continued to receive increasing attention due to their potential preventive and therapeutic effects vs cancer. The role of medicinal plants in cancer treatment has been widely investigated [15]. As earlier described, the products derived from plants are good sources for bioactive compounds' discovery as well as drug conception and development [16,17].

The traditional use of the currently studied plant in North African alternative medicine has been documented in the earlier data, which showed the use of *C. colocynthis* for the treatment of diabetes and hypertension [18].

In the present research work, we investigated the chemical composition, antioxidant and cytotoxic activities of *C. colocynthis* ethanolic extract. Regarding the antioxidant activity, the DPPH scavenging method was used to screen this activity. The findings obtained showed that the ethanolic extract possesses a promising antioxidant power with an IC_{50} value of 4.56 mg/mL. These results are highly justified by the presence of bioactive compounds in the plant extract characterized with a GC-MS (Table 1), which showed the presence of polyphenols and flavonoids as responsible agents for the antioxidant activity [19]. Isoorientin 3-*O*-methylether, isosaponarin, and isovitexin isolated from *C. colocynthis* possess a strong antioxidant power with an IC_{50} value ranging from 5.62×10^{-4} to 7.13×10^{-2} mg/mL [20]. The antioxidant power of *C. colocynthis* is in consent with those reported in the earlier data [21]. It was also revealed that the antioxidant activity is attributed to the cucurbitacin glycoside content in the ethanolic extract, as shown in the earlier literature [22].

In the current research work, we also investigated the toxic effects of *C. colocynthis* on both HT-29 and MDA-MB-231 cell lines. The results obtained showed that the studied extract possesses interesting cytotoxic effects with IC_{50} values of 170.34 and 132.31 µg/mL, respectively. These findings were in agreement with the earlier data which showed an important antiproliferative effect on human breast cancer cells induced by cucurbitacin, isolated from the leaves of *C. colocynthis* [22]. The cytotoxic effects of the fruit pulp of *C. colocynthis* against cancer breast cell lines MCF-7 were studied [23]. Some chemical compounds revealed in the current extract such as ethylbenzene and tetrachloroethylene could determine the cytotoxic effect of *C. colocynthis* on HT-29 and MDA-MB-231 cell lines as to whether their effects are due to a single molecule or due to a potential synergy between them, and therefore further studies that may lead to determining the responsible compounds for the current activities are still appreciated.

5 Conclusion

The present research work provides interesting data about the chemical characterization, antioxidant and cytotoxic effects of *C. colocynthis* ethanolic extract. Based on the results obtained, *C. colocynthis* seed extract may be used as a promising weapon to fight cancer and free radicals' damage.

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