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Chemical Profiling, Antioxidant, Antiproliferative, and Antibacterial Potentials of Chemically Characterized Extract of *Citrullus colocynthis* L. Seeds

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Abstract: Background: *Citrullus colocynthis* L. (*C. colocynthis*) is commonly known as colocynth. It belongs to the family *Cucurbitaceae* that is frequently used in alternative medicine in the north of Africa. The aim of the study: the present research was undertaken to investigate the chemical composition, antioxidant, antiproliferative, and antibacterial potentials of *C. colocynthis* seed extract. Material and methods: the chemical composition of *C. colocynthis* seed organic extract was characterized using gas chromatography/mass spectrometry (GC-MS). The antioxidant property was carried out using both β-carotene bleaching and DPPH assays. The antibacterial effect was effectuated using the agar disc diffusion method. The antiproliferative activity vs. human colorectal adenocarcinoma cell line (HT-29) and human breast adenocarcinoma cell line (MDA MB 231) were carried by WST-1 test. The chemical analysis showed the presence of interesting potentially bioactive compounds. The studied plant extract exhibited antioxidant potential with IC_{50} value of 2. 22 mg/mL (β-carotene bleaching) and 8.98 ± 0.619 mg/mL (DPPH). Concerning the antiproliferative activity, the seed extract was effective in MDA-MB-231 and HT-29 cancer cells with IC_{50} values 86.89 ± 3.395 and 242.1 ± 17.9 µg/mL, respectively, whilst the extract of *Citrullus colocynthis* seeds was non-toxic in healthy human dermal fibroblasts. Regarding the antibacterial test, the extract was effective in Gram-positive bacteria only. Conclusion: The outcome of this research indicated that the extracts from *C. colocynthis* seeds may compose a promising source with interesting compounds that can be used to fight cancer, free radicals damage, and bacterial infections.

Keywords: *Citrullus colocynthis* L. seeds; chemical composition; antiproliferative; antioxidant; antibacterial

1. Introduction

Plants have populated the planet for millions of years and have served humans and animals to meet their nutritional and medicinal requirements. Their uses have largely evolved with the discovery of their therapeutic properties [1]. Medicinal plants are defined as a vegetable that has at least a part (bark, leaves, roots, fruits) with medicinal properties [2]. Herbal medicine has been used as remedies in different forms (decoction, infusion, ingredients) to prevent and treat diseases. The development of aroma has started in ancient

times for cosmetic purposes [3]. Aromatic and medicinal plants have been used in various dietary and therapeutic practices since prehistoric times [4].

Recently, people have returned to alternative medicine based on natural products due to its efficacy in the treatment and the prevention of diseases with negligible side effects. For example, the resistance of microbes to synthesized drugs is a new problem that has led to the use of plant derivatives [5]. The use of medicinal plants plays an important role in the health care system. It is estimated that 50 to 75% of people throughout the world use traditional medicine for medications [6]. It was reported that over 950 species have been used to cure a wide variety of illnesses in Morocco [7].

C. colocynthis belongs to the family *Cucurbitaceae* and has a large genetic diversity. It is a perennial plant that commonly spreads in tropical soil and can survive hard ecological conditions [8,9]. *C. colocynthis* fruit is recognized by a globular aspect, with different colors along with soft pulp and edible seeds [10]. Early civilizations have reported potential pharmacological activities of *C. colocynthis*. It has traditionally been used in the treatment of a bundle of diseases such as cough, constipation, leprosy, diabetes, asthma, and toothache [11,12]. In Mediterranean countries, *C. colocynthis* fruits have been used in the treatment of pulmonary and urinary infections [13]. *C. colocynthis* was observed early to exert potential hepatoprotective effects [14]. In Morocco, *C. colocynthis* seeds are commonly used as antidiabetic and antihypertension agents [15,16].

Phytochemical studies have reported that *C. colocynthis* contains different chemical families such as carbohydrates, flavonoids, alkaloids, and phenolic acids [17].

The nutritional composition of *C. colocynthis* seeds was investigated in earlier works by Milovanović and Pićurić-Jovanović (2005), who reported that the dry weight of seeds consisted of testa and kernel with 52.3% and 47.7%, respectively; the moisture content was found at 54.5%; the oil content ranged from 22.1–53.5%; the protein content was 21.8%; and the fatty acid content in the oilseed consisted of a majority of unsaturated fatty acid (77.4%), including linoleic (18:2) acid (62.2%) [18].

The current research work was undertaken to study the phytochemical composition, antioxidant, antibacterial, and antiproliferative potentials of *C. colocynthis* seeds since no previous work has investigated the pharmacological activities of seeds from *C. colocynthis* growing in the north of Morocco up to this date.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

The plant was harvested in March 2015 from Morocco (Tangier city) and was authenticated by Dr. M. Bakkali before being deposited in the herbarium #LMB 06/04. Next, the fruits were washed with distilled water and dried at room temperature. Seeds were salvaged and ground before being macerated with hexane for 72 h. The whole mixture obtained was filtered using a Whatman filter and the extraction solvent was removed using a rotary set to 45 °C to obtain an oily paste. The obtained extract was meticulously saved at 4 °C until further use.

2.2. Antioxidant Activity

2.2.1. β-Carotene Bleaching Assay

The antioxidant activity was performed using bleaching of a beta-carotene assay. Briefly, 0.140 mg of β-carotene was solubilized in 0.70 mL of chloroform before being added to 200 µL of the β-carotene solution with 1.40 mg of linoleic acid and 14.00 mg of Tween 40. Thereafter, the solution was meticulously stirred with 3.5 mL of water before being added to the microtitration plates in 200 µL previously supplemented with 8 µL of different concentrations of the studied extracts (0.3125–10 mg/mL). The absorbance of each concentration was measured immediately using spectrophotometry at 470 nm. Afterward, samples were incubated at 50 °C for 120 min and the oxidation was assessed by reading the absorbance at 470 nm. BHT was used as a standard reference. The antioxidant property was given as an inhibition percentage [19].

2.2.2. DPPH Assay

The antioxidant effect test was done according to the previously reported protocols with slight modifications [19]. The plant extract and BHT (butylated hydroxytoluene) were tested with concentrations ranging from 1 to 14 mg/mL. Briefly, 150 µL of each concentration (1,2, 4,6, 8, 10,12, and 14 mg/mL) were dropped in wells supplemented with 50 µL of previously prepared 1 mM DPPH solution. The prepared microplates were saved at an ambient temperature in darkness for 31 min. The reading of absorbance was effectuated at 517 nm. BHT was considered as a standard antioxidant product.

2.3. Antiprolifertaive Activity

2.3.1. Cell Culture

MDA-MB-231 and HT-29 cancerous lines were used for screening the potential antiproliferative effect of *C. colocynthis* seed extract. Cells were obtained from UFR of pharmacy, Reims, France. The antiproliferative study was conducted according to protocols reported in earlier work [20].

2.3.2. Cell Viability Assay

The viability of cells was studied according to the assay as described in earlier work [21]. In this research work, the antiproliferative effect of *C. colocynthis* seed extract was investigated using MDA-MB-231 and HT-29 cells lines. These cancerous cells were kindly provided by Dr. L'Houcine Ouafik (laboratory of oncology—Marseille, France). A culture medium (MDEM) including 1% glutamine, 10% fetal calf serum, and 1% antibiotic (streptomycin/penicillin) was used to grow cells at 37 °C. For testing, cancerous cells were seeded on 96-well plates at a density of about 8000 cells per well. When the incubation period was finished, 10 µL of culture medium were replaced with an equivalent volume of *C. colocynthis* extract with concentrations ranging from 15.6 to 500 µg/mL. Next, the treated plates were incubated again for 72 h. Afterward, the WST-1 agent was added to the plates with further incubation for 4 h at 37 °C. For comparison purposes, the effect of *C. colocynthis* seed extract on healthy human dermal fibroblasts cultured under sub-similar conditions to those of cancer cells was investigated [22]. The reading of cell viability was conducted by using Wallac Victor X3 multiplate reader. In this protocol, non-treated cells were used as a negative control. The IC_{50} value (concentration required for killing of 50% of the cell population) was calculated from plotting the inhibition percentage vs. concentrations (µg/mL).

The percentage of antiproliferative activity was done as follows:

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

The concentration responsible for 50% cell inhibition (IC50) was performed from the dose-response curve.

2.4. Antibacterial Activity

Bacterial strains were used in the current research work including Gram-positive *Enterococcus faecalis* 471, *Listeria monocytogenes*, and *Staphylococcus aureus* 476; and Gram-negative *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium*. Bacterial growth was carried out at 37 °C on solid Müeller–Hinton Agar medium and peptone liquid medium.

The antibacterial effect was evaluated using the disc diffusion method. Briefly, 100.00 µL of inoculum of each bacterial suspension was seeded in Petri dishes with 20 mL of MHA medium. After 5 min, a disk of sterile Whatman paper with 0.5 cm in diameter was impregnated with 20 µL of the solubilized extract before being deposited on the plate surface. Afterward, dishes were placed at room temperature for 1 h and incubated again at 37 °C for a further 24 h to be ready for reading.

2.5. Identification of Constituents by GC-MS Analysis

The identification of phytochemical compositions of the *C. colocynthis* seed extract was performed by GC-MS. Briefly, 1 µL was injected for analysis into gas chromatography (GC/MS) equipped with a Thermo Fischer capillary column directly coupled to the mass spectrometry system and a column with HP-5MS fused silica capillary (30 m × 250 µm). The analysis was performed under the following GC/MS conditions: initial temperature of 50 °C/2 min, speed of 11 °C/min to a final temperature of 200 °C, hold for 0 min, ramp of 6 °C/min to 240 °C, hold for 1 min, carrier gas; helium (1 mL/min). Solvent delay: 4.00 min; injection temperature: 280 °C; detection temperature: 250 °C; scan: 40 to 450 Da. The identification of the extract phytochemicals was carried out by comparing the retention indices with those of the references obtained from the databases along with the calculation of retention indices (RI) [23–25].

2.6. Statistical Analysis

Data obtained in the current research were expressed using the means of duplicate bioassays ± SD. The obtained significant difference was performed using a *t*-test. Statistically, a significant difference was considered when $p < 0.05$

3. Results and Discussion

3.1. Antiproliferative Effect of C. colocynthis Seeds

Generally, the findings showed that both cancerous cells HT-29 and MDA-MB231 were sensitive to hexane extract of *C. colocynthis* seeds. The IC_{50} value of *C. colocynthis* seeds on MDA-MB231 cell lines was determined at 86.89 ± 3.395 µg/mL. Meanwhile, the IC_{50} value in inhibiting HT-29 was determined at 242.1 ± 17.9 µg/mL. The MDA-MB231 cell lines found to be more sensitive to the plant seed extract when compared to HT-29 ($p < 0.05$) (Figures 1 and 2).

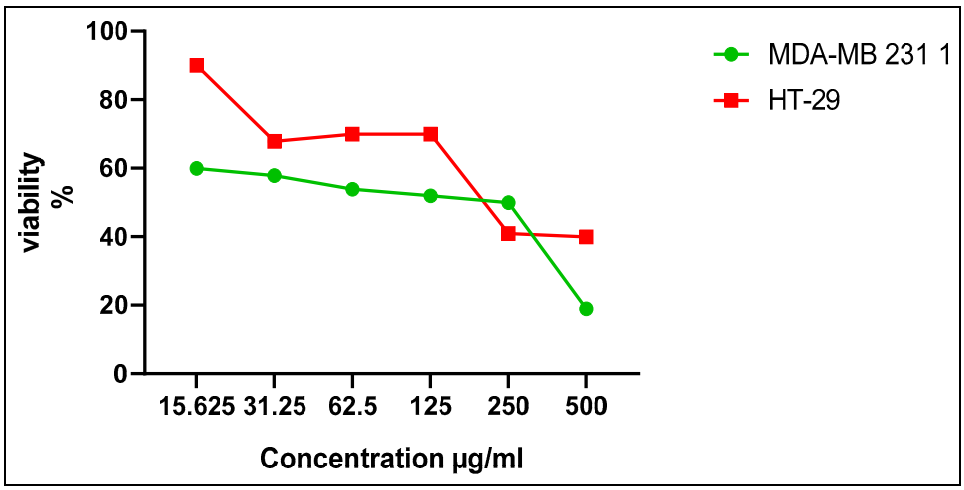


Figure 1. Curve of dose-response of cell viability assay after 72 h of treatment with hexane extract.

MDA-MB-231 cell lines were found to be more vulnerable to *C. colocynthis* seed organic extract than HT-29. Meanwhile, the extract of *C. colocynthis* seeds showed safety in in normal human dermal fibroblasts. The observed difference in IC_{50} values could be related to the difference in the treated cell lines (drug-resistant cell lines). The obtained findings in the current research conformed with a previous report, which showed that acetone pulp extract of *C. colocynthis* possessed cytotoxic effects on MCF-7, MDA-MB-231, and SiHa cancer lines [22,26]. In this sense, our hexane extract was majorly constituted of 2,4-dimethylhept-1-ene; (E)-hept-2-enal; 2-Pentenal, (E)-: 2,4,6-Trimethyloctane; Octane, 2,4,6-Trimethyl, and Undecane, which would be involved in the obtained results of antiproliferative activities; however, the *C. colocynthis* acetone extract reported in this literature is

mainly constituted of oxalic acid; 2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone; cyclobutyl octadecyl ester; Octadecatrienal, which might be involved in the cytotoxic effect on MCF-7, MDA-MB-231, and SiHa cancer lines. Moreover, both extracts of acetone and hexane were active towards MDA-MB-231 cell lines, so we can confirm that solvents with different polarities can result in different bioactive compounds from *C. colocynthis*.

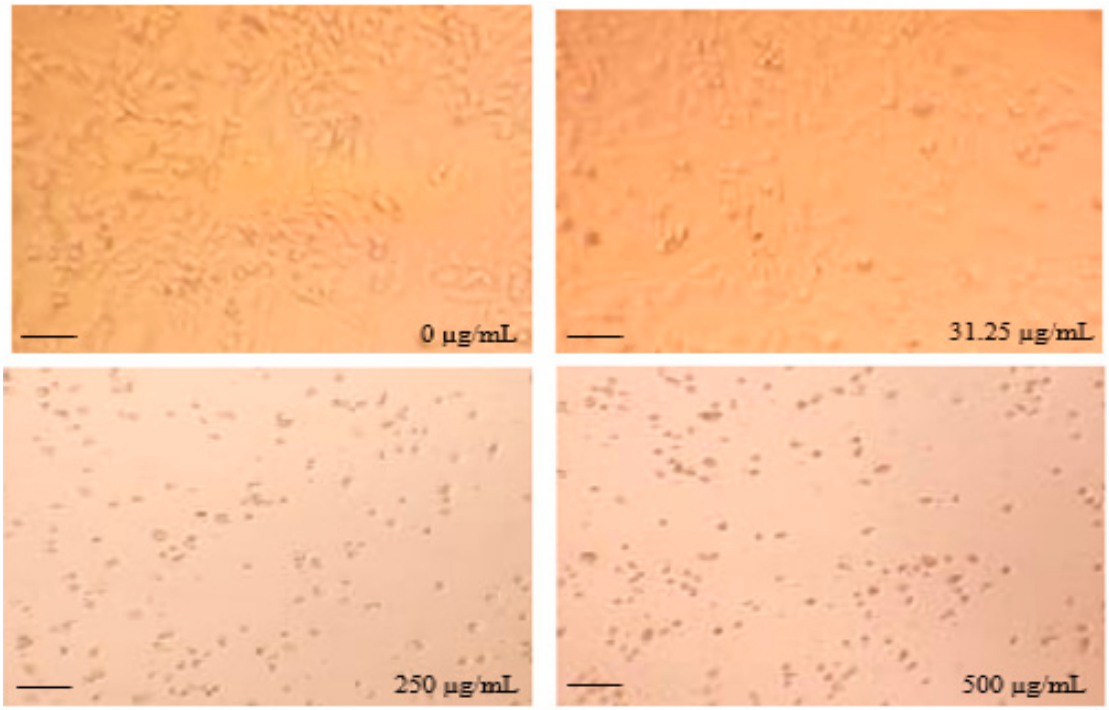


Figure 2. Photograph of MDA-MB231 cancer cell lines after 72 h of post-treatment with *C. colocynthis* seed extract (Scale bare = 0.5 cm).

3.2. Antioxidant Effect

In the current research study, hexane extract of *C. colocynthis* was screened for potential antioxidant activity using β-carotene bleaching and DPPH assay. As reported in Figure 3, the *C. colocynthis* organic extract exhibited potent antioxidant activity with an IC_{50} value of 2.22 mg/mL in beta-carotene bleaching. The hexane extract of *C. colocynthis* seeds was also studied in terms of antioxidant activity using DPPH assay as presented in Figure 4. The antioxidant activity increases with increasing extract concentration (Figure 4). Therefore, the studied extract exhibited antioxidant properties in a dose-dependent manner. The IC_{50} value of the organic studied extract was estimated at 8.98 ± 0.619 mg/mL.

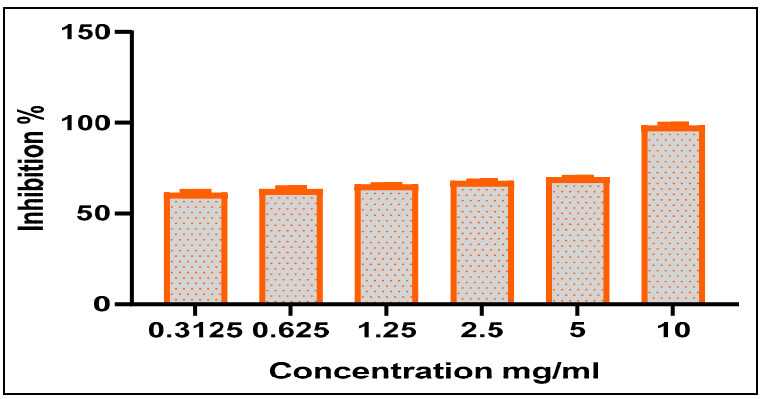


Figure 3. Beta-carotene bleaching percentage of hexane extract of *C. colocynthis* seed.

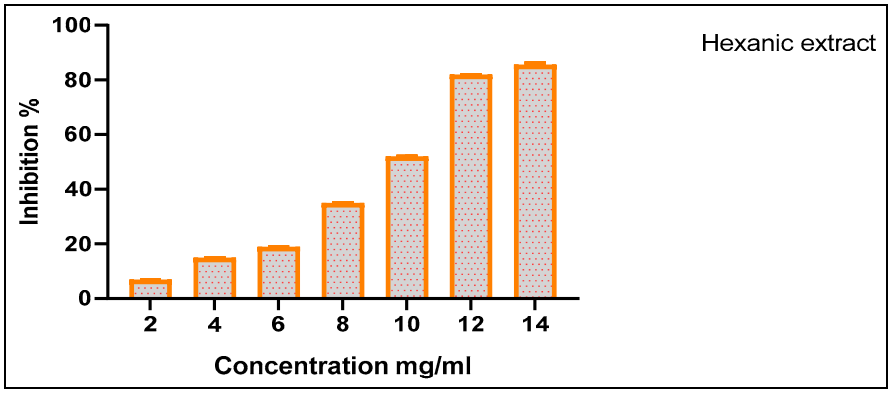


Figure 4. Scavenging activity of *C. colocynthis* hexane extract using DPPH assay.

The antioxidant activity of *C. colocynthis* seed extract effect was found more important. It is thus fitting that the present findings were in agreement with earlier work that reported free radical scavenging effects of *C. colocynthis* [27]. Many researchers have attributed the antioxidant property of *C. colocynthis* extract to the flavonoids content in its seeds and fruits, i.e., isosaponarin, isovitexin, and isoorientin3-O-methylether isolated from *C. colocynthis* possessed an important antioxidant activity with an IC_{50} value ranging from 5.62×10^{-4} to 7.13×10^{-2} mg/mL [28]. The reported findings in the current work were in accordance with earlier literature, which investigated the scavenging activity of *C. colocynthis* hexane extract [29].

3.3. Antibacterial Activity

The antibacterial effect of the tested extract varied as a function of the target microorganism. The studied extract did not affect Gram-negative bacterial strains whilst the hexane extract generated a clear growth inhibition zone on *E. faecalis* 471 strains with a diameter of inhibition zone reaching 3 mm (Gram-positive). However, *S. aureus* as a Gram-positive was not sensitive to the *C. colocynthis* extract.

Bacterial infections have presented a great challenge because of the eventual resistance of bacteria to modern drugs, hence the development of alternative drugs remains one of the most effective solutions to mitigate the extensions of bacterial infections. Desiring to contribute to the bacterial infection palliation, we tested *C. colocynthis* for its potential antibacterial activity in the current study. In this sense, the tested seed extract of *C. colocynthis* was active on some positive bacterial strains such as *E. faecalis* 471 and *L. monocytogenes*. However, no effect was observed on Gram-negative bacteria nor *S. aureus*. These findings were in agreement with previously reported studies, which highlighted a poor effect of *C. colocynthis* ethanolic extract on Gram-negative bacteria [30]. However, the aqueous and acetone extracts of *C. colocynthis* were active on both Gram-positive and Gram-negative bacteria, as reported in earlier data [31]. In this sense, the differences can be attributed to solvent polarities used for extraction. The antibacterial activity could probably due to the presence of cucurbitacin molecules since the cucurbitacin E was effective against *M. tuberculosis* H37Rvat [32].

3.4. Chemical Analysis of C. colocynhtis Seed Extract

The finding of chemical analysis of *C. colocynthis* seed extract revealed the presence of many interesting compounds majorly consisting of 2,4-Nonadienal, Tetradecane, hexadecane, pentadecane, cinnamic acid derivaties, Linalool, and butylated hydroxyanisole (Figure 5; Table 1).

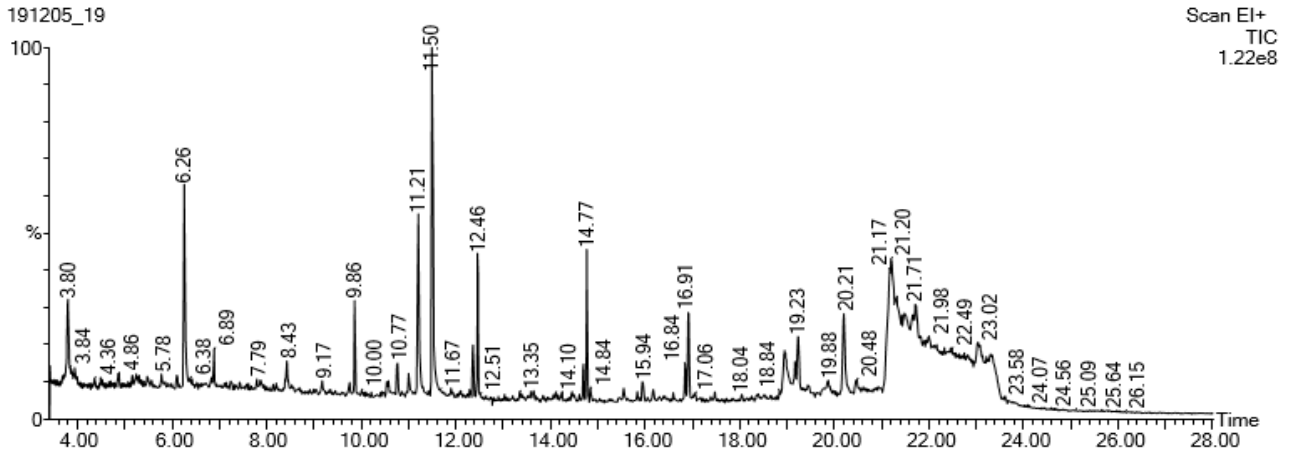


Figure 5. Chromatogram of chemicals identified in *C. colocynthis* seed extract.

Table 1. Chemical compounds identified in *C. colocynthis* seed extract.

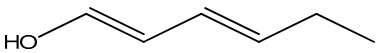
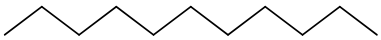
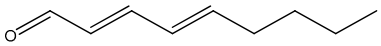
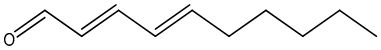
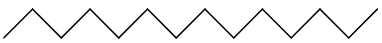
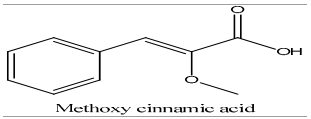
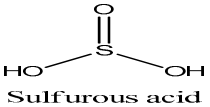
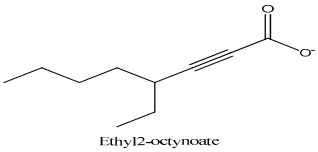
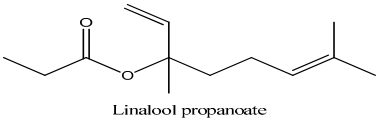
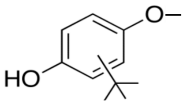
RT (min)	Compound	RI	Area (%)	Formula	Chemical Structure
3.80	Hexanal	801	4.54	$C_6H_{12}O$	 Hexanal
6.26	Hexadienol	916	3.61	$C_6H_{10}O$	 Hexadienol
9.86	Undecane	1100	2.04	$C_{11}H_{24}$	 Undecane
11.21	2,4-Nonadienal	1187	2.34	$C_9H_{14}O$	 2,4-Nonadienal
11.50	2,4-Decadienal	1295	7.79	$C_{10}H_{16}O$	 2,4-Decadienal
12.46	Tetradecane	1400	3.29	$C_{14}H_{30}$	 Tetradecane
14.77	Hexadecane	1600	1.27	$C_{16}H_{34}$	 Hexadecane
16.91	Methoxy cinnamic acid	1700	1.28	$C_{10}H_{10}O_3$	 Methoxy cinnamic acid
19.23	Sulfurous acid	1200	1.43	$C_{12}H_{26}$	 Sulfurous acid
20.21	Ethyl2-octynoate	1283	1.66	$C_{10}H_{16}O_2$	 Ethyl2-octynoate

Table 1. *Cont.*

RT (min)	Compound	RI	Area (%)	Formula	Chemical Structure
21.17	Linalool propanoate	1337	14.29	$C_{13}H_{22}O_2$	 Linalool propanoate
21.20	Pentadecane	1500	7.15	$C_{15}H_{32}$	 Pentadecane
21.71	Nonadienal	1381	15.39	$C_{13}H_{24}O_2$	 Nonadienal
23.02	Butylated hydroxy anisol	1489	4.28	$C_{11}H_{16}O_2$	
Total				70.36%	

The pharmacological activities investigated in this study are frequently related to the identified compounds in the *C. colocynthis* seed extract since the chemical analysis showed many potentially bioactive compounds with pharmacological activities, including antioxidant and antibacterial activities as reported in earlier works [30–35]. In this sense, Jasmonic acid played a crucial role in defense against insects, pathogenic microorganisms, kin aging, as well cancer development [36,37]. 2,4-Nonadienal is previously reported to possess antifungal, antidiarrheal, and antioxidant activities [38–40]. Tetradecane and hexadecane as well as pentadecane are alkanes possessing antifungal and antibacterial effects [41]. Cinnamic acid occurs naturally in flora. Cinnamic acid derivatives are reported to possess a wide spectrum of biological activities including antioxidant and anticancer activities [42]. Linalool is one of the major compounds in the *C. colocynthis* seed extract, with antioxidant and antimicrobial properties [43,44]. Butylated hydroxyanisol is also reported to have antioxidant power in previous work [45].

Our extract was generally rich in compounds belonging to alkanes, hydrocarbons, phenolic, and fatty acids. The presence of these compounds in our *C. colocynthis* seeds extract is probably correlated to its antioxidant, antiproliferative, and antibacterial effects. The identified compounds can react individually or in synergy and even with potential potentiation effects [46].

4. Conclusions

The present study evidenced the phytochemical composition, antioxidant, antibacterial, and acute toxicity testing of *C. colocynthis* extract. The studied plant extract possesses an interesting chemical composition with antioxidant, antiproliferative, and antibacterial properties. Therefore, *C. colocynthis* was used with the hope to contribute to the development of effective drugs against cancer and free-radical-related diseases, alongside bacterial infections.

Author Contributions: M.B., K.B., H.B.: writing—original draft; S.H., S.G., A.L.: formal analysis; A.M.S., A.A., H.K.A., N.A.A.: writing—reviewing and editing; M.E.M., L.B.: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Lehmann, H.; Pabst, J.-Y. La phytovigilance: Impératif médical et obligation légale. *Ann. Pharm. Fr.* **2016**, *74*, 49–60. [CrossRef]
2. Bouayyadi, L.; El Hafian, M.; Zidane, L. Étude floristique et ethnobotanique de La flore médicinale dans la région du gharb, maroc. *J. Appl. Biosci.* **2015**, *93*, 8770–8788. [CrossRef]
3. Lardry, J.-M.; Haberkorn, V. L'aromathérapie et les huiles essentielles. *Kinésithérapie Rev.* **2007**, *7*, 14–17. [CrossRef]
4. Roulier, G. *La Sante Au Feminin: Hygiene, Prevention et Traitements Naturels des Maladies de La Femme*; Editions Dangles: St-Jean de Braye, France, 1988; ISBN 978-2-7033-0329-9.
5. Couic-Marinier, F.; Lobstein, A. Les huiles essentielles gagnent du terrain à l'officine. *Actual. Pharm.* **2013**, *52*, 18–21. [CrossRef]
6. Bellakhdar, J.; Claisse, R.; Fleurentin, J.; Younos, C. Repertory of standard herbal drugs in the moroccan pharmacopoea. *J. Ethnopharmacol.* **1991**, *35*, 123–143. [CrossRef]
7. Jamil, F.; Mostaf, E. An overview on ethnobotanico-pharmacological studies carried out in Morocco, from 1991 to 2015: Systematic review (Part 1). *J. Ethnopharmacol.* **2020**, *267*, 113200.
8. Hadizadeh, I.; Peivastegan, B.; Kolahi, M. Antifungal activity of nettle (*Urtica dioica* L.), colocynth (*Citrullus colocynthis* L. Schrad.), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi* L.) extracts on plants pathogenic fungi. *Pak. J. Biol. Sci.* **2009**, *12*, 58. [CrossRef]
9. Mehta, A.; Srivastva, G.; Kachhwaha, S.; Sharma, M.; Kothari, S.L. Antimycobacterial activity of *Citrullus colocynthis* (L.) Schrad. against drug sensitive and drug resistant mycobacterium tuberculosis and MOTT clinical isolates. *J. Ethnopharmacol.* **2013**, *149*, 195–200. [CrossRef]
10. Torkey, H.M.; Abou-Yousef, H.M.; Abdel Azeiz, A.Z.; Hoda, E.A.F. Insecticidal effect of cucurbitacin E glycoside isolated from *citrullus colocynthis* against aphis craccivora. *Aust. J. Basic Appl. Sci.* **2009**, *3*, 4060–4066.
11. Abo, K.A.; Fred-Jaiyesimi, A.A.; Jaiyesimi, A.E.A. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J. Ethnopharmacol.* **2008**, *115*, 67–71. [CrossRef] [PubMed]
12. Adam, S.E.I.; Al-Yahya, M.A.; Al-Farhan, A.H. Response of najdi sheep to oral administration of *citrullus colocynthis* fruits, nerium oleander leaves or their mixture. *Small Rumin. Res.* **2001**, *40*, 239–244. [CrossRef]
13. Marzouk, B.; Marzouk, Z.; Décor, R.; Mhadhebi, L.; Fenina, N.; Aouni, M. Antibacterial and antifungal activities of several populations of tunisian *citrullus colocynthis* Schrad. Immature fruits and seeds. *J. Mycol. Médicale* **2010**, *20*, 179–184. [CrossRef]
14. Tabani, K.; Birem, Z.; Halzoune, H.; Saiah, W.; Lahfa, F.; Koceir, E.A.; Omari, N. Therapeutic effect of alkaloids and glycosides of colocynth seeds on liver injury, associated with metabolic syndrome in wistar rats, subject to nutritional stress. *Pak. J. Pharm. Sci.* **2018**, *31*, 277–290. [PubMed]
15. Ziyyat, A.; Legssyer, A.; Mekhfi, H.; Dassouli, A.; Serhrouchni, M.; Benjelloun, W. Phytotherapy of hypertension and diabetes in oriental Morocco. *J. Ethnopharmacol.* **1997**, *58*, 45–54. [CrossRef]
16. Tahraoui, A.; El-Hilaly, J.; Israili, Z.H.; Lyoussi, B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in South-Eastern Morocco (Errachidia province). *J. Ethnopharmacol.* **2007**, *110*, 105–117. [CrossRef]
17. Jeon, J.-H.; Lee, H.-S. Biofunctional constituent isolated from *citrullus colocynthis* fruits and structure–activity relationships of its analogues show acaricidal and insecticidal efficacy. *J. Agric. Food Chem.* **2014**, *62*, 8663–8667. [CrossRef]
18. Milovanović, M.; Pićurić-Jovanović, K. Characteristics and composition of melon seed oil. *J. Agric. Sci. Belgrade* **2005**, *50*, 41–47. [CrossRef]
19. Bourhia, M.; Laasri, F.E.; Moussa, S.I.; Ullah, R.; Bari, A.; Saeed Ali, S.; Kaoutar, A.; Haj Said, A.A.; El Mzibri, M.; Said, G. Phytochemistry, antioxidant activity, antiproliferative effect, and acute toxicity testing of two Moroccan aristolochia species. *Evid. Based Complement. Alternat. Med.* **2019**, *2019*. [CrossRef]
20. Tannin-Spitz, T.; Bergman, M.; Grossman, S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. *Biochem. Biophys. Res. Commun.* **2007**, *364*, 181–186. [CrossRef]
21. Bourhia, M.; Laasri, F.E.; Aourik, H.; Boukhris, A.; Ullah, R.; Bari, A.; Ali, S.S.; El Mzibri, M.; Benbacer, L.; Gmouh, S. Antioxidant and Antiproliferative Activities of Bioactive Compounds Contained in Rosmarinus Officinalis Used in the Mediterranean Diet. Evid. Based Complement. *Alternat. Med.* **2019**, *2019*. [CrossRef]
22. Al-Hwaiti, M.S.; Alsbou, E.M.; Abu Sheikha, G.; Bakchiche, B.; Pham, T.H.; Thomas, R.H.; Bardaweel, S.K. Evaluation of the anticancer activity and fatty acids composition of "Handal"(*Citrullus colocynthis* L.) seed oil, a desert plant from South Jordan. *Food Sci. Nutr.* **2021**, *9*, 282–289. [CrossRef]
23. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [CrossRef]
24. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
25. Amrati, F.E.-Z.; Bourhia, M.; Saghrouchni, H.; Slighoua, M.; Grafov, A.; Ullah, R.; Ezzeldin, E.; Mostafa, G.A.; Bari, A.; Ibenmoussa, S. Caralluma europaea (Guss.) NE Br.: Anti-inflammatory, antifungal, and antibacterial activities against nosocomial antibiotic-resistant microbes of chemically characterized fractions. *Molecules* **2021**, *26*, 636. [CrossRef] [PubMed]

26. Chowdhury, K.; Sharma, A.; Kumar, S.; Gunjan, G.K.; Nag, A.; Mandal, C.C. Colocynth extracts prevent epithelial to mesenchymal transition and stemness of Breast Cancer Cells. *Front. Pharmacol.* **2017**, *8*, 593. [CrossRef] [PubMed]
27. Benariba, N.; Djaziri, R.; Bellakhdar, W.; Belkacem, N.; Kadiata, M.; Malaisse, W.J.; Sener, A. Phytochemical screening and free radical scavenging activity of *citrullus colocynthis* seeds extracts. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 35–40. [CrossRef]
28. Delazar, A.; Gibbons, S.; Kosari, A.R.; Nazemiyeh, H.; Modarresi, M.; Nahar, L.; Sarker, S.D. Flavone C-glycosides and cucurbitacin glycosides from citrullus colocynthis. *Daru* **2006**, *14*, 109–114.
29. Hussain, A.I.; Rathore, H.A.; Sattar, M.Z.; Chatha, S.A.; ud din Ahmad, F.; Ahmad, A.; Johns, E.J. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* (L.) from the pakistani flora. *Ind. Crops Prod.* **2013**, *45*, 416–422. [CrossRef]
30. Memon, U.; Brohi, A.H.; Ahmed, S.W.; Azhar, I.; Bano, H. Antibacterial screening of citrullus colocynthis. *Pak. J. Pharm. Sci.* **2003**, *16*, 1–6. [PubMed]
31. Marzouk, B.; Marzouk, Z.; Décor, R.; Edziri, H.; Haloui, E.; Fenina, N.; Aouni, M. Antibacterial and anticandidal screening of tunisian *citrullus colocynthis* Schrad. from Medenine. *J. Ethnopharmacol.* **2009**, *125*, 344–349. [CrossRef]
32. Bourhia, M.; Messaoudi, M.; Bakrim, H.; Mothana, R.A.; Sddiqui, N.A.; Almarfadi, O.M.; El Mzibri, M.; Gmouh, S.; Laglaoui, A.; Benbacer, L. *Citrullus Colocynthis* (L.) Schrad: Chemical Characterization, Scavenging and Cytotoxic Activities. *Open Chem.* **2020**, *18*, 986–994. [CrossRef]
33. Srivastava, G.; Jain, R.; Vyas, N.; Mehta, A.; Kachhwaha, S.; Kothari, S.L. Antimicrobial Activity of the Methanolic Extract, Fractions and Isolated Compounds from *Citrullus colocynthis* (L.) Schrad. *Int. J. Pharma Bio Sci.* **2013**, *4*, 825–833.
34. Rahimi, R.; Amin, G.; Ardekani, M.R.S. A review on *citrullus colocynthis* Schrad.: From traditional Iranian medicine to modern phytotherapy. *J. Altern. Complement. Med.* **2012**, *18*, 551–554. [CrossRef]
35. Ahmed, M.; Ji, M.; Qin, P.; Gu, Z.; Liu, Y.; Sikandar, A.; Iqbal, M.F.; Javeed, A. Phytochemical screening, total phenolic and flavonoids contents and antioxidant activities of *Citrullus colocynthis* L. and *Cannabis sativa* L. *Appl. Ecol. Env. Res.* **2019**, *17*, 6961–6979. [CrossRef]
36. Michelet, J.F.; Olive, C.; Rieux, E.; Fagot, D.; Simonetti, L.; Galey, J.B.; Dalko-Csiba, M.; Bernard, B.A.; Pereira, R. The anti-ageing potential of a new jasmonic Acid Derivative (LR2412): In Vitro evaluation using reconstructed epidermis episkin^{TM}. *Exp. Dermatol.* **2012**, *21*, 398–400. [CrossRef]
37. Russo, A.; Espinoza, C.L.; Caggia, S.; Garbarino, J.A.; Peña-Cortés, H.; Carvajal, T.M.; Cardile, V. A new jasmonic acid stereoisomeric derivative induces apoptosis via reactive oxygen species in human prostate cancer cells. *Cancer Lett.* **2012**, *326*, 199–205. [CrossRef] [PubMed]
38. Kobaisy, M.; Tellez, M.R.; Webber, C.L.; Dayan, F.E.; Schrader, K.K.; Wedge, D.E. Phytotoxic and fungitoxic activities of the essential oil of kenaf (*hibiscus cannabinus* L.) leaves and its composition. *J. Agric. Food Chem.* **2001**, *49*, 3768–3771. [CrossRef]
39. Zavala-Sanchez, M.A.; Pérez-Gutiérrez, S.; Pérez-González, C.; Sánchez-Saldivar, D.; Arias-García, L. Antidiarrhoeal activity of nonanal, an aldehyde isolated from artemisia ludoviciana. *Pharm. Biol.* **2002**, *40*, 263–268. [CrossRef]
40. Saidana, D.; Mahjoub, M.A.; Boussaada, O.; Chriaa, J.; Chéraif, I.; Daami, M.; Mighri, Z.; Helal, A.N. Chemical composition and antimicrobial activity of volatile compounds of tamarix boveana (tamaricaceae). *Microbiol. Res.* **2008**, *163*, 445–455. [CrossRef]
41. Yogeswari, S.; Ramalakshmi, S.; Neelavathy, R.; Muthumary, J. Identification and comparative studies of different volatile fractions from monochaetia kansensis by GCMS. *Glob. J. Pharmacol.* **2012**, *6*, 65–71.
42. Pontiki, E.; Hadjipavlou-Litina, D.; Litinas, K.; Geromichalos, G. Novel cinnamic acid derivatives as antioxidant and anticancer agents: Design, synthesis and modeling studies. *Molecules* **2014**, *19*, 9655–9674. [CrossRef] [PubMed]
43. Duarte, A.; Luís, Â.; Oleastro, M.; Domingues, F.C. Antioxidant properties of coriander essential oil and linalool and their potential to control *Campylobacter* spp. *Food Control* **2016**, *61*, 115–122. [CrossRef]
44. Herman, A.; Tambor, K.; Herman, A. Linalool affects the antimicrobial efficacy of essential oils. *Curr. Microbiol.* **2016**, *72*, 165–172. [CrossRef] [PubMed]
45. Iverson, F. Phenolic antioxidants: Health protection branch studies on butylated hydroxyanisole. *Cancer Lett.* **1995**, *93*, 49–54. [CrossRef]
46. El Fakir, L.; Bouothmany, K.; Alotaibi, A.; Bourhia, M.; Ullah, R.; Zahoor, S.; El Mzibri, M.; Gmouh, S.; Alaoui, T.; Zaid, A. Antioxidant and understanding the anticancer properties in human prostate and breast cancer cell lines of chemically characterized methanol extract from berberis hispanica Boiss. & reut. *Appl. Sci.* **2021**, *11*, 3510.