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Inhibitory Activity of Leaves Extracts of *Citrullus colocynthis* Schrad. On HT29 Human Colon Cancer Cells

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AB, BM and FM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BM, FM, CAJ, SG, KB, LA and ZM managed the analyses of the study and the literature searches. Author AB had primary responsibility for the final content. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Citrullus colocynthis* is a plant endemic in Asia, Africa and in the Mediterranean basin. It is used in folk medicine against infections, inflammations and cardiovascular and immune-related diseases. There are further evidences of the use of *Citrullus colocynthis* Schrad in the treatment of cancer in traditional practices. The present study aimed to determine the potential antiproliferative effects of different *Citrullus colocynthis* leaf extracts on human cancer cells.
Methodology: Antiproliferative and antioxidant effects on HT-29 human colon cancer cells were detected by MTS assay and a modified protocol of the alkaline Comet assay. *In vitro* antioxidant activities of different leaf extracts were evaluated through DPPH, β-carotene/linoleic acid and reducing power assays.
Results: The leaf chloroform extract exhibited the higher cell growth inhibitory activity without induction of DNA damage; it showed to be able to significantly decrease DNA damage induced by H_2O_2 (100 µM). This antioxidant activity seems to be comparable to that of vitamin C (1 mM). Ethyl acetate, acetone and methanol leaf extracts showed to be the most effective in reducing the stable free DPPH radical (IC_{50} =113 µg/ml), in transforming the Fe^{3+} to Fe^{2+} (IC_{50} = 134 µg/ml) and in inducing linoleic acid oxidation with an inhibition of 31.9 %.
Conclusion: Our results confirm the antiproliferative potential of *Citrullus colocynthis* Schrad. on human cancer cells.

Keywords: Antiproliferative; DNA damage; antioxidant; DPPH assay, β-carotene/linoleic acid assay; reducing power assay.

1. INTRODUCTION

Citrullus colocynthis Schrad. (cucurbitaceae), is a desert plant native to the Mediterranean basin and Asia. It is a medicinal plant widely used in the folk medicine of these areas for treatment of many diseases such as rheumatisms, hypertension, dysentery, diabetes, dermatological problems and gynaecological or pulmonary infections [1,2]. Moreover, there are evidences of the use of fruits [1] or of a combination of *Citrullus colocynthis* Schrad flowers, leaves and roots in the treatment of cancer [2].

Citrullus colocynthis Schrad. contains cucurbitacins glucosides, terpens predominantly found in the cucurbitaceae family. The cucurbitacins have demonstrated many biological effects, such as immunostimulant [3], anti-microbial [4], antioxidant, analgesic and anti-inflammatory [5–7] properties, and efficient activities against hepatic diseases [8] and hyperglycaemia [9]. In addition, cucurbitacins have shown a growth inhibitory activity on cancer cells through different mechanisms [10,11]. Since the presence of cucurbitacins along with other biologically active molecules may explain the efficacy of *Citrullus colocynthis* Schrad. in the treatment of cancer, scientific studies are needed to support this idea. Thereafter, the aim of our study was to evaluate the ability of different extracts of *Citrullus colocynthis* Schrad. leaves to induce cell growth inhibition on a human colon cancer cell line (HT29). Since the oxidative stress can lead to inhibition of the proliferation, we evaluated the *in vitro* antioxidant effect of the leaf extracts and, for the chloroform extract, which proved to be the most antiproliferative one, we evaluated the genotoxic and antioxidant activity on the HT29 cell line.

To the best of our knowledge, comparisons between in-vitro and cell-based antioxidant assays on *Citrullus colocynthis* Schrad. leaf extracts have never been reported.

2. MATERIALS AND METHODS

2.1 Plant Material

Citrullus colocynthis Schrad. leaves were collected in August 2007 from nearby Medenine village, El-Araidha region, Sidi Makhlouf municipality, Tunisia. The taxonomic identification of the plant material was confirmed by a plant taxonomist, Marzouk, Z., in the biological laboratory of the Faculty of Pharmacy of Monastir-Tunisia- according to the flora of Tunisia [12]. A voucher specimen (C.C-01.01) has been deposited in this laboratory.

2.2 Preparation of Extracts

Collected plant materials were dried; the leaves were separated from the stems and ground in a

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grinder with a 2 mm mesh. Different solvents in ascending polarity (petroleum ether, chloroform, ethyl acetate, acetone and methanol) were used for Soxhlet extraction to fractionate the soluble compounds from the leaves. The extraction was performed with dried powder placed inside a thimble made by thick filter paper, loaded into the main chamber of the Soxhlet extractor, which consisted of an extracting tube, a glass balloon and a condenser. The total extracting time was 6 h for each solvent continuously refluxing over the sample. The resulting extracts were evaporated at reduced pressure to obtain the crude extracts.

2.3 *In vitro* Antioxidant Activity

2.3.1 DPPH assay

The in vitro antioxidant activity of *Citrullus colocynthis* Schrad. leaf extracts and control substances was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [13]. Fifty microliters of various concentrations of the sample in methanol (leaf extracts and control substances) were added to 2 ml of a 60 µM methanolic solution of DPPH. Absorbance measurements were read at 517 nm, after 20 min of incubation time at room temperature. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula: IP (%) = $(A_{blank}-A_{sample}/A_{blank}) \times 100$; where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentage against extract concentration. Synthetic antioxidant reagents BHT and quercetine were used as a positive control and all tests were carried out in triplicate and were reported as means ± Standard Deviation (SD).

2.3.2 Reducing power determination

The reductive potential of the *Citrullus colocynthis* leaf extracts and of the standards (BHT and quercetine) was determined according to the method of Oyaizu [14]. The different methanolic samples were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$; 2.5 ml, 1%). The mixture was then incubated at 50℃ for 20 min. Afterwards 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicate increased reducing power. The assay was carried out in triplicate and IC_{50} were reported as means±SD.

2.3.3 β-Carotene / linoleic acid bleaching assay

The β -carotene/linoleic acid test evaluates the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O_2). The amount of the remained β-carotene gives an estimation of the antioxidant potential of the sample.

The method described by Miraliakbari [15] was used with minor modifications. A mixture of β-carotene and linoleic acid was prepared by mixing 0.5 mg β-carotene in 1 ml chloroform (HPLC grade), 25 µl linoleic acid and 200 mg Tween 40. The chloroform was then completely evaporated under vacuum and 100 ml of oxygenated distilled water was subsequently added to the residue and mixed gently to form a clear yellowish emulsion. The extracts and positive controls (BHT and quercetine) were individually dissolved in methanol (2 g/l) and 350 µl of each of them were added to 2.5 ml of the above emulsion in test tubes and mixed thoroughly. The test tubes were incubated in a water bath at 50℃ for 2h. Negative control (blank) contained only methanol. The absorbance values were measured at 470 nm through an ultraviolet and visible (UV–Vis) spectrometer. Antioxidant activities (inhibition percentage, IP %) of the samples were calculated using the following equation: IP % = $(A_{\beta\text{-carotene after 2 h}} / A_{\text{initial β-carotene}}) * 100$; where $A_{\beta\text{-carotene after 2 h}}$ is the absorbance values of β-carotene after 2 h assay and $A_{\text{initial β-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiment.

All tests were carried out in triplicate and inhibition percentages were reported as means ± SD.

2.4 Biological Activities on HT-29 Cells

2.4.1 Maintenance of cell line

For *in vitro* cellular studies we employed the human colon adenocarcinoma cell line HT-29, used widely as a model for colon cancer.

The HT-29 cell line, kindly obtained from the Northern Ireland Center for Food and Health, was maintained in 25 cm^2 flasks with 5 ml of Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% foetal bovine serum (FBS), 1% L-glutamine and 0.5% penicillin/streptomycin in a humidified atmosphere containing 5% CO_2 at 37℃ in an incubator. Every 3 days the cells were sub-cultured by splitting the culture with fresh medium.

2.4.2 Antiproliferative effect

The antiproliferative activities of leaf extracts was measured by MTS assay (Promega, Madison, WI) [16].

HT-29 cells were seeded ($5x10^4$ cell/ml) in 96-well flat-bottom plates. After 24 h of incubation, HT-29 cells were treated, in quadruplicate, with increasing concentrations of leaf extracts or vehicle control and incubated for 24 h. Subsequently, 20 µl of MTS solution was added directly to culture wells and after 4h of incubation the absorbance at 450 nm with a 96-well plate reader (MULTISKAN EX, Thermo Electron Corporation, Vantaa, Finland) was recorded.

2.4.3 Cell viability

The Trypan blue exclusion method is used to determine the number of viable cells in a cell suspension. The assay is based on the principle that dye passes only through the permeabilized membranes of dead cells. Briefly, after 24h of treatment with 500 µg/ml leaf chloroform extract, cells were trypsinized and resuspended in DMEM; a 1:1 dilution of the cell suspension was obtained using a 0.4% trypan blue solution (BioWhittake®, Lonza). The dilution was loaded on a counting chamber of a haemocytometer in order to evaluate the percentage of viable cells [17].

2.4.4 Genotoxicity

Alkaline comet assay was performed according to standard methods [18] with minor modifications. DNA damage was evaluated on HT-29 cell line. Cells were seeded at 4 x 10^5 cell/ml in 6-well plates in DMEM supplemented with 1% glutamine, 0.5% penicillin/streptomycin and 10% foetal bovine serum. After seeding (24h), cells were treated with a range of concentrations of leaf chloroform extract (0.5÷10 µg/ml) that did not induce high cytotoxicity (viability>70%). After 24h of incubation at37℃, cells were trypsinized and resuspended in DMEM at a concentration of 5 x 10^4 cell/ml; the suspensions were centrifuged (1 min, 800 g) to recover the cells and the cell pellets were resuspended in 90 µl Low Melting Agarose 0.7% (LMA), transferred onto degreased microscope slides previously dipped in 1% Normal Melting Agarose (NMA) for the first layer. The agarose was allowed to set for 15 min at 4℃ before the addition of a final layer of LMA. Cell lysis was carried out at 4℃ overnight by exposing the cells to a buffer containing 2.5 M NaCl, 100 mM Na_2EDTA, 8 mM Tris-HCl, 1% Triton X-100 and 10% DMSO, pH 10. The electrophoretic migration was performed in an alkaline buffer (1 mM Na_2EDTA, 300 mM NaOH, 0℃) at pH>13 (DNA unwinding: 20 min; electrophoresis: 20 min, 0.78Vcm-1, 300mA). DNA was stained with 75 µl ethidium bromide (10 µg/ml) before the examination at 400x magnification under a Leica DMLS fluorescence microscope (excitation filter BP 515-560 nm, barrier filter LP 580 nm), using an automatic image analysis system (Comet Assay III– Perceptive Instruments Ltd, UK). Tail fluorescence percentage (TI, tail intensity) and DNA migration (TL, tail length) provided representative data on DNA damaging effects. For each sample, coded and evaluated blind, 100 cells were analyzed and the median values (+SD) of TI and TL were calculated. Nuclei with a completely disintegrated head region, named hedgehogs, were only registered as percentage (GC, ghost cell).

2.4.5 Oxidative DNA damage assay

A modified protocol of the Comet assay was used to study the antioxidant effect of the extracts [19]. The amount of DNA damage caused by an oxidative damage-inducing agent on cells pre-treated or not with leaf chloroform extract was evaluated. Cells were seeded at 4 x 10^5 cell/ml in 6-well plates in DMEM supplemented with 1% glutamine, 0.5% penicillin/streptomycin and 10% foetal bovine serum. After seeding (24 h), HT-29 cells were incubated with the extract (5 µg/ml) or with a known antioxidant agent, vitamin C (1mM), and incubated for 24h. After incubation, the cells were trypsinized and resuspended in DMEM (supplemented with 1% glutamine, 0.5% penicillin/streptomycin and 10% foetal bovine serum) at a concentration of $1x10^5$ cell/ml for a further treatment in suspension, before perform Comet assay, with 100µM hydrogen peroxide (H_2O_2) on ice for 5 min. After this, the

suspensions were centrifuged twice (1 min, 800 g) to wash and recover the cells. The slides were prepared and analyzed as reported above.

2.5 Statistical Evaluation

The data were analyzed using the statistical and graphical functions of SPSS 18 (SPSS Inc., Chicago, IL, USA). Differences were assessed using analysis of variance (ANOVA), followed by Bonferroni's post-hoc test as appropriate. Moreover, the Pearson's correlation coefficient of various data set about the *in vitro* antioxidant activity was calculated to assess their dependence. Significance was accepted at p =.05 level.

3. RESULTS

3.1 *In vitro* Antioxidant Activity

Free radical scavenging properties of leaf different extracts of *Citrullus colocynthis* Schrad., expressed as IC_{50} (µg/ml) for the DPPH and the reducing power assay and as inhibition percent (%) for the β-carotene/linoleic acid test, are given in Table 1. Free radical DPPH scavenging activity and reducing power assay of the extracts are concentration dependent. The most polar extract (methanol extract) was able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-coloured diphenylpicry-lhydrazine with an IC_{50} of 113.366 µg/ml, to reduce the Fe^{3+} to Fe^{2+} with an IC_{50} of 124.173 µg/ml and to exhibit a linoleic acid oxidation inhibition of 31.916% in the β-carotene/linoleic acid test. On the other hand, petroleum ether and chloroform extracts showed the lower antioxidant activities.

Dependence of these *in vitro* antioxidant assays data sets was characterized by calculating their Pearson's correlation (R_P) dividing the covariance of the two variables by the product of their standard deviations. The Pearson's correlation value is between -1 and 1 indicating the degree of linear dependence between the variables as well as the positive or negative nature of dependence. The closer the coefficient is to either -1 or 1 the stronger the correlation between the variables.

Our results pointed out a significant strong and positive correlation (R_P =.956) between DPPH assay and reducing power assay. Otherwise, we observed a significant strong and negative correlation between DPPH assay and β-carotene/linoleic acid test (R_P = -.903) and between reducing power assay and β-carotene/linoleic acid test (R_P = -.891).

3.2 Cytotoxic and Antiproliferative Effects

Proliferation of HT-29 cells was significantly inhibited by *Citrullus colocynthis* leaf extracts in a dose-dependent manner. Antiproliferative activity was expressed as the concentration able to inhibit of 50% cells growth (IC_{50}). Based on the IC_{50} values (Fig. 1), the inhibitory effects of the leaf extracts on HT-29 cell proliferation are in the decreasing order: acetone extract (368 µg/ml) > petroleum ether extract (355 µg/ml) > methanol extract (348 µg/ml) > ethyl acetate extract (296 µg/ml) > chloroform extract (160 µg/ml).

In order to distinguish between a cytotoxic or cytostatic effect of the chloroform extract, the most antiproliferative one, we evaluated the number of viable cells (Trypan Blue exclusion method) after a 24h treatment at the highest tested concentration (500 µg/ml) and we observed a cytotoxic effect.

In addition, preliminary data (data not reported) on HT-29 cell line, after a treatment of 24h with the leaf chloroform extract (500 µg/ml), suggest the apoptosis pathway involvement.

Table 1. *In vitro* antioxidant activity of *Citrullus colocynthis* leaf extracts

Extract	DPPH scavenging activity (IC_{50})	Reducing power (IC_{50})	β-carotene/ linoleic acid test (%)
PE	239.82±5.48	478.10±25.51	19.98±0.17
Chl	202.88±2.82	461.69±20.05	20.77±0.39
EA	180.69±1.77	392.62±2.59	21.48±0.62
A	128.94±0.48	134.17±2.54	25.88±1.07
M	113.37±0.58	124.27±3.51	31.92±2.30
Q	1.04±0.01	2.32±0.09	55.89±1.45
BTH	4.40±0.10	7.35±0.13	89.00±2.07

** PE: Petroleum ether; Chl: Chloroform; EA: Ethyl acetate; A: Acetone; M: Methanol; Q: Quercetine; BHT*

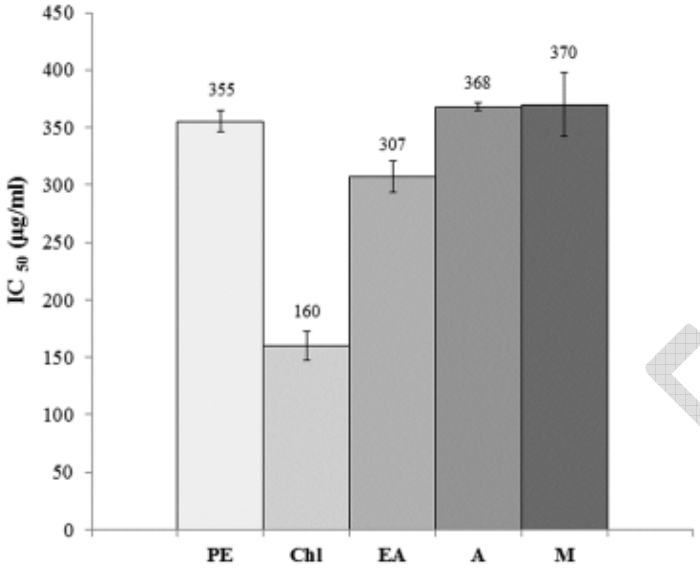

Fig. 1. Antiproliferative effect of *Citrullus colocynthis* on HT-29 after 24h in vitro treatment with different leaf extracts (0.5;5.0;50.0;500.0 µg/ml)

IC_{50}: 50% cell growth inhibitory concentration
PE: Petroleum ether; Chl: Chloroform; EA: Ethyl acetate; A: Acetone; M: Methanol.
Mean ± S.D.= Mean values ± Standard deviation of means of three experiments

3.3 Genotoxicity

Genotoxicity was measured using single-cell gel electrophoresis (Comet assay) in the alkaline version (pH > 13). This assay detects single-strand breaks and alkali-labile sites, such as apyrimidinic and apurinic sites, that are formed when bases are lost, and oxidized bases. Chloroform leaf extract did not induce any DNA damage on HT-29 cell after 24h treatment (Table 2).

3.4 Oxidative DNA Damage Protection

The antioxidant activity of chloroform leaf extract was evaluated on 24h pre-treated HT-29 cell line as reduction of DNA migration induced by H_2O_2.

The extract showed a significant decrease of DNA damage induced by H_2O_2 (Fig. 2). In particular at 5 µg/ml the chloroform leaf extract presented a protective antioxidant activity comparable to that of vitamin C (1 mM) (Fig. 2).

4. DISCUSSION

Traditional medical information could be a successful starting point to develop new anticancer strategies. Some worldwide used drugs employed in chemotherapy were isolated from plants that grow in particular environments such as taxol from the Pacific yew tree; camptothecin from the Chinese “happy tree” *Camptotheca acuminata* and combretastatin, from the South African bush willow [20]. The potential of ethno-drugs as to be proven with stringent scientific approach. *Citrullus colocynthis* Schrad. is an interesting traditional medicinal plant that showed different biological activities. In this study, we tried to evaluate the ability of *C. colocynthis* leaf extracts to control colon cancer cell proliferation in relation to their antioxidant properties.

During the common cellular pathways there is a physiological release of reactive oxygen/nitrogen species (ROS/RNS), such as superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radical, involved in mechanisms such as energy supply, detoxification and signalling. Endogenous antioxidant mechanisms, including enzymatic defences, contribute to their efficient removal. When they fail, free radicals induce an oxidative damage. These oxidative species are known to lead to DNA, lipid and protein damage compromising cellular processes such as proliferation, apoptosis and senescence [21,22]. Consequently, exposure to free radicals potentially increases the risk of a wide array of human diseases, such as cardiovascular diseases and cancer. For this reason there is a continuous search for new medical preparations to counteract the effects of oxidative stress.

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Table 2. Tail fluorescence percentage (TI, tail intensity), DNA migration (TL, tail length) and Ghost cell (GC) percentage detected by Comet assay on HT-29 after 24h in vitro treatment with the chloroform leaf extract

Extract concentration (µg/ml)	TI (%)	TL (µm)	GC (%)
0.0	0.14±0.01	18.58±4.70	3.5±0.71
0.5	0.51±0.10	21.26±1.60	14.5±3.54
1.0	0.42±0.27	21.26±2.07	8.5±2.12
5.0	0.32±0.03	18.83±1.03	6.5±2.83
10.0	0.27±0.01	17.93±0.34	7.0±0.01

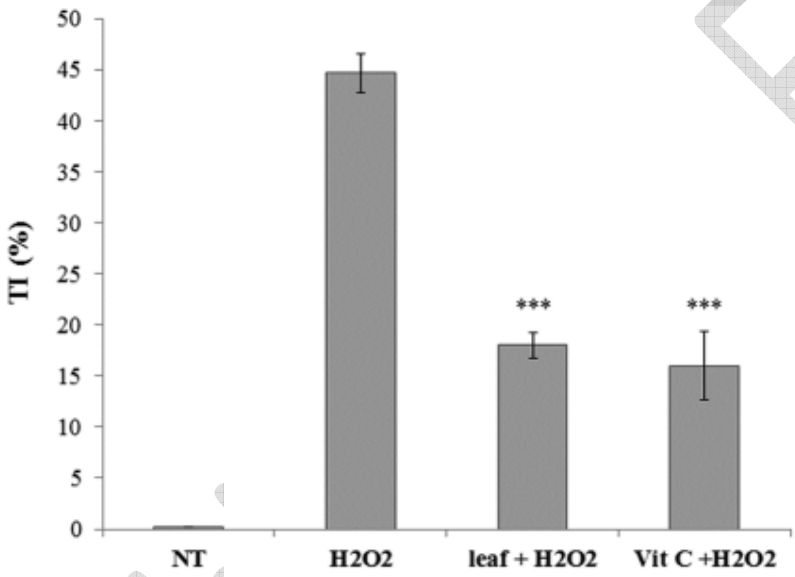

Fig. 2. Antioxidant activity of leaf chloroform extract (5 µg/ml) or vitamin C (1 mM) detected by comet assay on 24h pre-treated HT29 cell line as reduction of DNA migration induced by H_2O_2.
TI (%): tail fluorescence percentage.

Vegetables contain, in addition to vitamins and minerals, many microconstituents, such as carotenoids, glucosinolates, indoles, isothiocyanates, etc. These compounds are now recognized as being biologically active thanks to their ability to supplement cellular defence systems, in particular they showed a high ability to counteract the oxidative stress [23].

In many cases the exact composition and the bioavailability of active molecules is not known. Furthermore, the content of beneficial molecules varies with genetic strain, growth and storage conditions of the plant. For this reason assessing the true impact of such constituents on human health could be difficult.

Several assays are used for the evaluation of the antioxidant activities, these methods were applied to quench and scavenge reactive oxygen species, which comprise both free radicals (O_2•−, OH•, HO_2•, and RO•) and non-radical (molecular) forms (H_2O_2 and $^1O_2$) [24]. Numerous analyses such as total antioxidant activity, DPPH and ABTS assays, ROS quenching assay, metal chelating, reductive potential, β-carotene linoleate system and linoleic acid method are the most commonly used for the determination of antioxidant activities of plant extracts [25]. These assays are able to detect the three major mechanisms by which antioxidants can deactivate radicals: Hydrogen atom transfer (HAT), electron transfer (ET) and combination of both HAT and ET [26]. While HAT measures the ability of an antioxidant to quench free radicals by hydrogen donation, ET detects the ability of antioxidant to transfer one electron to reduce radicals, metals and carbonyls [27]. Majer [28] found that the different methods used to determine the antioxidant activity are not

interchangeable and sometimes they are not in correlation because antioxidants may have a different reactivity in the various methods. Nevertheless, in our study, we find a good correlation among *in vitro* assays: A significant strong and positive correlation between DPPH and reducing power assay and a significant strong and negative correlation between them and β-carotene/linoleic acid test. Since the DPPH assay detect both hydrogen atom and electron transfer, the reducing power assay detects only electron transfer and β-carotene/linoleic acid test only hydrogen atom transfer, the dependency relationship among these *in vitro* antioxidant assays suggest that *Citrullus colocynthis* Schrad. leaves, namely polar extracts (acetone and methanol ones), have *in vitro* antioxidant properties acting as electron donors.

The antioxidant activity of leaf extracts, observed in HT-29 cell line, didn't correlate with *in vitro* results. The methanol extract, the *in vitro* most active one, isn't the more effective on cells. On the contrary the chloroform extract, one of the less active *in vitro*, is the one that induces the higher cell response. Furthermore, it has shown an antioxidant *in vivo* activity on HT-29 cell line at 5 µg/ml, that is a concentration tenfold lower than the *in vitro* active one (50 µg/ml, data not shown) and much lower than the antiproliferative one (160 µg/ml).

This behaviour underlines a complex relationship between antioxidant activity and inhibition of proliferation. The antioxidant concentration, detected by Comet assay, seems to re-establish cell homeostasis since a cell proliferation variation isn't evidenced. On the contrary, higher extract concentrations result in a remarkable ROS depletion in cells, that can culminate in a cytotoxic effect as suggested by the oxidative balance under which a high reduction in intracellular ROS levels can induce a decrease in cell proliferation [29].

Antioxidant and antiproliferative activities of *C. colocynthis* leaf polar extracts could be related to the presence of phenolic compounds. In a previous paper [4], reporting the anticandidal and antibacterial effects of aqueous and acetone-water extracts, we showed that only leaves posses a content of coumarins and tannins among the different plant organs.

Furthermore, recently Chawech et al. [30] identified two new tetracyclic cucurbitane-type glycosides together with four known cucurbitacins, derived from an ethyl acetate leaf extract. Some of them showed a peculiar antiproliferative activity on colorectal cell lines (HT29 and Caco-2) without cytotoxic effects on mammalian normal cells. Cucurbitacin glucosides are known being able to scavenge hydroxyl radical, superoxide anion, and singlet oxygen[7]. Moreover, their antiproliferative action on human breast cancer cells increases when they are tested in combination [11], indicating that the complex relationship between the molecules present in the phytocomplex has to be taken into account when evaluating the biological activities of leaf extracts and, in a broader perspective, on vegetable extract in general.

5. CONCLUSION

In conclusion, our data confirm that *Citrullus colocynthis* Schrad fitocomplex could be an important source of natural atiproliferative agents. Furthermore, our study can be considered as a report on the complex relationships existing between antioxidant and antiproliferative properties of extracts prepared from *Citrullus colocynthis* Schrad. leaves. Pending further analysis and experimentation, these natural extracts may have potential applications in the food and pharmaceutical industries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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