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Full Length Research Paper

Gas chromatography-mass spectrometry (GC-MS) analysis and *in vitro* evaluation of antioxidant and antimicrobial activities of various solvent extracts from *Citrullus colocynthis* (L.) roots to control pathogen and spoilage bacteria

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***Citrullus colocynthis* (L.) schrad is an important medicinal plant belonging to the family of Cucurbitaceae. In the present study we evaluated *in vitro* the antimicrobial and the antioxidant properties of various solvent extracts from *C. colocynthis* roots as well as the latter phytochemical characterisation. Total phenolic and flavonoid contents were determined. The highest content of total phenolic was found in extracts obtained with ethyl acetate (205 mg GAE/g) followed by methanol (85 mg GAE/g). The major phenolic compounds in ethyl acetate extract were identified by gas chromatography-mass spectrometry (GC-MS). A total of 12 compounds were characterized including oleic acid, linoleic acid, caffeic acid, hexadecanoic acid and octadecanoic acids. The antioxidant activities were evaluated using 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) and lipid peroxidation methods. These tests showed that the ethyl acetate extract exhibited the best performance. The different extracts were also tested against a panel of pathogen, food-borne and spoilage bacteria and fungi in order to evaluate the antimicrobial properties of this plant. Among the tested extracts, only the ethyl acetate extract exhibited bactericidal and fungicidal activities. To our knowledge, this is the first report on the chemical composition, antioxidant and antimicrobial activities of phenolic extracts from *C. colocynthis* roots. The results of the present work indicate that *C. colocynthis* extracts could be used as natural antioxidant and antimicrobial agents in the food preservation and human health.**

Key words: *Citrullus colocynthis*, antibacterial activity, antifungal activity, antioxidant activity, phenolics composition.

INTRODUCTION

Microorganisms, including fungi, Gram-positive and negative bacteria, are among the agents that can induce various human infections and food deterioration during storage and processing. To overcome these problems, a wide range of synthetic antimicrobial agents have been used as the sodium or calcium benzoate in food preservation or methicillin in human infectious diseases (Mathur and Singh, 2005). However, considering the dramatically increasing resistance of bacteria towards these conventional antibiotics and their interactions with the food chain, the need to develop additional natural antibiotics is considered as a public health priority (Chopra, 2007). Additionally, lipids, proteins, DNAs and RNAs undergo gradual changes due to the formation of toxic free radicals and auto-oxidation by reactive oxygen species (ROS) into the organisms or the food. The reactive free radicals, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and peroxyl radical ($ROO^{\bullet}$), are particularly reactive and have long been recognized to

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contribute to oxidative damage and the development of pathological events such as cancer, inflammation, rheumatoid arthritis and cardiovascular diseases, as they were reported in previous works (Ames et al., 1993). Therefore, when the natural human protective systems against reactive oxygen species are overrun, exogenous antioxidative compounds must be delivered to maintain balance between oxidant and antioxidant. On the other hand, to prevent food oxidation, several synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ), are currently used as food supplements and stabilizers (Fernández-López et al., 2005). Nevertheless, previous studies have mentioned the inconvenience of these synthetic antioxidants due to their possible toxicity and injurious properties for human health (Botterweck et al., 2000). However, most consumers prefer additive free foods or a safer approach like the utilization of more effective antioxidants and antimicrobials from natural origins. Accordingly, plant extracts and their derived secondary metabolites, such as phenolic components, offer the opportunity in this regard (Wallace, 2004).

Citrullus colocynthis (L.) schrad is an important medicinal plant belonging to the family of Cucurbitaceae. *C. colocynthis* is a small scarbid perennial creeping herb with prostate or climbing stem, bearing smooth spherical fruits which are mottled green when young and somewhat yellow when ripe (Shah and Qadry, 1985). *C. colocynthis* is traditionally used as an antidiabetic medication in tropical and subtropical countries (Diwan et al., 2000). On its ethnopharmacological use, its root is given in abdominal enlargements, in coughs and asthmatic attacks of children. In order to investigate the potentiality of the antibacterial screening of *C. colocynthis*, the crude ethanolic extracts of fruits, leaves, stems and roots were examined in Gram-positive and negative bacilli (Memon et al., 2003). A powder of the root is given for three days in doses of 45 grains, well mixed with castor oil as a remedy for intestinal inflammation or tumours (Dastur, 1962).

In this work, we aimed to study the biological activities of *C. colocynthis*, based on ethnopharmacological use. The antimicrobial activity was evaluated against different microorganisms, including Gram-positive and negative bacteria and fungi. The antioxidant potential was studied by two distinct assays: scavenging of 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) radicals and beta-carotene bleaching assay. Moreover, we characterized by gas chromatography-mass spectrometry (GC-MS), the phenolic acids components of *C. colocynthis* and discussed their possible health benefits.

MATERIALS AND METHODS

DPPH, BHT, linoleic acid, β-carotene, polyoxyethylenesorbitan monolaurate (Tween-20), ascorbic acid, gallic acid, quercetine, Folin–Ciocalteu's, *N*,*o*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine reagents were purchased from Sigma (Sigma, France). For antimicrobial tests, Mueller Hinton agar and potato dextrose agar was purchased from Bradford (Bradford, France).

C. colocynthis roots were collected from Chebba (Tunisia, 35.23°N and 11.11°E) in November 2011. After the botanical identification of the species, a voucher specimen (LBPes 06) was deposited in the herbarium of the Bio-pesticides team (LPIP) (Center of Biotechnology of Sfax, Tunisia) for future reference.

Preparation of extracts

The dried *C. colocynthis* roots were ground to fine powder using a grinder. Then the powdered plant material (100 g) was extracted twice using the following sequence of solvents with increasing polarity; hexane, ethyl acetate, methanol and water at 30°C for 24 h each. The sample was then filtered through filter paper in a Buchner funnel. The filtered solution was evaporated at reduced pressure (Rotary Evaporator Buchi R 200, Switzerland) at 40°C and then dissolved in appropriate solvent. The resulting four solutions were concentrated *in vacuo* to dryness to give n-hexane extract, HE (0.7 g), ethyl acetate extract, EtOAcE (4 g), methanol extract, MeOHE (2 g) and water extract (1.5 g). The stock solutions were kept at 4°C in dark until further analysis. The dry extract was stored at -18°C.

GC-MS identification of phenolic acids

Sample preparation and derivatization

Silylation is an ideal procedure for the GC analysis of non-volatile and thermolabile compounds. This method has been applied to the silylation of the phenolic extract and standards. For the silylation procedure, a mixture of BSTFA (200 µl), 200 µl extract of *C. colocynthis* in acetonitrile and 50 µl of pyridine were mixed and mechanically shaked for 2 min at room temperature and consecutively placed in a water bath, at 80°C for 60 min. At this point the samples were ready to be injected into the gas chromatography-mass spectrometer.

Gas chromatographic analysis

The trimethylsilyl derivatives of phenolic acids were identified using GC-MS methodology using an Agilent 6890 N Network GC system (Agilent Technologies) gas chromatograph, fitted with a splitless injector for a low background HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) supplied by Agilent. Detection was carried out with a 5975 B Inert MSD (Agilent Technologies). The GC-MS operation control and data processes were carried out by Chem-Station software package (Agilent Technologies). The injector temperature was 250°C. The oven temperature was held at 100°C for 1 min, increased to 260°C at a heating rate of 4°C/min, then to 325°C at 10°C/min and held for 1 min. The detector temperature was 250°C. The carrier gas used was helium at a flow rate of 1.0 ml/min. The sample volume in the direct injection mode was 1 µl. The conditions for electron impact ionization (EI) were an ion energy of 70 eV and a scanned mass range of 50 to 550 *m*/*z*. The identification of compounds was achieved by comparing the retention times with those of authentic compounds and the spectral data obtained from the Wiley and NIST libraries. Determination was carried out in duplicate.

Determination of total phenolics and flavonoids content

Total phenolics content was determined using the Folin-Ciocalteu method (Waterman and Mole, 1994) adapted to a microscale. Gallic

acid was used as standard. The absorbance of all the samples was measured at 760 nm using a Bio-Rad SmartSpec™ plus UV-Vis spectrophotometer, and the results are expressed in mg of gallic acid equivalent per g of dry plant extract (mg GAE/g). The flavonoids content in extracts was determined spectrophotometrically according to Quettier-Deleu et al. (2000). The flavonoids content was expressed in mg of quercitin equivalent per g of dry plant extract (mg QE/g).

Antioxidant activity

DPPH free radical scavenging activity

Radical scavenging activity was determined by spectrophotometric method based on the reduction of a methanol solution of DPPH (Blois, 1958). 0.5 ml of different concentrations of the extract in methanol was added to 1 ml of 0.1 mM DPPH solution in methanol. The mixture was shaken vigorously and left to stand at room temperature for 30 min in the dark. Then, the absorbance was measured at 517 nm against a blank by a spectrophotometer (Bio-Rad SmartSpec™ plus). DPPH radical scavenging activity (%) was calculated according to the formula:

DPPH radical scavenging activity (%) = $[(OD_{blank} - OD_{sample}) / OD_{blank}] \times 100$

OD_{blank} is the absorbance of the control reaction containing all reagents except the tested compound. OD_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Ascorbic acid was used as positive control. Tests were carried out in triplicate.

β-Carotene-linoleic acid bleaching assay

The antioxidant activity of the *C. colocynthis* extracts was evaluated by the spectrophotometric β-carotene bleaching test (Juntachote and Berghofer, 2005). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform [high performance liquid chromatography (HPLC grade)] to which 25 µl linoleic acid and 200 mg Tween 20 were added. Chloroform was completely evaporated by using a vacuum evaporator. Then, 100 ml of distilled water with oxygen (30 min at a flow rate of 100 ml min^{-1}) were added with vigorous shaking. Aliquots of 4 ml of this mixture were mixed with 200 µl of the extracts at different concentrations. The emulsion system was incubated for 3 h at room temperature. The same procedure was repeated with the synthetic antioxidant, BHT, as positive control, and a blank containing only 200 µl of ethanol. After this incubation period, absorbance of the mixtures was measured at 470 nm. Antioxidative capacity of the extracts was compared with those of BHT and blank. Antioxidant activity is expressed as percentage inhibition relative to control (0.2 ml ethanol) using the equation:

Antioxidant activity (%) = $[1 - (OD_0 - OD_t / OD'_0 - OD'_t)] \times 100$

Where OD_0 and OD'_0 are the absorbance values measured at zero time for the test sample and control, respectively, while OD_t and OD'_t are the corresponding absorbance values measured after incubation for 3 h.

Antimicrobial screening

Microorganisms and growth conditions

Nine bacterial and four fungal strains were used in this study. The tested pathogenic bacteria were: *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *S. epidermis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 1880, *Esherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* CIP 32147, *E. coli* ATCC 8739, *Salmonella enterica* (food isolate 824), *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6536 and *Listeria monocytogenes* (food isolate 2132). The fungi tested in the antifungal test were *Aspergillus niger* CTM 10099, *Aspergillus flavus* (food isolate), *Aspergillus nidulans* (food isolate), *Fusarium graminearum* (plant isolate), and *Alternaria alternata* CTM 10230.

The bacterial strains were grown on Mueller Hinton broth (Bio-Rad, France) at 37°C for 12 to 14 h and on potato dextrose agar (PDA) (1.5% agar) at 28°C for four days for fungi. Inocula were prepared from an overnight broth culture by their dilution in saline solution to approximately 10^8 colony-forming units, CFU/ml for bacteria and 10^5 spores/ml for fungus.

Agar-well diffusion assay

Antibacterial and antifungal tests were based on British Society for Antimicrobial Chemotherapy (BSAC) procedures. Agar plates were prepared using sterile Mueller-Hinton agar (Bio-Rad, France) for antibacterial strain and potato dextrose agar for antifungal tests. Microorganisms of standardised cultures (100 µl) were spread onto the surface of agar plates. Then, wells with 6 mm diameter were punched in the inoculated agar medium with sterile Pasteur pipettes and the extracts were added to each well. Negative controls, used to dissolve plant extracts, are ethanol 50% (water). Gentamicin (10 µg/well) was used as positive reference standard to determine the sensitivity of each bacterial strain. The plate was allowed for 2 h at 4°C to permit the diffusion of the extracts. The agar plates are incubated at 37°C for 24 h and the antibacterial activity was evaluated by measuring the zones of inhibition (clear zone around the well) against the test micro-organisms. All tests were replicated three times.

Statistical analysis

Experimental results of this study are mean ± S.D. of three parallel measurements. Analysis of variance was performed using Excel procedures.

RESULTS

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of *C. colocynthis* extracts from different solvents were examined and are presented in Table 1. These compounds showed differences in their total contents depending on solvents polarities. The highest content of total phenolics was found in extracts obtained with ethyl acetate (205 mg GAE/g) followed by methanol (85 mg GAE/g).

Whereas, the contents obtained with hexane and water extracts were much smaller. Total flavonoids varied from 20.3 to 48.5 mg QE/g. Ethyl acetate and water extracts had the highest levels with 48.5 and 25.0 mg QE/g, respectively.

Table 1. Total phenolic, flavonoid contents and IC_{50} values of the DPPH free radical scavenging and the β-carotene-linoleate assays of *C. colocynthis* crude extracts. Ascorbic acid and BHT were used as standards. Each value represents the mean ± S.D of three experiments.

Extract	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	IC_{50} (µg/ml)	
			β-carotene-linoleate model	DPPH activity
Hexane	12±1.3	ND	0	0
Ethyl acetate	205±4.5	48.5±1.2	25±0.2	3.80±0.7
Methanol	85±5.6	20.3± 3.1	15.5±1.2	19.5±3.8
Water	35±1.4	25.0± 2.9	15.3±0.6	1.85±0.3
Ascorbic acid	-	-	-	3.1±0.1
BHT	-	-	1.4±0.1	-

mg GAE /g, mg of Gallic acid equivalent per g of dry plant extract; mg QE/g, mg of quercitin equivalent per g of dry plant extract. The IC_{50} (µg/ml) values correspond to the amount of extract required to scavenge 50% of radicals present in the reaction mixture. -, Not tested; ND, not detected. DPPH, 2,2-Diphenyl-1-picrylhydrazyl free radical; BHT, butylated hydroxytoluene.

Table 2. Chemical composition of the mass spectra of silylated derivatives of the major peaks of phenolic compounds detected by GC-MS in of the ethyl acetate extract from *C. Colocynthis* roots.

Compound[a]	RT (min)[b]	Percent (%)
Benzoic acid	7.04	0.47
p-hydroxyphenylacetic acid	17.553	0.20
Hydrocinnamic acid	20.643	1.83
Vanillic acid	20.819	2.98
Protocatechuic acid	22.370	1.52
p-Coumaric acid	25.087	1.09
Hexadecanoic acid	26.895	20.5
Ferulic acid	28.709	4.22
Caffeic acid	29.813	6.62
Linoleic acid	31.325	10.5
Oleic acid	31.452	35
Octadecanoic acid	31.861	5.22

[a]Compounds listed in order of retention time; [b]Rt, retention time (as min). GC-MS, Gas chromatography–mass spectrometry.

GC-MS identification of phenolic acids

Ethyl acetate extract of *C. colocynthis* roots exhibited a high content of total phenolic compounds as analysed by GC-MS. The relative retention times (Rt) and mass spectra of the extract components were compared with those of authentic samples and the mass spectra from a data library.

As shown in Table 2, GC-MS analysis of the *C. colocynthis* ethyl acetate extract resulted in the identification of 12 compounds, with more than 90% similarity with the standard mass spectra in the library. This extract is characterized by the presence of oleic acid (35%), hexadecanoic acid (20.5%), linoleic acid (10.5), caffeic acid (6.62%), octadecanoic acid (5.22%), ferulic acid (4.22%) and vanillic acid (2.98%).

Antioxidant activity

DPPH free radical scavenging activity

The effect of the different *C. colocynthis* organic extracts on DPPH radical scavenging was compared to those of ascorbic acid used as positive control, and appreciated by the determination of the IC_{50} values. As shown in Figure 1, DPPH test revealed that increase in extracts concentration resulted in increase in free radical-scavenging activity in a dose dependent manner. Based on the IC_{50} values, the best activities were obtained with water extract (IC_{50} = 1.85 µg/ml), followed by ethyl acetate (IC_{50} = 3.80 µg/ml) and methanol extract (IC_{50} = 19.5 µg/ml) (Table 1). It is clear that water extract exhibited a more potent antioxidant activity than ascorbic acid used as standard (IC50 = 3.5 µg/ml).

β-*C*arotene-linoleic acid bleaching assay

The inhibitory effect of the different *C. colocynthis* extracts on lipid peroxidation was determined by the β-carotene-linoleic acid bleaching test. Figure 2 shows a various degree of the linoleic acid oxidation and subsequently the β-carotene bleaching after addition of the *C. colocynthis* organic extracts and the BHT used as positive control at different concentrations. This antioxidant activity was dose dependent as found in the DPPH test. All the *C. colocynthis* organic extracts inhibited the β-carotene oxidation and the water and methanol extract exhibited strong inhibition activities with IC_{50} values of 15.3 and 15.5 µg/ml respectively.

Antimicrobial activities

The *in vitro* antimicrobial activity of *C. colocynthis* extracts were qualitatively and quantitatively assessed by

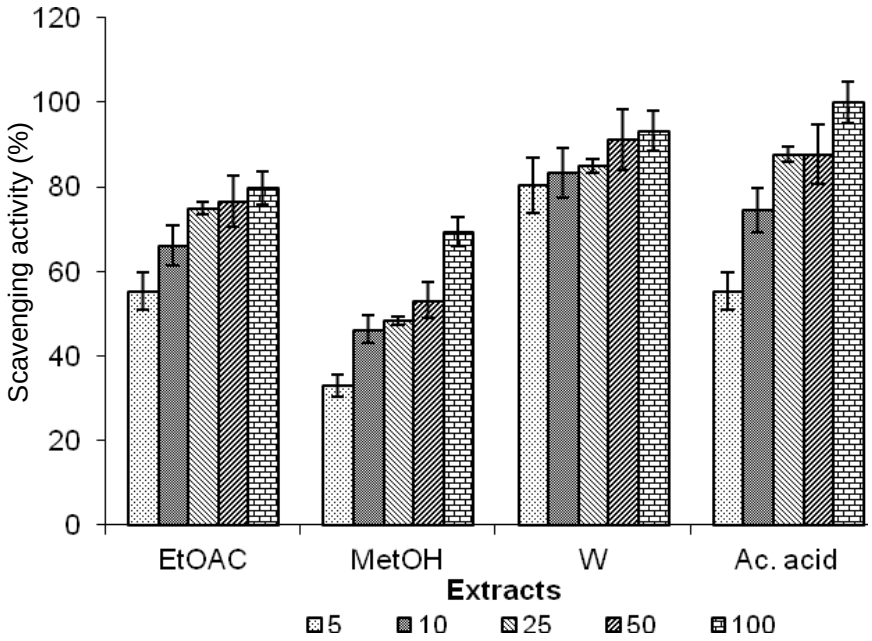

Figure 1. Scavenger effect of *C. colocynthis* extracts at different concentrations on the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Results are expressed as percentage decrement of absorbance at 517 nm with respect to control. Ascorbic acid was used as a standard. Each value represents the mean ± S.D. of three experiments.

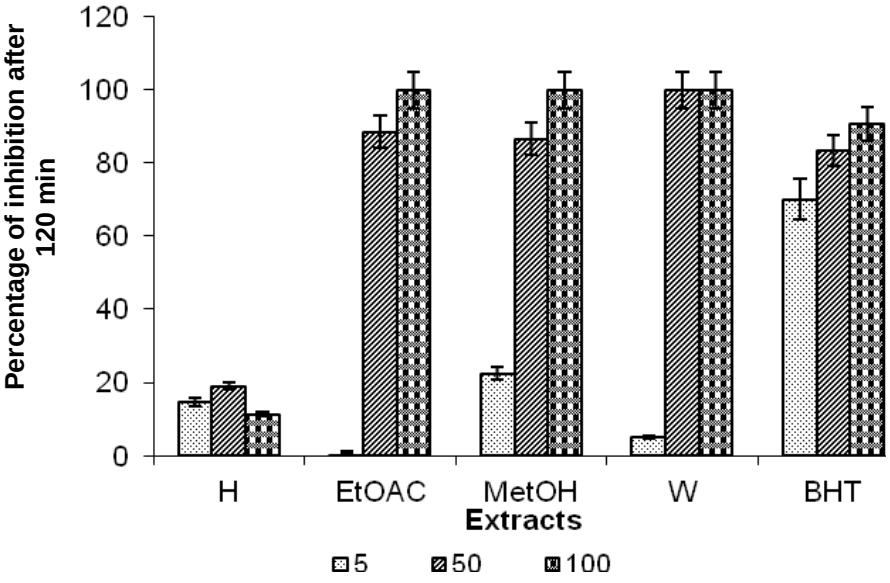

Figure 2. Antioxidant activities of *C. colocynthis* extracts at different concentrations measured by β-carotene bleaching method. BHT was used as reference antioxidant. BHT was used as standard. Values are means ± S.D (n = 3). BHT, Butylated hydroxytoluene.

the presence or absence of inhibition zones using well diffusion test. 21 microorganisms including fungi, Gram-positive and negative bacteria were screened using plants extracts at a concentration of 4 mg/well. The results summarized in Table 3 reveal stronger antibacterial and antifungal activities (92.3%) against almost all the tested micro-organisms using the ethyl acetate crude extract. However, hexane, methanol and

Table 3. Antimicrobial activities of *C. colocynthis* ethyl acetate extract against fungi, food borne and spoiling bacteria and the determination of the minimal inhibition concentration (MIC) in µg/ml.

Strain	Extract		
	Ethyl acetate[a]	Gentamicin[b]	MIC (µg/ml)
Bacterial strains			
Gram positive			
Bacillus subtilis ATCC 6633	13±0.5	20	0.312
Bacillus cereus ATCC 14579	12.6±0.6	20	0.156
Staphylococcus aureus ATCC 25923	15 ±1.7	25	0.625
Staphylococcus aureus ATCC 6536	17±0	16	0.625
Staphylococcus epidermis ATCC 12228	12.6±0.6	20	0.625
Enterococcus faecalis ATCC 29212	11.6±0.7	12	0.625
Micrococcus luteus ATCC 1880	10.5±0.5	20	0.625
Listeria monocytogenes (food isolate 2132)	14.5	15	0.625
Gram negative			
Salmonella enterica (food isolate)	13 ±1.1	18	1.25
Escherichia coli ATCC 25922	10.5±1.1	21	0.312
Escherichia coli ATCC 8739	09±0.5	20	2.5
Pseudomonas aeruginosa ATCC 27853	11.3±1.5	18	0.625
Pseudomonas aeruginosa ATCC 9027	11±1.2	18	0.625
Klebsiella pneumoniae CIP 32147	11.3±1.5	12	0.312
Fungal strains			
Aspergillus niger CTM 10099	11±0.5	-	1.25
Aspergillus flavus (food isolate)	12±1.4	-	2.5
Aspergillus nidulans (food isolate)	11±0.7	-	1.25
Fusarium graminearum (food isolate)	13±0.7	-	0.312
Alternaria alternata CTM 10230	15.5±0.7	-	0.625

Values are given as mean ± S.D of triplicate experiment. [a]Diameter of inhibition zones of ethyl acetate extract of *C. colocynthis* extract including diameter of disc 6 mm. [b]Gentamicin was used as a standard antibiotic at a concentration of 10 µg/ml. -, not detected.

water extracts were found to be inactive within the tested concentration range. Gram-positive bacteria appeared to be more sensitive to the extract than the Gram-negative ones.

The best activity was exhibited by ethyl acetate crude extract against *S. aureus*, one of the most common Gram-positive bacteria causing food poisoning. The evaluation of the antifungal activity of *C. colocynthis* extracts against five fungi strains, *A. niger, A. flavus, A. nidulans, F. graminearum and A. alternata* revealed that the ethyl acetate crude extract was the most active, and the best activity was found against *F. graminearum* and *A. alternate*, with inhibition zone of 13 to 15 mm and minimal inhibition concentration values of 312 and 625 µg/ml, respectively (Table 3). Also, the ethyl acetate extract of *C. colocynthis* exhibited an antifungal activity against *Aspergillus* species such as *A. niger, A. flavus, A. fumigatus* and *A. nidulans* which are responsible for spoilage of many foods and feeds. The inhibition zone and the minimal inhibition concentration values of the ethyl acetate extract against *Aspergillus* species were recorded as 11 to 12 mm and at 1.25 to 2.5 mg/ml, respectively.

DISCUSSION

In our study, the quantification of the phenolic and flavonoid amounts present in the different extracts of *C. colocynthis* revealed that the ethyl acetate was the best solvents to use for the extraction of *C. colocynthis* phenolics as compared with the other solvents due to their suitable polarities. Interestingly, the phenolic compounds of this plant were characterized and well investigated for the first time. In the present study, oleic, hexadecanoic, linoleic, caffeic, octadecanoic, ferulic and vanillic acids were identified as the main component of *C. colocynthis* by GC-MS. Several studies of the beneficial effects of these phenolics have been reported, such as the antimicrobial activity of the caffeic acids against a

panel of microorganisms (Coneac et al., 2008).

Antioxidants act as radical-scavengers and inhibit lipid peroxidation and other free radical mediated processes. Actually, there is a growing interest in the substitution of synthetic antioxidants used in food preservation, which involve toxic side effects, with natural ones. Polyphenols, with their redox properties can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Galato et al., 2001). In the present study, the antioxidant potential of *C. colocynthis* extracts was measured by different biochemical assays: scavenging activity on DPPH radicals and lipid peroxidation inhibition by the β-carotene-linoleate system. Based on the susceptibility of the different extracts to the free radicals, it is possible to conclude that the ethyl acetate extract of *C. colocynthis* have stronger and broader spectrum of antioxidant activity as compared with the other *C. colocynthis* extracts. In fact, the present investigation showed that the ethyl acetate extract exhibited significantly higher DPPH scavenging activity. This result demonstrates the hydrogen donating ability of these extracts, since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability.

Furthermore, these extracts could protect polyunsaturated fatty acids in food from lipid peroxidation. Our results demonstrate a good correlation between the antioxidant activity and the phenolic contents in the ethyl acetate and acetone extracts. Phenolic compounds seems to be responsible for the antioxidant activity by its `chain-breaking` property that can counteract peroxyl and alkoxyl radical generated during lipid peroxidation to prevent continual hydrogen abstraction and thus inhibiting chain propagation step. Previous studies have reported the same results for mulberry extract and culinary herbs (Arabshahi-Delouee and Urooj, 2007).

Apart from damage caused by the free radicals on food and human, another common problem is the contamination by microbes affecting the food quality and causing serious health problems. In our study, the results of the antibacterial screening showed that among the tested extracts, only the ethyl acetate and acetone extracts have potential antibacterial activity against a panel of human and food born pathogenic bacteria such as *L. monocytogenes, S. enterica*, *S. aureus, E. coli* and *B. subtilis*. Ethyl acetate extract exhibited a promising antibacterial effect by inhibition zones and minimal inhibition concentration (MIC) values for against the tested bacteria.

Gram-negative bacteria are generally less susceptible to plant extracts than the Gram-positive bacteria, since they have an outer membrane which plays the role of a barrier to the biomolecules (Chopra and Greenwood, 2001). Additionally, foods are prone to bio-deterioration by moulds and fungi during post-harvest processing, transport and storage (Chauhan, 2004). Contamination by *Aspergillus*, *Fusarium* and *Alternaria* species, with their respective mycotoxins is considered as a challenge for the pharmaceutical and food industries. Plant extracts are widely claimed to have a broad-spectrum antifungal activity and are considered as a main source for the search of lead compounds. The present study reports the capacity of the ethyl acetate crude extract of *C. colocynthis* to control *Aspergillus* strains, *F. graminearum* and *A. alternata* strains. In consequence, antibacterial activity might be related to the phenolic compounds. Therefore, *C. colocynthis* extracts could be considered as one of the sources of natural antibiotics against opportunistic pathogens and could be used as food anti-poisoning agents. The purification of the antimicrobial compounds from the ethyl acetate extracts, using bioactivity-directed fractionation and the characterization of the bioactive compounds needs to be pursued.

Conclusion

Plants dietary and health values have received an increasing attention during the last decade. Hence, plant extracts were more recommended in several oxidative stress-related diseases as cancer or microbial contamination due to the beneficial effect of the natural products as the phenolic compounds. In this study, we show that crude extracts of *C. colocynthis* exhibit a broad spectrum of biological activities such as antibacterial, antifungal and antioxidant activities. A special attention has been made for the characterisation by GC-MS of the phenolic composition of the ethyl acetate extract which have potential bioactivities and high content of phenolic compounds. Furthermore, our results show the potential effect of the ethyl acetate extract from *C. colocynthis* as a source for natural health products or natural food preservatives due to its high antibacterial and antifungal activities against a panel of food pathogenic bacteria and fungus. Moreover, we expect that *C. colocynthis* extracts may provide a candidate for a potential antioxidant agent. The above results are a good agreement of the popular use and experimentally observed effects of *C. colocynthis* and would promote the reasonable usage and exploitation of the biomolecules of this important plant.

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