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Antimicrobial, antibiofilm and antioxidant activities of *Citrullus colocynthis* fruit extracts

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ABSTRACT

According to its beneficial characteristics, the desert medicinal plant Citrullus colocynthis L. is commonly used in traditional medicine. The objective of this study is to investigate the antioxidant, anti-hemolysis, antimicrobial, and antibiofilm activities of Citrullus colocynthis L. fruit extracts, and their content on phenolic compounds. Butanol and ethyl acetate extracts contain a high content of polyphenols (222.72 and 260.61 µg GA/mg Extract) and flavonoids (73.03 and 77.7µg CAT/mg E) compared to hydro-methanol and chloroform extracts. Ferulic acid, rutin, and numerous other unidentified substances were identified in ethyl acetate and butanol extracts, according to RP-HPLCPDA analysis. These extracts exhibited the highest antioxidant activity in DPPH assay (65.09 µg/mL <IC_{50}>89.29 µg/mL), FRAP (99.7 µg/mL < $A_{0.5}$ >119.37 µg/mL), CUPRAC (19.41µg/mL < IC_{50}>26.63 µg/mL) and ABTS (77.43 µg/mL < IC_{50}> 86.87 µg/mL). Compared to the other extracts, the butanol extract revealed the best protective effect from hemolysis in red blood cells against AAPH-induced oxidative stress (IC_{50}=19.11 µg/mL). In addition, Gram-positive and -negative bacteria were more sensitive to butanol and ethyl acetate extracts, mainly Staphylococcus aureus ATCC 6538 and MicrococcusluteusATCC9341 with zone inhibition diameters ranging between 11 mm and 13 mm, and MIC values between 0,312 mg/mL and 0,625 mg/mL. These extracts exhibited an interesting anti-adhesion activity (35%-58%) against Staphylococcus aureus, Listeria monocytogenes, and Escherichia coli, which indicates their likely antibiofilm effect. Extracts had no specific antifungal activity against Candida albicans According to the obtained results colocynthis, a potential therapeutic herb can be used to treat infectious diseases and oxidative stress.

Keywords **:** Antibacterial, antibiofilm**,** antioxidant activity, *Citrullus colocynthis*, polyphenols

INTRODUCTION

The main cause of many chronic and neurodegenerative diseases, including cancer, diabetes, cardiovascular disease, Alzheimer, and autoimmune diseases, is free radical production, which antioxidants quickly neutralize **(**Thèriault *et al.*, 2006**)**. Several scientific studies have been performed to reveal the therapeutic effects of phytochemicals on health by determining their impact on oxidative stress, and assessing their potential to increase enzymatic antioxidants, reduce peroxides, scavenge free radicals, and chelate transition metals (Li *et al*., 2009). A vast number of medicinal plants used in phytotherapy that have been evaluated for their antibacterial, antioxidant, and anti-inflammatory effects represent an inexhaustible source of natural antioxidants (Brewer, 2011, El-Kadi *et al.*, 2021).

Many species of the Cucurbitaceae family, including *Citrullus colocynthis*, are traditionally used as anti-diabetic remedies. Having smooth, and spherical fruits that are mottled green when young

and yellowish when mature, colocynth is a creeping annual herb that is native to India, West Asia, tropical Africa, and the Mediterranean region (Gurudeeban *et al*., 2010; Banjo *et al.*, 2021). In Asian and African countries, the fruits are traditionally used to treat infectious diseases, inflammation, ulcers, hepatitis, kidney illnesses, and diabetes (Azzi *et al*., 2012; Banjo *et al*., 2021). The anti-diabetic and antioxidant activities of *C. colocynthis* extracts have been established in many *in vivo* and *in vitro* research (Nmila *et al*., 2000; Kumar *et al*., 2008; Benariba *et al*., 2009; Benariba *et al.*, 2012; Benariba *et al.*, 2013a; Ckekroun *et al*., 2015; Ckekroun *et al*., 2017; Shahzadi *et al.*, 2022). The present study's main objective is to identify the polyphenols of the hydromethanolic extract, and its fractions ethyl acetate and butanol, prepared from *Citrullus colocynthis* fruits that were collected in Tlemcen, in the west of Algeria, and to investigate their antimicrobial, antioxidant, and antibiofilm activities.

MATERIALS AND METHODS

Plant materials

Citrullus colocynthis fruit was harvested in Naâma, in western Algeria. Its authenticity was confirmed by Tlemcen University's Laboratory for Ecology and Management of Natural Ecosystems. Once the fruits were cleaned and dried at room temperature, they were crushed into small pieces and kept for further extraction.

Preparation of extracts

For 48 hours at room temperature, 10 grams of crushed fruit were macerated in 100 mL of a water-methanol mixture (20/80 V/V). A vacuum concentration process was performed on the resultant solution after it was filtered. To obtain the dry hydromethanol extract, a portion of this extract was evaporated until it was completely dry. In a liquid-liquid funnel extraction, the remaining hydromethanol was fractionated by chloroform, ethyl acetate, and n-butanol. Ethyl acetate and butanol extracts were obtained by evaporating organic phases under a vacuum.

Total polyphenols content

The Folin-Ciocalteu method was used to determine the total polyphenol content in colocynthis fruit extracts according to Vermerris and Nicholson (2006). At various concentrations, 100 µL of samples or the standard (gallic acid) were combined with 2 mL of freshly prepared Na_2CO_3 (2%) solution. The mixture was incubated at room temperature for 5 min before being added to 100 µL of Folin-Ciocalteu reagent (0.2 N). At 700 nm optical density measurement was performed, and the results are expressed as mg of gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content

$AlCl_3$ reagent was used to measure flavonoid content according to Ardestani and Yazdanparast (2007). 500 µL of samples or catechin (standard) were combined with 150 µL of $NaNO_2$ solution (15%), and 2 mL of distilled water. A subsequent incubation at room temperature for 6 min was followed by the addition of 150 µL of $AlCl_3$ (10%) and 2 mL of NaOH (4%). Following a second 15-minute incubation at room temperature, distilled water was added to the total volume to adjust it to 5 mL, and optical density was determined at 510 nm. As a result, the data were reported as mg of dry extract/g catechin equivalent (mg Cat eq/mg).

High-performance liquid chromatography (HPLC-DAD) analysis of phenolic compounds

RP-HPLC-PDA was applied to separate and identify the phenolic component of the butanol and the ethyl acetate extracts *of C. colocynthis*. This analysis was conducted on an Eclipse ODS Hypersil C18 column and a Perkin Elmar Flexar system with a binate pump transmission method (150 mm x 4.6 m). Each sample was injected at a flow rate of 1 mL/min using 20 µl. A binary solvent system containing acetic acid 2% (A) and acetonitrile (B) formed the mobile phase. A 10% B (Acetonitrile) gradient was applied for 5 minutes, followed by 10% B for 25 minutes, followed by 15% from 90% to 100% B, followed by 15 minutes of equilibration. Filters of 0.22 m Millipore were used before the HPLC injection of samples and mobile phases. For component analysis, the chromatograms of extracts obtained at 280 nm were chosen (El-Haci *et al*., 2020; Adjdir *et al*., 2021a; Adjdir *et al.*, 2021b).

Antioxidant activity

Free radical scavenging activity: DPPH assay

C. colocynthis extracts were investigated for their ability to scavenge free radicals via the DPPH assay according to Ataoui *et al.* (2005). 1.95 mL of DPPH was incubated with 50 µL of each extract for 30 minutes, after which the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid were used as standard antioxidant molecules. The results are expressed as percent DPPH inhibition, and the IC50 value was calculated from the linear regression curve.

$$\text{Free radical scavenging activity (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Ferric Reducing power assay

The iron-reducing capacity of *C. colocynthis* extract was evaluated according to the method described by Karagözler *et al.* (2008). 1 mL of each extract was combined with 2.5 mL phosphate buffer (0.05 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After standing at 50°C for 20 min, the mixture was cooled down to room temperature, and the absorbance of the samples was measured at 700 nm. Then, 2.5 mL of trichloroacetic acid solution (10%) was added to the medium and centrifuged at 3000rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL $FeCl_3$ (1%) solution to measure the absorbance at 700 nm. The results are presented as A0.5 (sample concentration in an absorbance range of 0.5).

Cupric reducing antioxidant capacity (CUPRAC)

96-well microplates were filled with 40 µL of extracts at various concentrations, all of which were combined with 50 µL of $CuCl_2$ (10 mM). Following the addition of 60 µL of ammonium acetate buffer (1M, pH 7.0) and 50 µL of neocuproin solution (7.5 mM), the medium was then filled to a final volume of 200 µL. Following 60 min of incubation, absorbance was measured at 450 nm (Apak *et al*., 2004).

ABTS cation radical scavenging assay

The $ABTS^+$ radical is produced by combining ABTS 7 mM (2,2'-azino-bis(3-ethylbenzothia zoline-6-sulfonic acid) and potassium persulfate (2.45 mM) and incubated in the dark at room temperature for 12 hours. Then, 160 µL of the $ABTS^+$ solution (with an absorbance of 0.700-0.025 at 734 nm) was mixed with 40 µL of the extracts, and the absorbance was measured at 734 nm after 10 minutes of incubation (Re *et al*., 1999; Dong *et al*., 2015). The trapping capacity of $ABTS^+$ was determined according to the following formula:

$$ABTS^+ \text{ scavenging effect (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Total Antioxidant Capacity

According to the process prescribed by Prieto *et al.* (1999), 0.2 mL of various concentrations of extract of *C. colocynthis* were mixed with 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate to determine their total antioxidant capacity (TAC). Following 90 minutes of incubation at 95°C, each tube's absorbance was measured at 695 nm. Gallic acid milligram equivalents per gram of extract (mg GAE/gE) were used to express the total antioxidant ability. The antioxidant activity was expressed in milligram equivalents of gallic acid per gram of extract (mg GAE/gE).

AAPH (2,2'-Azobis-2-amidinopropane dihydrochloride) induced hemolysis in erythrocytes

Using AAPH as a peroxyl radical generator, the capacity of *C. colocynthis* fruit extracts to prevent free radical-induced hemolysis was assessed according to Xiaoping Yuan *et al.* (2005).

Preparation of erythrocytes

Blood was obtained at the city hospital's blood transfusion service from healthy volunteers. In a

laboratory, erythrocytes were removed from the plasma by centrifugation at 3000g for 5 minutes at 4°C, and the pellet was then washed three times with a cold (4°C) solution of phosphate-buffered (10 mM sodium phosphate, 125 mM NaCl, pH=7.4). After each step, the washed erythrocytes recovered from the pellet were then carefully taken from the supernatant, and at the end, a final concentration of 5% (v/v) in PBS was used to resuspend the washed erythrocytes that were recovered from the pellet.

Erythrocyte hemolysis assay

During the hemolysis test, 100 µL of the erythrocyte suspension is mixed with 100 µL of extracts (20-100 µg/mL), and the mixture is then incubated at room temperature for 15 min. Incubation was followed by adding 200 µL of AAPH solution (100 mmol/L) and the tubes were incubated for 3 h tubes at 37°C; then, the medium was diluted with 8 mL of PBS before centrifugation at 2000g for 10 minutes. The optical density of the resulting supernatant was measured at 540 nm. The results were expressed as a hemolysis percentage according to the formula as follows:

$$\text{Hemolysis inhibition (\%)} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Antimicrobial activity

The antimicrobial activity of *Citrullus colocynthis* fruit extracts was evaluated on bacterial and fungal pathogenic strains of American Type Culture Collection (ATCC) using microdilution and disk diffusion methods. Among the gram-negative bacterial strains investigated were *Citrobacter freundii* ATCC 8090, *Acinetobacter baumanii* ATCC 19606, *Enterobacter cloacae* ATCC 1304, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 13311, *Proteus mirabilis* ATCC 35659, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853. Six Gram-positive bacterial strains were also tested, *Bacillus cereus* ATCC 25921, *Enterococcus faecalis* ATCC 49452, *Staphylococcus aureus* ATCC 6538, *Micrococcus luteus* ATCC 9341, *Listeria monocytogenes* ATCC 15313, *Bacillus subtilis* ATCC 6633, as well as *Candida albicans* reference strains are used ATCC 26790, ATCC 10231, and IP 444. Extracts with significant antibacterial effects were tested for antibiofilm activity using an anti-adhesion bioassay against *Staphylococcus aureus*, *Listeria monocytogenes*, and *E. coli*.

Disk diffusion method

According to CLSI (2012), disk diffusion tests were conducted. 1 mL of each bacterial suspension was prepared from a suspension containing10^8 CFU/mL (0.5 McFarland standard; OD = 0.08-0.10 λ = 625 nm), then was cast onto the solid medium Mueller-Hinton agar. Discs of sterile filter paper (6 mm) containing 10 µL of colocynth extracts (512µg/mL) were puted on the agar surface. Then, Under adequate cultivation conditions, Petri Plates were incubated for 24 hours. the antibacterial action of each extract corresponded to the lowest concentration that create a zone of inhibition around the disk, refred to the positive control we used gentamicin. All experiments were carried out in triple.

Minimum Inhibitory Concentration (MIC)

The microdilution method using Borth medium (CLSI, 2009) was applied to extracts that showed antibacterial activity according to the previous method. 100 µL of bacterial suspensions containing 10^6 CFU (0.08-0.13/ λ =625 nm) were mixed with 100 µL of extracts (0.02-10 mg/mL) using 96-well microplate. In the control, only culture medium and bacterial suspension were used. The MIC is the lowest concentration of extracts that will completely inhibit microbial growth within 24 hours of incubation of the microplate at 37°C (no turbidity after 24 hours).

Antibiofilm activity: Bacterial adhesion assay

Adhesion is a necessary step in the formation of a biofilm (Guo *et al*., 2021). *Citrullus colocynthis* fruit extracts were used to test their anti-adhesion properties using an adhesion assay on a polystyrene microplate as reported by Agarwal *et al.* (2011) and Aissaoui *et al.* (2021). 100 µL of each concentration of extracts (0.0156 to 2 mg/mL) was mixed in 96-well microplates containing 10^8 CFU/mL of the bacterial strains. Cells can attach to a surface after 3 hours of incubation at 37°C without shaking. After incubation, To completely remove the medium as well as non-adherent bacteria, each well was rinsed three times with 200 µL of distilled water. Afterward, the adherent cells were stained for 1min

at room temperature with crystal violet (0.5%) and fixed for 30 minutes in methanol. Then, 200 µL of ethanol-acetone (80/20%) decolorization solution was added to each well for 15 minutes after excess crystal violet staining was removed with distilled water. Inhibition of bacterial adhesion expressed in percentage was achieved by the absorbance measurement of adherent cells at 595 nm.

$$anti\text{-}adhesion\ effect\ (\%) = \frac{A\ control - A\ sample}{A\ control} \times 100$$

Statistical analysis

The results of independent experiments that were carried out in trials are reported in means ± SEM. The mean differences were determined using the Student test "*t*" and a $P \leq 0.05$ is statistically significant.

RESULTS AND DISCUSSION

Polyphenol and flavonoid content

The polyphenol and flavonoid content in *Citrullus colocynthis* fruit extracts are reported in Table 1. The highest levels of total polyphenols, 260.61 µg eq AG/mg E and 77.7 µg eq Cat/mg E, and flavonoids 222.72 µg eq AG/mg E and 73.03 µg eq Cat/mg E were observed in the butanol and ethyl acetate extracts. These results are in line with Chekroun *et al.* (2015) study which reported that in the butanol extract of C. colocynthis fruit extract a total polyphenols and flavonoids content ranged between 221.85 µg eq AG/mg E and 61.20 µg eq Cat/mg E, respectively. However, Kumar *et al.* (2008) demonstrated a high total polyphenols and flavonoids content in the methanolic extract of *C. colocynthis* fruit which is in the order of 740 mg eq AG/100 g dry extract and 130 mg eq Cat/100 g dry extract, respectively. The content of phenolic compounds in a plant is related to the season of harvest, and the used parts of the plant. Current studies revealed a high level of polyphenols and flavonoids in aqueous and organic extracts in the fruit and the seeds than in the root of *C. colocynthis* extracts (Benariba *et al.*, 2013b; Al-Nabli *et al.*, 2022).

Phenolic compound identification

C. colocynthis extracts contained both ferulic acid and rutin in the ethyl acetate and butanol extracts based on the results of RP and HPLC-PDA chromatographic analysis (Figure 1). In addition, butanol extract contains more unidentified components than ethyl acetate extract. In contrast to previous analyses that have been published in the literature, this analysis is the first to identify phenolic compounds from *C. colocynthis* harvested in Algeria. The reverse phase HPLC identification of *C. colocynthis* from the Pakistani flora revealed the presence of phenolic acids including, ferulic, vanillic, p-coumaric, gallic, and p-hydroxybenzoic acids, as well as flavonoid, catechin, myricetin, and quercetin (Hussain *et al*., 2013).

Antioxidant Activity

The results of the antioxidant activity of *C. colocynthis* extracts expressed in IC_{50} values are reported in Table 2. With an IC_{50} of 65.09 µg/mL for butanol and 89.29 µg/mL for ethyl acetate, respectively, the butanol extract significantly increased the ability to scavenge DPPH. Furthermore, these two extracts demonstrated a substantial impact on the ABTS assay (65.09 IC50 > 89.26 µg/mL), FRAP (99.7 µg/mL A0.5 > 119.37 µg/mL), and CUPRAC (19.41 µg/mL A0.5 > 26.63 µg/mL), except for the total antioxidant capacity assay, where the chloroform extract. In addition, the chloroform extract demonstrated a significant value of 47.6 µg GAA/mg in comparison to the remaining extracts. Our results are in line with the results of Chekroun *et al*. (2015) which documented that the butanol extract of *C. colocynthis* fruit had a significant ability to scavenge DPPH (IC_{50} = 61 µg/mL) and moderate ferric-reducing activity. However, according to Kumar *et al.* (2008) and Benariba *et al.* (2013b), the fruit and seed extracts in methanolic and ethyl acetate showed DPPH scavenging action at high concentrations (0.350 mg/mL < IC_{50} > 2500 mg/mL). No results are published regarding the antioxidant power of colocynth using the ABTS and CURAC tests. According to the bibliography, flavonoids have an anti-oxidant effect due to their ability to transfer their hydroxyl groups to neutralize free radicals and produce FLO•, an effect primarily attributed to the 3',4'-orthodihydroxy group on the B ring, the 4-carbonyl group on the C ring, and the 5-OH and 3-OH groups on the C ring (Chira *et al.*, 2008; Benariba *et al.*, 2013b El Kadi *et al*., 2021).

Table 1: Total polyphenols content in *Citrullus colocynthis* fruit extracts

	Total polyphenol (μg GA/mg E)	Total flavonoid (μg CAT/mg E)
Hydro-methanol	203.94 ± 8.97	60.33 ± 2.52
Chloroform	117.27 ± 5.68	42.4 ± 1.44
EthylAcetate	222.72 ± 4.54	73.03 ± 2.05
Butanol	260.61 ± 13.88	77.7 ± 3.01

Table 2: *Citrullus colocynthis* fruit extracts have antioxidant activity expressed as IC_{50} and $A_{0.5}$.

	DPPH (IC_{50}μg/mL)	ABTS (IC_{50}μg/mL)	FRAP ($A_{0.5}$μg/mL)	CUPRAC ($A_{0.5}$μg/mL)	TAC (μg GAA/mg)
Hydromethanol	147.41±2.21	NT	130.75±2.35	-	103.54±2.67
Chloroform	274.28±13.51	159.12±2.1	277.58±7.92	57.69±2.98	47.6±1.38
Ethyl acetate	89.29±1.41***	86.87±1.26***	119.37±9.73**	26.63±3.43***	114.89±3.67
Butanol	65.09± 8.4**	77.43±3.36**	99.7±5.46***	19.41±1.94***	123.97±5.45
BHA[a]	1.61±0.04	1.81±0.10	14.53±5.76	3.64±0.19	-
BHT[a]	5.32±0.13	1.29±0.30	54.17±1.76	9.62±0.87	-
Ascorbic acid[a]	1.26±0.01	-	19.16±3.47	-	-

IC_{50} and $A_{0.5}$ values expressed asmeans ± SD (n=3)p <0.01; ***p <0.001**
([a]) standard molecule BHA: Butylated Hydroxyanisol.
(-) Not tested.

Table 3: Effect of *Citrullus colocynthis* fruit extracts on AAPH-induced hemolysis

	Hemolysis inhibition (%)				IC_{50} (μg/mL)
	20μg/mL	40 μg/mL	80 μg/mL	100 μg/mL	
Butanol	51.21±2.69	52.13±3.88	53.66±0.47	56.21±2.11	19.11±0.5***
Ethyl acetate	41.83±1.91	43±2.09	45.71±3.07	50.21±1.46	99.64±2.92
Hydro-methanol	35.88±1.38	37.42±0.95	37.46±3.44	41.63±8.06	>100
Quercetin[a]	53±1.39	53.87±0.55	57.37±0.83	58.75±0.63	18.27±0.2
Ascorbic acid[a]	59.5±0.57	59.88±0.69	64.5±0.81	68.63±0.51	16.32±0.05

[a] standard molecule: Quercetin, Acid ascorbic.
IC_{50}value expressed as means ± SD (n=3).
*****p <0.001; ***significance is compared to the positive control (quercetin).**

Effect of *C. colocynthis* extracts on AAPH-induced hemolysis

Upon incubation of red blood cell suspension with AAPH as a peroxyl radical generator, lipids and proteins in the cell membrane are oxidized, which leads to hemolysis (Sekiya *et al.*, 2005; Ilavenil *et al.*, 2011). The effect of colocynth extracts and antioxidant molecules (ascorbic acid and quercetin) on the inhibition of AAPH-induced hemolysis is reported in Table 3. A dose-dependent inhibition of hemolysis was observed with the investigated extracts, as well as the butanol extract showed the most protective effects with an IC_{50} of 19.11 μg/mL, which is equivalent to ascorbic acid (IC_{50} = μ16.32 g/mL) and quercetin (IC_{50} = 18.27 μg/mL). However, the IC_{50} observed in the hydromethanol and ethyl acetate extracts are close to 100 μg/mL. Since no studies on the protective impact of colocynth extracts on red blood cells against AAPH-induced hemolysis have been published, we have no way to compare our results to the existing research. Polyphenols and flavonoids present in butanol extract are thought to play an active role in its anti-hemolytic properties, which have already shown in the bibliography their ability to scavenge the radical species that cause lipid peroxidation and hemolysis of red blood cells (Sekiya *et al.*, 2005**)**. The liposolubility of flavonoids, allows them to bind to phospholipids

Table 4: Antimicrobial activity of *Citrullus colocynthis* fruit extracts determined by microdilution and disk diffusion method

		Diameter of inhibition zone (mm)						MIC (mg/mL)					
		Hydromethanol	Chloroform	Ethyl acetate	Butanol	Gentamicin	Amphotericin B	Hydromethanol	Chloroform	Ethyl acetate	Butanol	Gentamicin	Amphotericin B
Gram-positive	***E. coli* ATCC 8739**	6±0	6±0	8±0.4	8±0.6	22	-	2.5	-	1.25	1.25	0.32	-
	***K. pneumoniae* ATCC 700603**	6±0	6±0	9±0.6	7±0	19	-	2.5	-	1.25	0.625	4.16	-
	***P. aeruginosa* ATCC 27853**	6±0	6±0	6±0	7±0	12	-	2.5	-	2.5	1.25	0.78	-
	***A. baumanni* ATCC 19606**	9±0.6	6±0	10±0.6	11±0.7	35	-	2.5	-	1.25	0.625	0.78	-
	***C. freundii* ATCC 8090**	6±00	6±0	6±0	7±0	18	-	1.25	-	1.25	1.25	0.19	-
	***P. mirabilis* ATCC 35659**	9±0.9	7±0.4	11±0.6	11±0.9	25	-	5	-	1.25	0.625	0.19	-
	***S. typhimirium* ATCC 13311**	6±0	6±0	6±0	6±0	22	-	2.5	-	2.5	2.5	0.65	-
	***E. cloacae* ATCC 13047**	7±0.4	6±0	7±0	9±0.8	21	-	1.25	-	2.5	1.25	2.6	-
Gram-negative	***S. aureus* ATCC 6538**	10±0.8	7±0.7	11±0.8	13±0.9	32	-	1.25	-	0.625	0.312	0.19	-
	***B. cereus* ATCC 25921**	6±0	6±0	6±0	6±0	20	-	5	-	2.5	1.25	0.19	-
	***B. subtilis* ATCC 6633**	10±0.6	7±0.4	9±0.6	10±0.7	22	-	1.25	-	1.25	0.625	5.20	-
	***E. faecalis* ATCC 49452**	7±0.8	6±0	6±0	7±0.4	21	-	2.5	-	1.25	0.625	0.78	-
	***L. monocytogenes* ATCC 15313**	8±0.6	7±0.4	10±0.4	9±0.6	22	-	2.5	-	2.5	1.25	2.21	-
	***M. luteus* ATCC9341**	11±0.4	8±0.6	12±0.8	13±0.9	22	-	1.25	-	0.625	0.625	0.12	-
Yeast	***C. albicans* ATCC 10231**	11±0.8	6±0	12±0.9	15±0.8	-	32	≥50	-	50	25	-	4
	***C. albicans* IP444**	8±0.6	6±0	10±0.6	10±0.6	-	30	25	-	25	25	-	8
	***C. albicans* ATCC26790**	7±0	6±0	13±0.8	8±4	-	30	≥50	-	50	25	-	2

Data are expressed as mean ± SE values (n = 3) ; (-) Not tested.

and membrane proteins by hydrogen bonds, and react as antioxidants against free radicals produced at the red blood cell membrane, which reflects the ability of polyphenols to prevent red blood cell hemolysis (Blasa *et al.*, 2007; Paiva-Martins *et al.*, 2009; Ramchoun *et al.*, 2015).

Antimicrobial activity

Table 4 illustrates the antibacterial effect of *C colocynthis* fruit extracts. Several extracts were observed to be effective against Gram-positive and negative bacteria, but the butanol extract exhibited

Table 5: Bacterial antiadhesion activity (%) of *Citrullus colocynthis* fruit extracts

		Extract concentrations (mg/mL)							
		0.0156	**0.031**	**0.0625**	**0.125**	**0.25**	**0.5**	**1**	**2**
***S. aureus* ATCC 6538**	**Butanol**	10.97± 0.12	16.52± 0.74	25.59± 0.68	32.18± 0.99	46.36± 0.73	50.80± 0.74	51.17± 0.62	51.29± 0.67
	Ethyl acetate	8.75± 0.12	12.95± 0.25	19.3± 0.92	28.98± 0.62	35.08± 0.55	41.62± 0.55	41.8± 0.36	43.09± 0.31
	Hydro-methanol	8.08± 0.67	12.15± 0.06	14.72± 0.3	22.5± 0.55	28.91± 0.55	34.59± 0.18	36.31± 0.55	37.05± 1.04
***L. monocytogenes* ATCC 15313**	**Butanol**	10.73± 0.37	13.35± 0.3	18.48± 0.42	25.49± 0.37	31.4± 0.67	39.51± 0.97	48.84± 0.3	49.39± 0.12
	Ethyl acetate	12.68± 0.12	13.05± 0.12	18.9± 0.37	29.39± 0.85	32.93± 0.85	37.74± 0.43	41.46± 0.24	41.52± 0.19
	Hydro-methanol	7.87± 0.43	10.98± 0.24	15.49± 1.34	19.7± 0.55	27.13± 1.15	33.23± 0.55	34.63± 0.12	35.18± 0.3
***E. coli* ATCC 8739**	**Butanol**	13.66± 0.18	15.86± 0.95	19.59± 0.3	28.64± 0.12	34.62± 0.77	41.72± 1.24	41.89± 1.18	41.95± 1.0
	Ethyl acetate	8.46± 0.06	16.09± 0.83	16.57± 0.11	23.25± 0.53	27.81± 0.82	33.73± 0.36	36.27± 0.3	36.80± 0.35
	Hydro-methanol	9.4± 0.3	12.6± 0.3	15.08± 0.41	18.05± 1	20.65± 0.41	28.93± 0.89	33.25± 1.53	32.25± 0.65

the highest activity. In the disk diffusion method, inhibition zones were determined to have diameters between 7 and 13 mm, and the diameters were detected mostly against *Proteus mirabilis* ATCC 35659, *Staphylococcus aureus* ATCC 6538, and *Micrococcus luteus* ATCC 9341. Also, the butanol extract exhibited the highest MIC values against *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumanii* ATCC 19606, and *Proteus mirabilis* ATCC 35659, ranging from 0.312 to 1.25 mg/mL. *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 6538 were the most sensitive strains to butanol (MIC = 0.312 mg/mL) and ethyl acetate extracts (MIC = 0.625 mg/mL). For antifungal activity, ethyl acetate and butanol extracts showed low activity against Candida albicans strains, with inhibition zone diameters ranging from 10-15 mm and MIC values between 25-50 mg/mL (Table 4), compared to the control antifungal molecule, amphotericin B (32 mm, 2-8 mg/mL).

According to Shahraki-Mojahede *et al.* (2021), *P. aeruginosa* was more sensitive to the ethyl acetate extract than the methanol extract of *C. colocynthis*, MIC = 0.62 mg/mL and 1.25 mg/mL, respectively. Furthermore, the aqueous and methanolic extracts of colocynth leaves showed low antibacterial and antifungal effects (Gurudeeban *et al.*, 2010). Whereas, the aqueous and organic extracts of different parts from *C. colocynthis* (roots, stems, leaves, fruits, and seeds) inhibit the growth of the following strains, *E. coli* (MIC =

mg/ml), *C. albicans, C. kreusei, C. glabrata,* and *C. parapsilosis* (Merzouk *et al.*, 2011; Al-Snafi, 2016). In addition, organic extracts from fresh leaves of *C. colocynthis* ethanol, chloroform, and petroleum ether are more active against *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus*, and slightly active against *Klebsiella pneumoniae* and *Salmonella typhi* (Paul, 2008). In the present study, the antibacterial activity of butanol and ethyl acetate extracts could be explained by the high content of polyphenols and flavonoids in these extracts. According to the literature, Gram-positive bacteria are more sensitive to phenolic compounds compared to Gram-negative bacteria; this sensitivity is related to the absence of the protective hydrophobic lipopolysaccharides of the bacterial outer membrane (Pitchamuthu *et al.*, 2012; Mezni *et al.*, 2015). In addition, polyphenols inhibit the production of nucleic acids, cell walls, and energy, as they can directly affect microbial metabolism by inhibiting the activity of some crucial enzymes, including RNA polymerase, alcohol

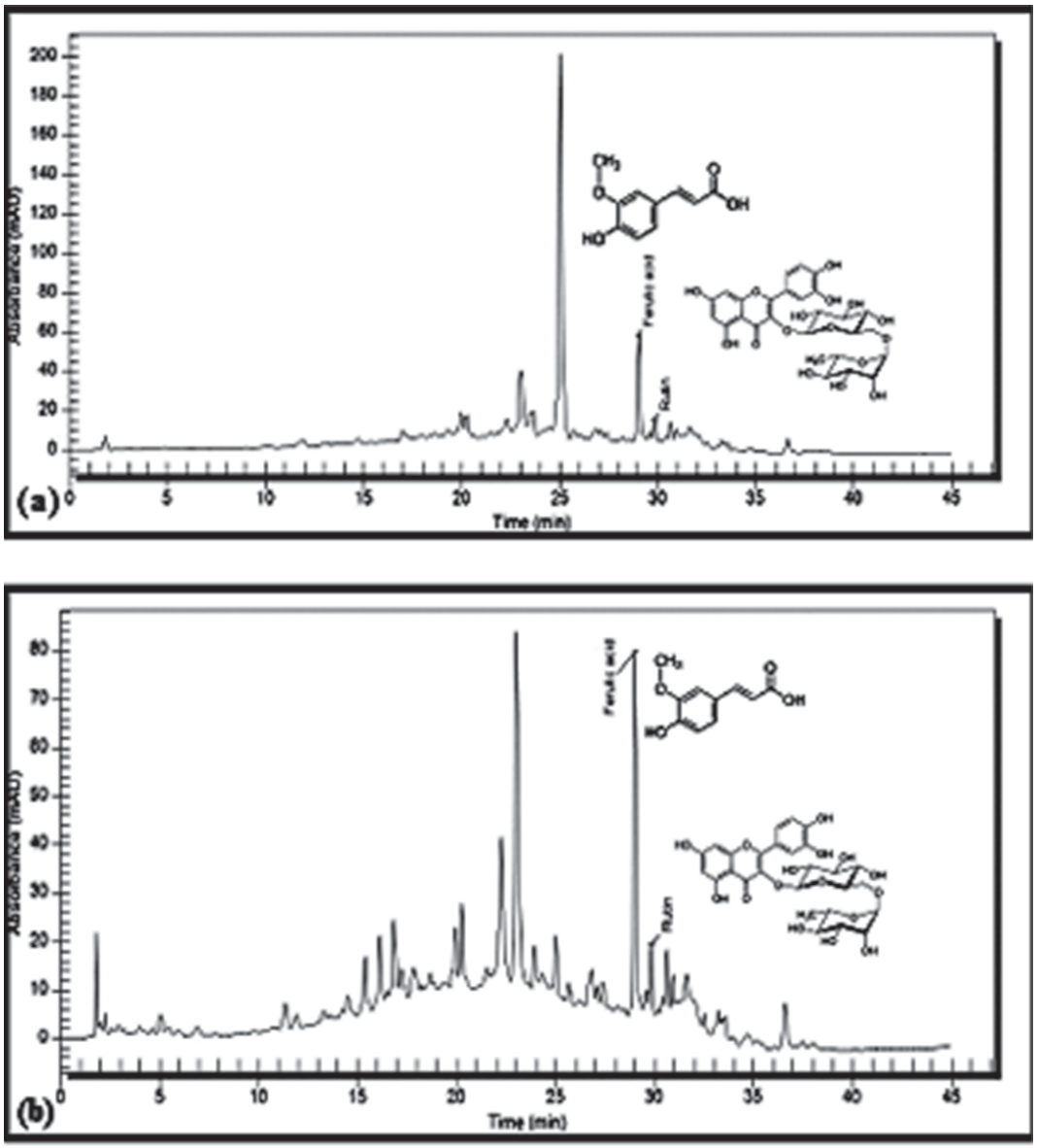

Fig. 1: *Citrullus colocynthis* fruit extracts' high-performance liquid chromatography profile (at 280 nm). (a) ethyl acetate extract (b) n-butanol extract

dehydrogenase, thioredoxin reductase, urease, and dihydrofolate reductase (Simões *et al.,* 2012; Khameneh *et al.,* 2019; Slobodníková *et al.,* 2016; Prakash *et al*., 2020; Almi *et al.,* 2022).

Our study is the first to investigate *Citrullus colocynthis*' anti-bacterial adhesion activity on polystyrene microplates as an informative test on its antibiofilm activity. The results (Table 5) revealed that ethyl acetate, butanol, and hydromethanol extracts significantly reduced the adhesion of *L. monocytogenes* ATCC 15313, *E. coli* ATCC 8739, and *S. aureus* ATCC 6538 at an inhibition rate ranging between 28% and 50%. These findings demonstrate the ability of colocynth to prevent the development of biofilm on the plastic surface. According to Satyavani *et al.* (2011) *Citrullus colocynthis* silver nanoparticles inhibited significantly biofilm-forming bacteria including *P. aeruginosa* (8 mm), *E. coli* (10.1 mm), *P. vulgaris* (9 mm), *V. paraheamolyticus* (10.1 mm), and *L. monocytogens* was the most inhibited (8 mm). The antibiofilm activity of our extracts can be linked to their polyphenol and flavonoid content. In the literature, several flavonoids and phenolic acids

such as quercetin, rutin, catechin, gallic, ferulic, and caffeic acids inhibit biofilm formation by *Listeria monocytogenes*. The anti-biofilm effect of polyphenols lies in their ability to inhibit the synthesis of extracellular polymeric substances, initial adhesion, cell motility, and modification of the physicochemical characteristics of bacteria, all of which are crucial steps in the formation of biofilms (Vazquez-Armenta *et al*., 2018). Polyphenols, without affecting bacterial growth, can inhibit biofilm formation by modifying regulation mechanisms such as quorum sensing or other bacterial systems (Slobodníková *et al*., 2016).

CONCLUSION

In conclusion, phenolic compounds such as ferulic acid, rutin, and other unidentified compounds identified in *C. colocynthis* fruit extracts, might promise the potent antioxidant and antimicrobial properties of these extracts, and their ability to inhibit bacterial adhesion to a plastic surface, which is subsequently the initial stage of biofilm development. *C. colocynthis* may have several benefits, including the potential to aid in the treatment of diseases related to oxidative stress and the prevention of diseases caused by oxidative stress. It can also be used as an antimicrobial source for the treatment of bacterial infections.

REFERENCES :

Adjdir Sara, Nabila Benariba, Imad Abdelhamid El Haci, Khaoula Ouffai, Fouzia Atik Bekkara and Rabah Djaziri.2021*a*. Antioxidant activity and phenolic compounds identification of *Micromeria inodora* (Desf.) Benth. from Western Algeria, *Natural Product Research*, **35** (17): 2963-2966.

Adjdir, S., Benariba, N., Adida, H., Kamila, G., El, I. A., Haci, M. Terki, Z. Fekhikher, H. Benramdane, F. Atik-Bekkara and R. Djaziri. 2021b. Phenolic Compounds and Antimicrobial Activity of Ziziphus jujuba Mill. Fruit from Tlemcen (Algeria). *Journal of Biochemical Technology*, **12**(1): 40-44.

Agarwal, R.K, Singh, S, Bhilegaonkar, K.N. and Singh, V.P. 2011. Optimization of microtitre plate assay for the testing of biofilm formation ability in defferent *Salmonella* serotypes. *International Food*, **18**(4) : 1493-1498.

Aissaoui, M., Rahmoun, M., Latrache, H., Barek, S., Elassri, A., Bensouici, C., El Haci, I., Choukchou-Braham, N. 2021. Structural characterization, antioxidant, and antibiofilm activities of *Coffea canephora* green seeds. *Journal of Complementary and Integrative Medicine*, **18**(1):107-112.

Almi, D., Sebbane, H., Lahcene, S., Habera, F., Laoudi, K. and Mati, A. 2022. Antibacterial and Antioxidant Activities of Various Extracts and Essential Oil from Dried Leaves of *Artemisia herba-alba* Asso of Tamanrasset (South Algeria). *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **8** (1):47-55

Al-Nablsi, S., El-Keblawy, A., Ali, M.A., Mosa, K.A., Hamoda, A.M., Shanableh, A., Almehdi, A.M. and Soliman, S.S. 2022. Phenolic Contents and Antioxidant Activity of *Citrullus colocynthis* Fruits, Growing in the Hot Arid Desert of the UAE, Influenced by the Fruit Parts, Accessions, and Seasons of Fruit Collection. *Antioxidants*, **11**(4): 656.

Al-Snafi, A.E. 2016. Chemical constituents and pharmacological effects of *Citrullus colocynthis*-A review. *IOSR Journal of Pharmacy*, **6**(3):57-67.

Apak, R., Guclu¨, K., Ozyurek, M. and Karademir, S. 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, Using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. *Journal of Agricultural and Food Chemistry*, **52**:7970-7981.

Ardestani, A. and Yazdanparast, R. 2007. Inhibitory effects of ethyl acetate extract of Teucrium polium in vitro protein glycolxydation. *Food and Chemical Toxicology*, **45**:2402-2411.

Atoui, A.K., Mansouri, A., Boskou, G. and Kefalas, P. 2005. Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food Chemistry*, **89**(1):27-36.

Azzi, R., Djaziri, R., Lahfa, F., Sekkal, F.Z., Benmehdi, H. and Belkacem, N.2012. Ethnopharmacological survey of medicinal plants used in the traditional treatment of diabetes mellitus in the North Western and South Western Algeria. *J. Med Plants Res*., **6**(10):2041-50.

Banjo, T.T., Aina, Y.O. and Falade, F.A. 2021. Phytopharmacotherapeutic and Antimicrobial Attributes of Bitter Apple (*Citrullus colocynthis*)-A Review. *Covenant Journal of Physical and Life Sciences*, **9**(1):1-9.

Benariba, N., Djaziri, R., Bellakhdar, W., Belkacem, N., Kadiata, M., Malaisse, W. J., and Sener, A. 2013b. Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. *Asian Pacific Journal of Tropical Biomedicine*, **3**(1):35-40.

Benariba, N., Djaziri, R., Hupkens, E., Louchami, K., Malaisse, W. J. and Sener, A. 2013a. Insulinotropic action of *Citrullus colocynthis* seed extracts in rat pancreatic islets. *Molecular Medicine Reports*, **7**(1): 233-236.

Benariba, N., Djaziri, R., Zerriouh, B.H., Bellakhdar, W., Hupkens, E., Boucherit, Z. and Malaisse, W. J. 2012. Short-and long-term effects of various *Citrullus colocynthis* seed extracts in normal and streptozotocin-induced diabetic rats. *International Journal of Molecular Medicine*, **30**(6):1528-1536.

Benariba, N., Djaziri, R., Zerriouh, B.H., Boucherit, K., Louchami, K., Sener, A. and Malaisse, W.J. 2009. Antihyperglycemic effect of *Citrullus colocynthis* seed aqueous extracts in streptozotocin-induced diabetic rats. *Met Funct Res Diab*, **2**:71-76.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M. P. and Piatti, E. 2007. Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chemistry*, 104(4):1635-1640.

Brewer, M.S. 2011. Natural antioxidants: sources, compounds, mechanisms of action and potential applications. *Comp Rev Food Sci Food Saf*; **10**:221-247.

Chakroun, E., Benariba, N., Adida, H., Asma, B., Rachid, A. and Djaziri, R. 2015. Antioxidant activity and phytochemical screening of two Cucurbitaceae: *Citrullus colocynthis* fruits and Bryonia dioica roots. *Asian Pacific Journal of Tropical Disease*, **5**(8): 632-637.

Chekroun, E., Bechiri, A., Azzi, R., Adida, H., Benariba, N. and Djaziri, R. 2017. Antidiabetic activity of two aqueous extracts of two cucurbitaceae: *Citrullus colocynthis* and Bryonia dioica. *Phytothérapie*, **15**(2) : 57-66.

Chira, K., Suh, J. H., Saucier, C. and Teissèdre, P. L. 2008. Les polyphénols du raisin. *Phytotherapie*, **6**(2), 75–82.

CLSI. 2009. Clinical and Laboratory Standards Institute. Methods for Antimicrobial Susceptibility Tests for Bacteria that Growth Aerobically, 8th Ed. Approved Standard M7-A8. Clinical and Laboratory Standards Institute Wayane, PA.

CLSI. 2012. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 9th Ed. Approvedstandard M07-A9. Clinical and Laboratory Standards Institute Wayane, PA.

Dong, J. W., Cai, L., Xing, Y., Yu, J. and Ding, Z. T. 2015. Re-evaluation of ABTS•+ assay for total antioxidant capacity of natural products. *Natural Product Communications*, **10**(12): 2169-2172.

El Haci, I.A., Mazari, W., Atik-Bekkara, F., Mouttas-Bendimerad, F. and Hassani, F. 2020. Bioactive compounds from the flower part of *Polygonum maritimum* L. collected from Algerian coast. *Current Bioactive Compounds*, **16**(4) : 543-545.

Elkadi, F. Z., Kanoun, K., Trouzine A., Tehami, W., LebgaHouaria, Boumaza, N. and Harir, N. 2021. Phenolic compound profiles and antioxidant activity of *Rutachalepensis* L. leaves, a spontaneous medicinal herb: influence of harvest zone (Western Algeria). *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, **7**(1) : 26-35.

Guo, H.N., Tong, Y. C., Wang, H. L., Zhang, J., Li, Z. X., Abbas, Z., Yang, T. T., Liu, M. Y., Chen, P. Y., Hua Z. C., Yan, X.N., Cheng, Q., Ahmat, M., Wang, J.Y., Zhang, L.L., Wei, X. B., Liao, X.D. and Zhang, R.J. 2021. Novel Hybrid Peptide Cathelicidin 2 (1-13)-Thymopentin (TP5) and Its Derived Peptides with Effective Antibacterial, Antibiofilm, and Anti-Adhesion Activities. *International Journal of Molecular Sciences*, **22**(21) : 11681.

Gurudeeban, S., Rajamanickam, E., Ramanathan, T. and Satyavani, K. 2010. Antimicrobial

activity of Citrullus colocynthis in Gulf of Mannar. *International Journal of Current Research*, **2**(1): 78-81.

Hussain, A. I., Rathore, H. A., Sattar, M. Z., Chatha, S. A., ud din Ahmad, F., Ahmad, A. and Johns, E. J. 2013. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* (L.) from the Pakistani flora. *Industrial Crops and Products*, **45**: 416-422.

Ilavenil, S.B., Kaleeswaran, B., Sumitha, P.D, Tamilvendan, D. and Ravikuma, S. 2011. Protection of human erythrocyte using Crinumasiaticum extract and lycorine from oxidative damage induced by 2-amidinopropane. *Journal of Saudi Biological Science*,**18** (2):181-187.

Karagözler, A.A., Erdag B., Emek YÇ and Uygun D.A. 2008. Antioxidant activity and proline content of leaf extracts from Dorystoechas hastata. *Food Chem*, **111**(2):400-407.

Khameneh, B., Iranshahy, M., Soheili, V. and Fazly Bazzaz, B.S. 2019. Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*, **8**(118): 1–28.

Kumar, S., Kumar, D., Saroha, K., Singh, N. and Vashishta, B. 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharmaceutica*, **58**(2): 215-220.

Li, W., Hydamaka, A.W., Lowry, L. and Beta, T. 2009. Comparison of antioxidant capacity and phenolic compounds of berries, chokecherry and seabuckthorn. *Cent Eur J Biol*., **4**:499-506.

Marzouk, B., Marzouk, Z., Mastouri, M., Fenina, N. and Aouni, M. 2011. Comparative evaluation of the antimicrobial activity of Citrullus colocynthis immature fruit and seed organic extracts. *African Journal of Biotechnology*, **10**(11): 2130-2134.

Mezni, F., Aouadhi, C., Khouja, M. L., Khaldi, A. and Maaroufi, A. 2015. *In vitro* antimicrobial activity of *Pistacia lentiscus* L. edible oil and phenolic extract. *Natural product research*, **29**(6):565-570.

Nmila, R., Gross, R., Rchid, H., Roye, M., Manteghetti, M., Petit, P. and Sauvaire, Y. 2000. Insulinotropic effect of *Citrullus colocynthis* fruit extracts. *Planta Medica*, **66**(05):418-423.

Paiva Martins, F., Fernandes, J., Rocha, S., Nascimento, H., Vitorino, R., Amado, F., Belo, L., Santos Silva, A. 2009. Effects of olive oil polyphenols on erythrocyte oxidative damage. *Molecular Nutrition & Food Research*, **53**(5):609-616.

Paul, J. and John Peter. 2008. Studies on Antimicrobial Efficiency of *Citrullus colocynthis* (L.) Schrad: A Medicinal Plant. *Ethnobotanical Leaflets*: Vol. 2008 : Iss. 1 , Article 128.

Pitchamuthu, A., Muthiah, G. and Rajaram, P. 2012. Preliminary study on the antimicrobial activity on *Enicostem malittorale* using different solvents. *Asian Pacific Journal of Tropical Medecines*, **5**:552-555.

Prakash, B., Kumar, A., Singh, P. P. and Songachan, L.S. 2020. Antimicrobial and antioxidant properties of phytochemicals: Current status and future perspective. Chapter 1; Book *Functional and Preservative Properties of Phytochemicals*. Academic Press. ISBN 978-0-12-818593-3. Pages 1-45.

Prieto, P, Pineda, M. and Aguilar, M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem*., **269** : 337–341.

Ramchoun, M., Khalid, S., Hicham, H., Chakib, A., Mohamed, B., Farid, K. and Souliman, A. 2015. Investigation of antioxidant and antihemolytic properties of Thymus satureioides collected from Tafilalet Region, south-east of Morocco. *Asian Pacific Journal of Tropical Biomedicine*, **5**(2): 93-100.

Re R, Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med*, **26**:1231-1237.

Satyavani, K., Ramanathan, T. and Gurudeeban, S. 2011. Green synthesis of silver nanoparticles by using stem derived callus

extract of bitter apple (*Citrullus colocynthis*). *Dig J Nanomater Biostruct*, **6**(3): 1019-1024.

Sekiya, N., Hikiami, H., Yokoyama, K., Kouta, K., Sakakibara, I., Shimada, Y. and Terasawa, K. 2005. Inhibitory Effects of Stephania tetrandra S. M OORE on Free Radical-Induced Lysis of Rat Red Blood Cells. *Biological and Pharmaceutical Bulletin*, **28**(4):667-670.

Shahraki-Mojahed, L., Behzadmehr, R. and Beigomi, Z. 2021. Antimicrobial effects of ethanol methanol and ethyl acetate Teucrium polium and *Citrullus colocynthis* extract on Pseudomonas aeruginosa. *Mic Environ.*, **1:** 26-32.

Shahzadi, I., Fazal, R.M., Hussain, Z., Yasin, R., Aziz, M.K., Samiullah, K., Malik, I.U., Waseem, M., Yar, A., Khan, S. H. and Ilyas, M. 2022. Anti-diabetic Effects of Aqueous plant (*Citrullus colocynthis*) Extract in Streptozotocin Induced Diabetic Mice. *Haya Saudi J Life Sci*, **7**(6):199-205.

Simões, M., Lemos, M. and Simões, L. C. 2012. Phytochemicals Against Drug-Resistant Microbes. In D. Springer (Ed.), *Dietary Phytochemicals and Microbes* (pp. 185–205).

Slobodníková, L., Fialová, S., Rendeková, K., Kováè, J. and Muèaji, P. 2016. Antibiofilm activity of plant polyphenols. *Molecules*, **21**(12):1717.

Thèriault, M., Caillet, S., Kermasha, S. and Lacroix, M. 2006. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. *Food Chem.*, **98**:490-501.

Vazquez-Armenta, F. J., Bernal-Mercado, A. T., Tapia-Rodriguez, M. R., Gonzalez-Aguilar, G. A., Lopez-Zavala, A. A., Martinez-Tellez, Hernadez-Oñate, M. A. and Ayala-Zavala, J. F. 2018. Quercetin reduces adhesion and inhibits biofilm development by Listeria monocytogenes by reducing the amount of extracellular proteins. *Food Control*, **90:** 266-273.

Vermerris, W. and Nicholson, R. 2006. Isolation and identification of phenolic compounds. Phenolic compound biochemistry. Springer Netherlands, pp.151-196.

Xiaoping, Y, Jing, W., Huiyuan, Y. and Feng, C. 2005. Free radical-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *Swiss Society of Food Science and Technology*, 877-883.