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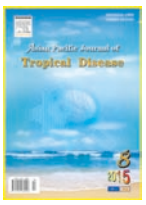

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Antioxidant activity and phytochemical screening of two Cucurbitaceae: *Citrullus colocynthis* fruits and *Bryonia dioica* roots

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ABSTRACT

Objective: To evaluate antioxidant activity and quantify total content of polyphenols and flavonoids in two Cucurbitaceae plant extracts, *Citrullus colocynthis* (*C. colocynthis*) fruits and *Bryonia dioica* (*B. dioica*) roots.
Methods: Qualitative and quantitative analysis of aqueous and butanolic extracts, prepared from *C. colocynthis* fruits and *B. dioica* roots was carried out using standard methods. Estimation of their antioxidant activity was determined by free radical scavenging activity assay using 2,2-diphenyl-l-picrylhydrazyl and ferric reducing antioxidant power assay.
Results: Preliminary phytochemical screening of the aqueous and organic extracts showed the presence of tannins, flavanoids, terpenoids, saponins and quinones. The content of phenolic compounds varies among the two species of cucurbits and even in the different extracts of the same species. The most important amount of total polyphenols and flavanoids expressed respectively as gallic acid and catechin equivalent per gram extract, was determined in butanolic extract of *B. dioica* roots (541.78 mg/g, 120.60 mg/g). The lowest values were noted in *C. colocynthis* fruit aqueous extract (219.58 mg/g, 46.13 mg/g). Butanolic extract of *B. dioica* roots exhibited an interesting free radical scavenging activity with IC_{50} = 2.25 μg/mL compared to IC_{50} = 1.5 μg/mL of ascorbic acid, followed by *B. dioica* roots aqueous extract (IC_{50} = 47.25 μg/mL), butanolic *C. colocynthis* fruit extract (IC_{50} = 61 μg/mL) and *C. colocynthis* fruit aqueous extract (IC_{50} = 241.25 μg/mL). A highest reducing power was observed in *B. dioica* roots butanolic extract compared to other extracts (*C. colocynthis* fruit aqueous and butanolic extracts and *B. dioica* roots aqueous extract).
Conclusions: The results indicate that the wealth of *B. dioica* roots extracts on polyphenols and flavonoids is more than *C. colocynthis* fruit extracts. These phytoconstituents ensure an interesting free radical scavenging activity and reducing power for the *B. dioica* roots extracts especially butanolic extract.

1. Introduction

Antioxidants are widely interesting because of their potential role in food industry and human health. In small quantities, they are able to inhibit or delay oxidation of easily oxidizable molecules[1]. Numerous studies showed that many natural and synthetic antioxidants were reported to prevent various diseases. However, for their efficiency and less side effects on health, natural antioxidants are more used and preferred relatively to synthetic antioxidants which increase liver damage and carcinogenesis[2]. Last decades, many scientific studies have been interested in medicinal plants, an important source of secondary metabolites as natural antioxidants, whose phenolic compounds, flavonoids and terpenes reported to be potent free radical scavengers[3]. In this context, a large number of medicinal plants used in traditional medicine around the world were analyzed for their antioxidant activity. Among these plants, many species of Cucurbitaceae family traditionally used as antidiabetic remedies are currently studied for their antioxidant capacity[4-6]. Cucurbitaceae includes 118 genera with 825 species[7]. The most important genus are *Cucurbita*, *Cucumis*, *Ecballium*, *Citrullus*, *Luffa*, *Bryonia*, *Momordica* and *Trichosanthes* distributed over the world, many of them are edible while others are medicinal or ornamental plants[8-10]. They grow in tropical, subtropical, arid deserts and temperate areas[11]. Pharmacological activity of many

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cucurbit species is related to their secondary metabolites content, essentially cucurbitacins, saponins, polyphenols, flavonoids, alkaloids and terpenoids[5,9,12,13]. In Algeria, *Citrullus colocynthis* (*C. colocynthis*) and *Bryonia dioica* (*B. dioica*), are the frequent Cucurbitaceae used for the treatment of infectious diseases, inflammation, hemorrhoids, diabetes and cancers[6,14-16]. The aim of the present study is to evaluate the antioxidant capacity of *C. colocynthis* fruit and *B. dioica* roots extracts (the parts of the plants used locally in traditional medicine), using two conventional methods: free radical scavenging activity assay [2,2-diphenyl-l-picrylhydrazyl radical (DPPH)] and ferric reducing antioxidant power assay (FRAP). A phytochemical screening and quantitative analysis of total polyphenols and flavonoids were also carried out on aqueous and organic plant extracts.

2. Materials and methods

2.1. Chemicals and reagents

DPPH, Folin-Ciocalteu reagent, gallic acid (GA), catechin, ascorbic acid, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), potassium ferricyanide ($K_3Fe(CN)_6$), trichloroacetic acid, ferric chloride ($FeCl_3$) and all organic solvents (hexane, methanol, chloroform, *n*-butanol) were purchased from Sigma-Aldrich chemie GmbH (Germany).

2.2. Plant materials

C. colocynthis fruits and *B. dioica* roots were collected respectively from Naâma and Mascara in the west of Algeria during spring and summer 2012. Botanical identification was performed in Laboratory of Ecology And Management of Natural Ecosystems at Tlemcen University (Algeria). In the laboratory, both fruits and roots were cleaned and dried at room temperature. Fruits were crushed into small pieces and roots were ground to fine powder using laboratory grinder (Mortar Grinder km 100, Retsch) and conserved for further extractions.

2.3. Preparation of extracts

2.3.1. Aqueous extracts

C. colocynthis fruit aqueous (CCFaq) extract was prepared as follows: 10 g of crushed fruits are infused for 1 h in 100 mL of distilled water at 50 °C, then boiled under reflux for 15 min. Regarding the *B. dioica* roots aqueous (BDRaq) extract, 10 g of powdered roots were extracted in 100 mL of distilled water at room temperature over night with continuous stirring. After that, preparations were filtered, centrifuged and evaporated to dryness.

2.3.2. Butanolic extracts

Both *C. colocynthis* fruits and *B. dioica* roots were defatted with hexane in Soxhlet for 3 h. After air drying, 10 g of the defatted samples were extracted twice in 100 mL of aqueous methanol mixture (v/v) at room temperature over night with continuous stirring. Extracts were then centrifuged at 4 316 r/min for 10 min and the supernatant was collected. After elimination of methanol, the aqueous phase was then transferred into a separating funnel to be extracted three times with an equal volume of chloroform to remove pigments. The recovered aqueous phase was extracted twice with *n*-butanol, which was completely evaporated to dryness under pressure[17]. Obtained extracts were *C. colocynthis* fruit butanolic (CCFn-but) extract and *B. dioica* roots butanolic (BDRn-but) extract.

2.4. Phytochemical screening

Prepared extracts were subjected to preliminary phytochemical screening in order to qualitatively determine some of the secondary metabolites: saponins, terpenoids, flavonoids, tannins, alkaloids, quinones, anthraquinones and reducing sugar using appropriate methods[6,16,18].

2.5. Total polyphenols content

The total polyphenols content of aqueous (CCFaq, BDRaq) and butanolic (CCFn-but, BDRn-but) extracts, prepared from *C. colocynthis* fruits and *B. dioica* roots was assessed by Folin-Ciocalteu reagent with GA as standard. Aliquots of test samples (100 μL) containing 1 mg/mL, were mixed with 2 mL of freshly prepared Na_2CO_3 (2%) solution. After 5 min, 100 μL of Folin-Ciocalteu reagent (0.2 N) were added to the mixture, all was left for 30 min in obscurity at room temperature and the reading was performed against a blank at 700 nm. A calibration curve was performed in parallel under the same operating conditions using GA as a positive control. The results are expressed as mg GA equivalent per gram of dry extract (mg GA eq/g)[6,19].

2.6. Total flavonoids content

The total flavonoids content of each aqueous (CCFaq, BDRaq) and butanolic (CCFn-but, BDRn-but) extracts of *C. colocynthis* fruits and *B. dioica* roots was determined by a colorimetric method. Each sample (0.5 mL) containing 1 mg/mL, was mixed with 2 mL of distilled water and 0.15 mL of $NaNO_2$ solution (15%). After 6 min, 0.15 mL of $AlCl_3$ (10%) and 2 mL of NaOH (4%) solutions were added. Immediately, water was added to bring the final volume to 5 mL and tubes were thoroughly mixed and incubated for 15 min at room temperature. The absorbance was measured at 510 nm. Results were expressed as catechin equivalent per gram of dry extract (mg Cat eq/g)[6,19].

2.7. Antioxidant activity

2.7.1. DPPH free radical scavenging activity

The free radical scavenging activity of *C. colocynthis* fruit and *B.*

dioica roots extracts was determined using DPPH according to the protocol of El-Haci *et al.* Briefly, 50 μL of each aqueous (CCFaq, BDRaq) and butanolic (CCFn-but, BDRn-but) extracts solutions at different concentrations were added to 1.95 mL of DPPH solution (6 × 10^{-5} mol/L in methanol). The mixture was vigorously shaken and incubated for 30 min in obscurity[20]. Absorbance was measured at 515 nm and ascorbic acid was used as antioxidant reference. The absorbance (A) of the control and samples was measured, and the free radical scavenging activity in percentage was determined as follow:

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

IC_{50} was determined graphically from nonlinear regression analysis, and the inverse of the value of IC_{50} means antiradical activity (ARA) value[21].

$ARA = 1/IC_{50}$

2.7.2. FRAP

FRAP assay measures the reduction of ferric iron to the ferrous form in the presence of the antioxidant components[22]. The reducing power of both *C. colocynthis* fruit and *B. dioica* roots extracts was evaluated according to the method of Karagözler *et al.* To 1 mL of each extract solution (CCFaq, CCFn-but, BDRaq, BDRn-but) at different concentrations (0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00 mg/mL), 2.5 mL of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1%) were added. The mixture was incubated at 50 ℃ for 20 min, after that 2.5 mL of trichloroacetic acid (10%) were added. The mixture was centrifuged at 3 000 r/min for 10min, then 2.5 mL of the supernatant were mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (1%)[23].

The absorbance was measured at 700 nm against a blank sample and ascorbic acid was used as antioxidant reference. Increased absorbance of the reaction mixture indicated increased reducing power.

3. Results

3.1. Phytochemical screening

Some characteristics of *C. colocynthis* fruit and *B. dioica* roots extracts were illustrated in Table 1. After evaporation to dryness, all extracts were obtained as powder (CCFaq, CCFn-but, BDRn-but), except for BDRaq extract which was recovered as hygroscopic paste. The extraction yield of aqueous extract from the roots of *B. dioica* was 11.26% than the yield of CCFaq 5.41%. Phytochemical analysis (Table 2) revealed the presence of the majority of phytoconstituents in CCFaq extract except anthraquinones. In its butanolic fraction (CCFn-but), we found mainly terpenoids, saponins and flavonoids, quinones at lesser degree. BDRaq extract is characterized by the presence of tannins, saponins, quinones, amines, flavonoids and terpenoids. Its butanolic fraction (BDRn-but) contained mainly tannins, saponins, terpenoids, flavonoids and quinones.

Table 1

Characteristics of *C. colocynthis* fruit and *B. dioica* roots extracts.

Extract	Physical aspect	Color	Yield (%)	Solubility
CCFaq	Powder	Brown	5.41	Water
CCFn-but	Powder	Brown	2.61	Water
BDRaq	Paste	Brown	11.26	Water
BDRn-but	Powder	Orange	1.90	Water

Table 2

Phytochemical screening of *C. colocynthis* fruit and *B. dioica* roots extracts.

	CCFaq	CCFn-but	BDRaq	BDRn-but
Flavonoids	++	+	+	+
Tannins	+	–	++	++
Alkaloids	++	–	–	–
Terpenoids	++	++	+	+
Saponins	+	++	++	++
Coumarins	++	–	–	–
Quinones	++	+	++	+
Anthraquinones	–	–	–	–
Amines	++	–	++	–

++: Strong positive test; +: Low positive test; –: Negative test.

3.2. Total polyphenols and flavonoids content

The results reported in Table 3 showed that the BDRn-but extract contains the higher rate of total polyphenols, 541.78 mg followed by BDRaq, CCFn-but and CCFaq extracts with 226.87, 221.85 and 219.58 mg GA eq/g dry extract respectively. Similarly, the rate of total flavonoids rises in the same extracts to 120.60, 61.20, 53.67 and 46.13 mg Cat eq/g dry extract in BDRn-but, CCFn-but, BDRaq and CCFaq extracts respectively. Results relatively expressed to 100 g of plant matter are dependent on extraction yield of each extract.

Table 3

Total polyphenols and flavonoids content of *C. colocynthis* fruit and *B. dioica* roots extracts.

Extract	Polyphenols[a]	Polyphenols[b]	Flavonoids[a]	Flavonoids[b]
CCFaq	219.58	1187.90	46.13	249.58
CCFn-but	221.85	579.04	61.20	159.73
BDRaq	226.87	2554.56	53.67	604.29
BDRn-but	541.78	1029.38	120.60	229.14

[a]: mg gallic acid (catechin) equivalent per g extract; [b]: mg gallic acid (catechin) per 100 g plant matter.

3.3. Antioxidant activity

3.3.1. Free radical scavenging activity on DPPH

The test is based on evaluation of different extracts (CCFaq, BDRaq, CCFn-but, BDRn-but) effect on scavenging free radicals using DPPH. Ascorbic acid is used as a reference molecule. According to the results (Table 4) we found that the free radical scavenging activity increased with concentration of reference molecule or tested extracts. The butanolic *B. dioica* roots extract (BDRn-but) shows an interesting free radical scavenger activity compared to the other extracts, 10μg/mL of this extract decrease 94% of the DPPH and the IC_{50} found was to be 2.25μg/mL. Butanolic *B. dioica* roots (BDRn-but) extract at high concentrations quickly reduces DPPH, for this

reason it has been tested at low concentrations from 1.25 to 10 µg/mL. The other three extracts, BDRaq, CCFn-but and CCFaq show IC_{50} values of 47.25, 61 and 241.25µg/mL respectively.

Table 4
DPPH inhibition percentage of ascorbic acid and *C. colocynthis* fruit, *B. dioica* roots extracts.

Extract	Concentration (µg/mL)	DPPH inhibition percentage (%)	IC_{50} (µg/mL)	ARA
Ascorbic acid	0.5	13.86	1.50	0.6667
	1.0	35.68		
	1.5	50.43		
	2.0	61.97		
	4.0	91.60		
CCFaq	200	44.97	241.25	0.0041
	225	48.13		
	250	50.79		
	300	67.28		
	350	81.63		
	400	91.95		
CCFn-but	25	32.68	61.00	0.0164
	50	43.34		
	75	52.82		
	125	68.06		
	175	76.12		
	250	85.74		
BDRaq	25	31.02	47.25	0.0212
	50	48.00		
	75	56.37		
	125	71.19		
	175	84.36		
	250	95.85		
BDRn-but	1.25	23.52		
	1.87	36.49		
	2.50	52.52	2.25	0.4444
	5.00	69.28		
	7.50	84.59		
	10.00	94.00		

3.3.2. FRAP

Results of the reducing power of *C. colocynthis* fruit and *B. dioica* roots extracts measured for the concentration up to 3 mg/mL increased with concentration as presented in Figure 1.

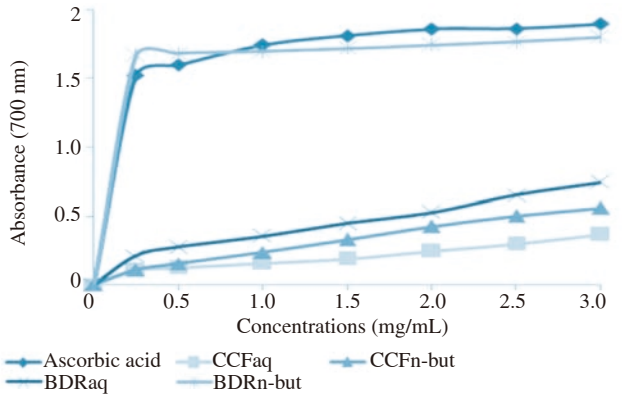

Figure 1. Reducing power of ascorbic acid, *C. colocynthis* fruit extracts and *B. dioica* roots extracts.

At the concentration of 3 mg/mL, CCFaq, CCFn-but, BDRaq and BDRn-but extracts showed a reducing power of 0.36, 0.55, 0.74 and 1.79 respectively. Among aqueous and organic extracts, butanolic *B. dioica* roots extract BDRn-but provided the highest reducing power, comparable with ascorbic acid (1.89 at 3 mg/mL).

4. Discussion

Scientific and pharmaceutical researches have recently interested in cucurbitaceae medicinal plants, which are mainly used in traditional medicine for their therapeutic properties as antidiabetic and anti-inflammatory remedies[6,14,24]. Thus, their therapeutic property is probably related to their content of phytochemicals.

This study and other previous studies Kumar *et al.* Hussain *et al.* Benariba *et al.* Barreira *et al.* and Rafael *et al.* illustrated the phytoconstituents contain and antioxidant activity of *C. colocynthis* fruits and *B. dioica* roots collected at maturity and prepared in various aqueous and organic extracts[4-6,25,26].

The qualitative analysis of the *C. colocynthis* fruit extracts revealed the presence of terpenoids, saponins, flavonoids and quinones and the absence of anthraquinones. These results are in confirmation with a recent study of Najafi *et al.* in which it was reported that the aqueous and ethanolic (80%) extracts of both *C. colocynthis* fruits and leaves contained flavonoids, glycosides and saponosides[12]. A number of chemical compounds were already identified in different extracts obtained from different parts of *C. colocynthis* (seeds, pulps, leaves and roots), particularly alkaloids ($C_{10}H_{15}NO_3$; $C_{20}H_{32}NO$; $C_{16}H_{24}NO_7$), sterols ($C_{29}H_{48}O$; $C_{29}H_{50}O$), cucurbitacins (I, E, L, J) and flavonoids such as quercetin, myrcetin, kaemoferol, isovitexin, iso-orientin, iso-orientin-3'-methylether and C-p-hydroxybenzyl derivatives (8-C-p hydroxybenzoylisovitexin, 6-C phydroxybenzoylvitexin and 8-C-p-hydroxybenzoyl isovitexin-4'-O-glucoside)[6].

In our phytochemical screening on *B. dioica* roots extracts we have revealed the existence of tannins, flavonoids, terpenoids, saponins and quinones. Recently, Barros *et al.* have reported that the phenolic profile of *B. dioica* shoots, identifying mainly C-glycosylated flavonoids[27]. Moreover, the study of Barreira *et al.* identified fourteen chemical compounds in the fruit of *B. dioica*, nine flavonols and five flavones, which kaempferol 3-O-neohesperidoside and apigenin-6-C-glucoside-8-C-glucoside were the main flavonol and flavone respectively[25]. Other several active chemical constituents of *B. dioica* plant extracts were recorded, they are grouped as polyphenols, saponins and alkaloids[16,28]. In addition, the roots contain seven new triterpene glycosides, bryoniosides, which have been isolated with two known triterpene glycosides, cabenoside D and bryoamarid[29].

The therapeutic properties of plant extracts, antioxidant, are generally related to the rate of phytoconstituents mainly polyphenols and flavonoids. The pivotal role of phenolic compounds as scavengers of free radicals is emphasized in several reports[30,31]. In fact, polyphenols and flavonoids are reported powerful antioxidants and have been consistently protective through scavenging numerous diverse reactive oxygen species including hydroxyl radical, peroxyl

radical, hypochlorous acids, superoxide anion, and peroxynitrite[32]. In this study, the level of total polyphenols determined vary in the different extracts of the cucurbit plants, in either *C. colocynthis* fruits as *B. dioica* roots, the organic extracts contain more than the aqueous extracts. Therefore, it suggests a high correlation between the rate of polyphenols in each plant extracts and the free radical scavenging activity as the ferric reducing power.

The results show that BDRn-but has an interesting scavenging activity (IC_{50} = 2.25 μg/mL) than the other plant extracts (BDRaq IC_{50} = 47.25 μg/mL, CCFn-but IC_{50} = 61.00 μg/mL, CCFaq IC_{50} = 241.25 μg/mL). As well as, reducing power assay revealed that BDRn-but showed the highest power to reduce iron followed by BDRaq, CCFn-but and CCFaq. This antioxidant activity of BDRn-but extract may be attributed to its high total content of polyphenols and flavonoids, 541.78 mg GA eq/g and 120.60 mg Cat eq/g dry extract respectively. The ability of BDRn-but to scavenge free radicals (DPPH) and reduce iron (FRAP) is related to polyphenols considered as electron donors, which can react with free radicals and convert them to more stable products.

Antioxidant activity of phenolic compounds such as flavonoids depends on their chemical structure, especially of the distribution of hydroxyl groups. The scavenging of free radicals is tightly linked to the configuration of the 3',4'-orthodihydroxy on the B cycle and the 4-carbonyl group on the C cycle, the 3-OH and 5-OH groups on the C cycle are also relevant to the antioxidant effect[6,33].

Various extracts from *C. colocynthis* seeds were also reported by Benariba *et al.* to contain polyphenols, 61.62–298.88 mg GA equivalent per g extract, and flavonoids, 24.49–219.18 mg catechin equivalent per g extract. Besides, the results of IC_{50} revealed that ethyl acetate extract represented the most important antioxidant activity with 350 μg/mL[6]. Furthermore, the antioxidant activity and phenolic content of *C. colocynthis* fruits collected from the desert area of Pakistan were investigated by Hussain *et al.* results of free radical scavenging activity assay (DPPH) revealed IC_{50} value of 8.15 μg/mL and 7.14 μg/mL for the hexane and ethanol extracts respectively; again the ethanol extract showed better reducing power than hexane extract according to FRAP; As well as, the total polyphenols and flavonoids values were reported as respect 0.84–0.46 mg/g of dry plant material for the hexane extract and 3.07–0.51 mg/g of dry plant material for the ethanol extract[5]. Moreover, the methanolic extract of *C. colocynthis* fruits collected from the Haryana area in India, contained 740 mg of GA equivalent per 100 g plant matter as polyphenols and 130 mg of catechin equivalent per 100 g plant matter as flavonoids. The highest free radical scavenging ability of this extract was observed at the concentration of 2 500 μg/mL[4]. Comparatively, the antioxidant capacity obtained in the *C. colocynthis* fruit extracts of previous studies is more important than the present study. This is probably related to ecological conditions and treatment (harvest, drying, and extraction methods) of the plant.

With respect to *B. dioica*, the property of various parts extracts (fruits, stems, leaves, flowers) to scavenge the free radicals and reduce iron has been evaluated using different assays. According to Rafael *et al.* the methanolic extract of *B. dioica* ripened fruits presented IC_{50} by DPPH assay and FRAP assay (1.21 ± 0.02) and (0.40 ± 0.00) mg/mL respectively[26]. The same is also reported by the results of Barreira *et al.* showing that the ethanolic extract of *B. dioica* ripened fruits was characterized by IC_{50} value of (1.5 ± 0.1) mg/mL in DPPH assay and (0.9 ± 0.1) mg/mL in FRAP assay[25]. Another study, documented the DPPH free radical scavenging activity of three parts of *B. dioica* methanolic extract, during flowering the polar subfraction of stems, leafs and flowers showed a IC_{50} value of (285.67 ± 9.38), (48.32 ± 3.86) and (31.25 ± 3.38) μg/mL respectively[34].

According to our knowledge, no earlier reports are available regarding the antioxidant activity of the roots extracts of *B. dioica*. Our study revealed that roots extracts of *B. dioica* are characterized by a high antioxidant activity, comparatively to other parts of the plant as reported by previous studies[25,26,34]. This property can be attributed to high amounts of polyphenols as evaluated by qualitative and quantitative phytochemical analysis.

In conclusion, the present study has demonstrated that *B. dioica* roots extracts contain relatively large amounts of polyphenols and flavonoids compared to *C. colocynthis* fruit extracts. Furthermore, the butanolic extract of *B. dioica* roots possesses an interesting free radical scavenging activity, which could be related to the total content of polyphenols and flavonoids. Thus, it seems pertinent to identify the majority chemical components of this butanolic extract of *B. dioica* roots and to document its other therapeutic effects in further phytochemical and pharmacological studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action and potential applications. *Comp Rev Food Sci Food Saf* 2011; **10**: 221-47.

[2] Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process* 2011; **89**: 217-33.

[3] Mathew S, Abraham TE. *In vitro* antioxidant activity and scavenging effect of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food Chem Toxicol* 2006; **44**: 198-206.

[4] Kumar S, Kumar D, Manjusha, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus*

colocynthis (L.) Schrad. methanolic fruit extract. *Acta Pharm* 2008; **58**: 215-20.

[5] Hussain AI, Rathore HA, Sattar MZA, Chatha SAS, Ahmad F, Ahmad A, et al. Phenolic profile and antioxidant activity of various extracts from *Citrullus colocynthis* (L.) from the Pakistani flora. *Ind Crops Prod* 2013; **45**: 416-22.

[6] Benariba N, Djaziri R, Bellakhdar W, Belkacem N, Kadiata M, Malaisse WJ, et al. Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. *Asian Pac J Trop Biomed* 2013; **3**(1): 35-40.

[7] Solmaz I, Sari N, Aka-Kacar Y, Yalcin-Mendi NY. The genetic characterization of Turkish watermelon (*Citrullus lanatus*) accessions using RAPD markers. *Genet Resour Crop Evol* 2010; **57**: 763-71.

[8] Jeffrey C. A review of Cucurbitaceae. *Bot J Linn Soc* 1980; **81**: 233-47.

[9] Schaefer H, Renner SS. Cucurbitaceae. In: Kubitzki K, editor. *Flowering Plants. Eudicots.* Berlin: Springer Verlag; 2011, p. 112-74.

[10] Renner SS, Pandey AK. The Cucurbitaceae of India: accepted names, synonyms, geographic distribution, and information on images and DNA sequences. *PhytoKeys* 2013; **20**: 53-118.

[11] Fokou E, Achu MB, Kansci G, Ponka R, Fotso M, Tchiegang C, et al. Chemical properties of some Cucurbitaceae oils from cameroon. *Pak J Nutr* 2009; **8**(9): 1325-34.

[12] Najafi S, Sanadgol N, Nejad BS, Beiragi MA, Sanadgol E. Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus. J Med Plant* 2010; **4**: 2321-5.

[13] Gurudeeban S, Ramanathan T, Satyavani K. Characterization of volatile compounds from bitter apple (*Citrullus colocynthis*) using GC-MS. *Int J Chem Anal Sci* 2011; **2**: 108-10.

[14] Azzi R, Djaziri R, Lahfa F, Sekkal FZ, Benmehdi H, Belkacem N. 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* 2012; **6**(10): 2041-50.

[15] Sebbagh N, Cruciani-Guglielmacci C, Ouali F, Berthault MF, Rouchb C, Sari DC, et al. Comparative effects of *Citrullus colocynthis*, sunflower and olive oil-enriched diet in streptozotocin-induced diabetes in rats. *Diabetes Metab* 2009; **35**: 178-84.

[16] Benarba B, Meddah B, Aoues A. *Bryonia dioica* aqueous extract induces apoptosis through mitochondrial intrinsic pathway in BL41 Burkitt's lymphoma cells. *J Ethnopharmacol* 2012; **141**: 510-6.

[17] Ncube B, Ngunge VN, Finnie JF, Van Staden J. A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv plants. *J Ethnopharmacol* 2011; **134**: 775-80.

[18] Khan AM, Qureshi RA, Ullah F, Gilani SA, Nosheen A, Sahreen S, et al. Phytochemical analysis of selected medicinal plants of Margalla Hills and surroundings. *J Med Plants Res* 2011; **5**(25): 6055-60.

[19] Belkacem N, Djaziri R, Lahfa F, El-Haci IA, Boucherit Z. Phytochemical screening and *in vitro* antioxidant activity of various *Punica granatum* l peel extracts from Algeria: a comparative study. *Phytothérapie* 2014; **12**: 372-9.

[20] El-Haci IA, Didi A, Atik Bekkara F, Gherib M. *In vitro* antioxidant activity and total phenolic contents in methanol crude extracts from the Algerian medicinal plant *Limoniastrum feei*. *Sci Study Res* 2009; **10**: 329-36.

[21] Maisuthisakul P, Suttajit M, Pongsawatmanit R. Assessment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. *Food Chem* 2007; **100**: 1409-18.

[22] Suàrez B, Alvarez AL, Garcia YD, Barrio G, Lobo AP, Parra F. Phenolic profiles, antioxidant activity and *in vitro* antiviral properties of apple pomace. *Food Chem* 2010; **120**: 339-42.

[23] Karagözler AA, Erda B, Emek YÇ, Uygun DA. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. *Food Chem* 2008; **111**(2): 400-7.

[24] Matsuda H, Nakashima S, Abdel-halim OB, Morikawa T, Yoshikawa M. Cucurbitane-type triterpenes with anti-proliferative effects on U937 cells from an Egyptian natural medicine, *Bryonia cretica*: structures of new triterpene glycosides, bryoniaosides A and B. *Chem Pharm Bull(Tokyo)* 2010; **58**: 747-51.

[25] Barreira JCM, Pereira E, Due=ñas M, Carvalho AM, Santos-Buelga C, Ferreira ICFR. *Bryonia dioica*, *Tamus communis* and *Lonicera periclymenum* fruits: characterization in phenolic compounds and incorporation of their extracts in hydrogel formulations for topical application. *Ind Crops Prod* 2013; **49**: 169-76.

[26] Rafael M, Barros L, Carvalho AM, Ferreira ICFR. Topical anti-inflammatory plant species: bioactivity of *Bryonia dioica*, *Tamus communis* and *Lonicera periclymenum* fruits. *Ind Crops Prod* 2011; **34**: 1447-54.

[27] Barros L, Dueñas M, Ferreira ICFR, Carvalho AM, Santos-Buelga C. Use of HPLC-DAD-ESI/MS to profile phenolic compounds in edible wild greens from Portugal. *Food Chem* 2011; **127**: 169-73.

[28] García-Herrera P, Sánchez-Mata MC, Cámara M, Tardío J, Olmedilla-Alonso B. Carotenoid content of wild edible young shoots traditionally consumed in Spain (*Asparagus acutifolius* L, *Humulus lupulus* L, *Bryonia dioica* Jacq. and *Tamus communis* L). *J Sci Food Agric* 2013; **93**: 1692-8.

[29] Ukiya M, Akihisa T, Yasukawa K, Toduda H, Toriumi M, Koike K, et al. Anti-inflammatory and antitumor-promoting effects of cucurbitane glycosides from the roots of *Bryonia dioica*. *J Nat Prod* 2002; **65**: 179-83.

[30] Dueñas M, Hernández T, Estrella I. Assessment of *in vitro* antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chem* 2006; **98**: 95-103.

[31] Kilani-Jaziri S, Bhouri W, Skandrani I, Limem I, Chekir-Ghedira L, Ghedira K. Phytochemical, antimicrobial, antioxidant and antigenotoxic potentials of *Cyperus rotundus* extracts. *South Afr J Bot* 2011; **77**: 767-76.

[32] Halliwell B, Gutteridge J. *Free radicals in biology and medicine*. 4th ed. Oxford: Oxford University Press; 2007.

[33] Wojdyło A, Oszmian´ski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 2007; **105**: 940-9.

[34] Gholivand MB, Piryaei M. The antioxidant activity, total phenolics and total flavonoids content of *Bryonia dioica* Jacq. *Biologija* 2012; **58**(3): 99-105.