



Full Length Article

Phytochemical Composition, Antioxidant Activity, Antiproliferative Effect and Acute Toxicity Study of *Bryonia dioica* Roots used in North African Alternative Medicine

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Received 14 October 2019; Accepted 18 November 2019; Published 04 February 2020

Abstract

Bryonia dioica (*B. dioica*) belongs to the Cucurbitaceae family. It is widely used in folk medicine for disease treatment. The current work was conducted to investigating the phytochemical composition, total polyphenolic and flavonoids contents, antioxidant activity, antiproliferative effect and acute toxicity of methanolic extract of *B. dioica* roots. Phytochemical composition of *B. dioica* roots was performed using GC-MS. Antioxidant activity was evaluated using DPPH assay. Antiproliferative effect against human cancer cell lines (T-24, HT-29, and HepG-2) was studied using WST-1 assay, and the acute toxicity was assessed with oral administration to mice. Phytochemical composition of the studied plant roots showed the presence of interesting phytoconstituents. The extract showed an important rate of total polyphenolic and flavonoid contents 430.643 ± 23.578 mg GAE/g, 7.216 ± 0.329 mg QE/g respectively. The studied extract possessed an important DPPH activity with an IC₅₀ value estimated at 0.975 ± 0.064 mg/mL. The plant extract also demonstrated a promising antiproliferative activity against T-24, HT-29 and Hep G-2, with an IC₅₀ of 14 ± 3 μ g/mL, 48 ± 4 μ g/mL, and 18 ± 2 μ g/mL respectively. Regarding acute toxicity results, the percentage of mortality extended to 100%. The current finding reported that the *B. dioica* roots possess interesting phytoconstituents consolidating free radical scavenging activities and antiproliferative effects without ignoring their toxic effect. © 2020 Friends Science Publishers

Keywords: *B. dioica* roots; Phytochemical composition; Antioxidant activity; Antiproliferative effect; Acute toxicity; GC-MS

Introduction

Since ancient times, herbs and plants have been utilized to cure many diseases and have been found to have medicinal and therapeutic importance in the prevention and treatment of diseases. This knowledge has been inherited from one generation to upcoming either verbally or in writing (Okeniyi and Garba 2012). Nowadays, although synthetic drugs are very available and highly effective, many people prefer to use traditional medicines that remain less

expensive and without major side effects. Several studies have shown that natural compounds, especially secondary metabolites isolated from plants, have many biological and medicinal activities such as antibacterial, antiviral, antifungal, anticancer, analgesic, anti-inflammatory and antitumoral. The universal role of herbs in disease treatment has been widely exemplified by their utilization in all major systems of modern medicine (Iqbal *et al.* 2015).

Bryonia dioica Jacq. is a perennial climbing vine

indigenous to Central and Southern Europe, North Africa, and western Asia. Belongs to the genus *Bryonia* some species may contain toxic compounds like cucurbitacins (Chekroun *et al.* 2015). Although it is widely used in folk medicine, it is taken orally in small amounts for the treatment of various inflammatory conditions, fevers, asthma, intestinal ulcers, bronchitis, arthritis, rubefacient, hypertension and anti-diabetic agent (Kadhim 2014). In Morocco, *Bryonia dioica* is commonly used to treat stomachache, colds and coughs, rheumatic pains and liver failure. It is used also as a hypotensive and anti-inflammatory agent. Especially *B. dioica* roots are used to treat dysentery purgative, intestinal ulcers (Bnouham *et al.* 2006a) and cancer (Yamani *et al.* 2015).

This work was undertaken to determine the scientific basis of traditional uses of *B. dioica* roots, to investigate the phytochemical composition, to assess the antioxidant activity, to evaluate *in vitro* the antiproliferative effect on some cancerous cell lines (T-24), (HT-29), (Hep G-2) and to study the acute toxicity of methanolic extract of *B. dioica* roots.

Materials and Methods

Plant material

B. dioica was collected in January 2016 from the Benslimane region, located at 60 km southeast of the Moroccan capital (Rabat). The plant was authenticated and a voucher specimen has been deposited under reference 1045 at the Scientific Institute of Mohammed V-Moroccan capital. The plant roots were cleaned and then dried in the shade at department temperature.

Preparation of plant extract

The powder of plant roots was extracted using Soxhlet at 40°C for 2 h. Methanol was used as an extraction solvent. The mixture was centrifuged, filtered and then concentrated in a rotary vacuum evaporator.

Identification of bioactive constituents by GC-MS

GC-MS screening of *B. dioica* roots extract was performed using a Claus 580 Gas chromatograph, according to the following standards: Oven: Initial temp 50°C for 2 min, ramp 11°C/min to 200°C, hold 0 min, ramp 6°C/min to 240°C, hold 1 min, Inj Bauto=0°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=4.00 min, Transfer Temp=280°C, Source Temp=250°C, Scan: 40 to 450Da, Column 30.0 m x 250 µm.

Total phenolic content determination

The total polyphenolic content of the methanolic extract

prepared from the *B. dioica* roots was determined following the method described in an earlier report (Belkacem *et al.* 2014), using the Folin-Ciocalteu reagent with slight modifications. Aliquots of test samples (250 µL) containing 1 mg/mL were added to 1.5 mL of Na₂CO₃ (7%) solution. After 5 min, 1.25 mL of Folin-Ciocalteureagent (0.2 N) was added. The mixture stored overnight at the temperature of the room, and the detection is carried out against a 765 nm blank. Gallic acid was used as a positive control.

Total flavonoids content

The total flavonoids content of the methanolic extract, prepared from *B. dioica* roots, was determined using colorimetric assay (Chekroun *et al.* 2015), with some modifications. A sample (1.5 mL) and 1 mg/mL was mixed with 2 mL of aluminum chloride (2%) reagent and incubated for 15 min at the temperature of the room. The detection was carried out by spectrophotometry versus a blank at 415 nm. Quercetin was used as a standard. The results were expressed in quercetin equivalent per gram of dry extract (mg QE/g),

Antioxidant activity

The antioxidant activity of *B. dioica* roots was performed using the method as described in earlier reports (Senhaji *et al.* 2017) with some modifications. The activity was evaluated *in vitro* by assessing the trapping power of the DPPH activity of the plant extract. 1 mL of methanolic extract at different concentrations ranging from 0.5 to 4 mg/mL was added to 500 µL of a methanolic solution of DPPH (0.005%). Ascorbic acid was used as a positive control. The absorbance (A) was performed spectrophotometrically, and the DPPH activity was expressed as a percentage as follows:

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

IC₅₀ was identified graphically from nonlinear regression analysis.

In Vitro Cytotoxicity Assay

Cell lines

cancerous cell (T-24) was cultured in McCoys5a, by the time (HT-29) and (HepG-2) were maintained in DMEM media, added with 10% fetal calf serum (Gibco, USA), 1% of the mixture of streptomycin/penicillin and 1% of Glutamine (Gibco, USA). Cells were cultivated at 37°C with 5% of CO₂ and 95% humidity.

Cell proliferation analysis assay

Antiproliferative effect of *B. dioica* roots extract on human

cancer cell lines T-24, HT-29, and Hep G-2 was evaluated under the metabolic activity of mitochondria by WST-1 assay. During the exponentially growing of cell lines on 96-well with a density of 8000 in each, the cultures were treated by various concentrations of crude extract, (12.5 to 400 $\mu\text{g}/\text{mL}$ in triplicate). After 72 h of incubation, 100 μL of the medium solution was taken out and 10 μL of WST1 solution was added again. Microplates were then re-incubated at 37°C for 4 h. the absorbance was registered at 590 nm using a Wallac Victor X3 multiwell spectrophotometer.

The percent of cytotoxicity was calculated in accordance with the following formula:

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

The antiproliferative effect of the current extract was identified by plotting against the viability percentage versus concentrations of *B. dioica* extract. In addition, $\text{IC}_{50}\%$ value, (the concentration which reduces the absorbance of 50% of treated cells), were determined graphically.

Animal material and study of acute toxicity

Suis Albino mice weighing 26 g breeding in the animal colony of the Faculty of medicine and pharmacy Hassan II, Morocco, were used to perform the acute toxicity study of the investigated plant extract. The mice were housed in the animal colony with respect the laboratory environmental conditions such as light/dark cycles (12/12 h) and temperature ($26 \pm 2^\circ\text{C}$). the mice were free for accessing to appropriate pellet diet.

Twelve mice were divided into four groups of three mice, one group was used as a control, and three treatments. The organic extract was given to mice with single doses of 2000 mg/kg. The group used as a positive control was received the volume of vehicle. Throughout the whole period of the experiment, the animals were observed for toxic symptoms. This work was directed with respect to (O.E.C.D.) Guidelines No. 425 (O.E.C.D., 2008).

Statistical analysis

Quantitative data were expressed as the average of triplicate experiments \pm SD (standard deviation). The significance of differences between the means was evaluated using ANOVA and GraphPad Prism 7 Software. The means of the reported data were subjected to comparison using Holm Sidak Test. Statistically, a significant difference was considered at $P < 0.05$.

Results

GC-MS analysis

The GC-MS analysis of *B. Dioica* revealed the existence of six compounds; 1,2,3-trimethylbenzene, Coumaran, 2-Formyl-9-[a dribofuranosyl] hypoxanthine, Maaliol,3-

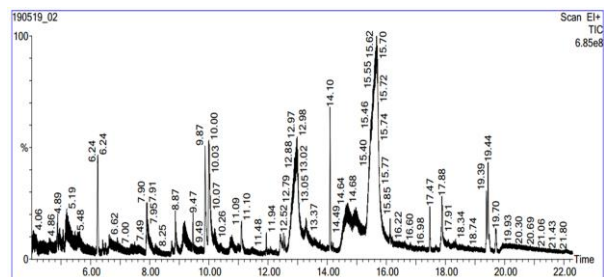


Fig. 1: GC-MS spectral chromatogram of *B. dioica* methanolic extract

methylglucose and Phthalic acid, di(2-propylpentyl) ester (Fig. 1; Table 1).

Total polyphenols and flavonoids content

The total polyphenolic content of the methanolic extract of *B. dioica* roots was determined from the gallic acid graph equation. The results of total polyphenolic content of methanolic extract of *B. dioica* roots showed a value of $430,643 \pm 23,578$ mg GAE/g. In the same way, the total flavonoid content was determined from the quercetin graph equation. The results of the total flavonoid content of the plant extract showed a value of 7.216 ± 0.329 mg QE/g.

Antioxidant activity

In the DPPH radical scavenging assay, the variable concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mg/mL) of the samples, as shown in Fig. 2, showed that the scavenging abilities increased with the increasing sample concentration and, therefore the assay represents a dose-dependent inhibition percentage.

The IC_{50} value of DPPH

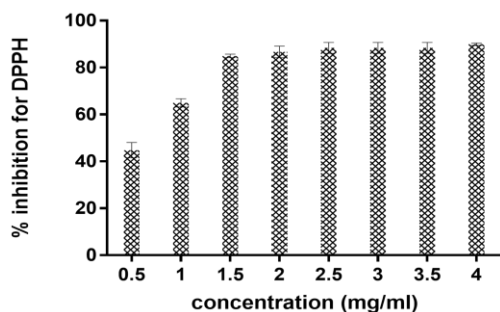
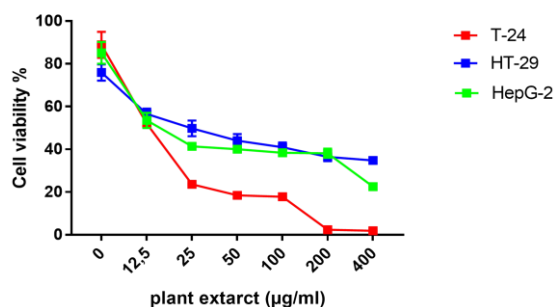
The percentage of inhibition of DPPH free radicals was expressed as the IC_{50} using ascorbic acid as a positive control. In the current research work, the results of DPPH IC_{50} value of *B. dioica* roots extract and ascorbic acid were estimated at 0.975 ± 0.06 and 0.06 ± 0.01 , respectively. The IC_{50} value of DPPH activity of methanolic extract of *B. dioica* roots revealed a significant difference compared to the IC_{50} value of DPPH radical scavenging activity obtained from ascorbic acid as control ($P^{**} < 0.05$).

In Vitro cytotoxicity assay

The antiproliferative effect of methanolic extract of *B. dioica* roots against T-24, HT-29 and Hep G-2 Cancer cell lines was evaluated by measuring spectrophotometrically the viability. As shown in Fig. 3, the crude extract exerts a remarkable antiproliferative effect on treated cell lines and reduces the cell viability in a dose-dependent manner after 72 h of cell treatment (concentration adopted 12.5 to 400 $\mu\text{g}/\text{mL}$).

Table 1: Phytochemicals identified in the methanolic extract of *B. dioica* roots by GC-MS analysis

S. No	Retention time (min)	Compound name	Molecular formula	Cas	Peak area %
1	6,243	1, 2,3-trimethylbenzene	C ₉ H ₁₂	526-73-8	1,877
2	9,875	Coumaran	C ₈ H ₈ O	496-16-2	2,124
3	12,913	2-Formyl-9-[α -d-ribofuranosyl]	C ₁₁ H ₁₂ N ₄ O ₆	125425-35-6	2,614
4	14,097	hypoxanthine	C ₁₅ H ₂₆ O	527-90-2	1,712
5	15,669	Maaliol	C ₇ H ₁₄ O ₆	146-72-5	29,412
6	19.438	3-methylglucose	C ₂₄ H ₃₈ O ₄	70910-37-1	1,198

**Fig. 2:** DPPH inhibition percentage of *B. dioica* roots extract**Fig. 3:** Cell viability after 72 h of treatment with the methanolic extract of *B. dioica* roots

Taking into account our data, bladder cancer cell lines T-24 was more sensitive to the effect of *B. dioica* roots extract than colon cell lines HT-29 and liver cell lines Hep G-2. For a global comparison of IC₅₀ values obtained from the plant extract versus the three cell lines treated, there was a significant difference between T-24 and HT-29 cell lines with IC₅₀ values of $14 \pm 3 \mu\text{g/mL}$ and 48 ± 4 respectively ($P^{**} < 0.05$). The plant extract revealed also a significant difference between Hep G-2 and HT-29 with an IC₅₀ approximately and respectively of 18 ± 2 and 48 ± 4 ($P^* < 0.05$). On the other hand, there was no significant difference between T-24 and Hep-G2 cell lines with an IC₅₀ approximately and respectively of 14 ± 3 and 18 ± 2 ($P > 0.05$).

Acute toxicity study

During the first day of treatment, significant signs of toxicity were observed on mice treated with 2000 mg/kg of the organic extract of *B. dioica* roots. shortness of breath, hypoactivity, abnormal locomotion, reversal reflection,

salivation, and occasional convulsion were the major clinical symptoms registered on the treated mice compared to the control group. The mortality rate was extended to 100% on the first day of treatment.

Discussion

With increasing attention to side effects generated habitually by the use of synthetic anticancer agents and the emergence of cancer cell resistance, the search for new, efficient and less toxic effects is becoming highly important (Yuan *et al.* 2014). The scientific research draws attention to compounds of natural origin, which considered to have a less secondary effect compared to current treatments with synthetic drugs. Nowadays the medicinal plants are considered as an excellent source of anti-cancer substances (Cragg and Newman 2005). The medicinal herbs are largely exploited for developing novel therapeutic agents. Herein, our study highlighted phytoconstituents, acute toxicity profile, the antioxidant and antiproliferative activity of organic extract of Moroccan *B. dioica* roots.

The results of the current research work showed that the qualitative screening of *B. dioica* roots extract revealed the presence of interesting chemical components, such as polyphenols, saponins, flavonoids, alkaloids, tannins and absence of anthraquinone, sterols, and terpenes. Our results were in agreement with other reported in previous literature (Chekroun *et al.* 2015), it informed that the extract of Algerian *B. dioica* roots contains tannins, flavonoids, terpenoids, saponins, and the absence of anthraquinones. On the other hand, the phenolic profile of *B. dioica* roots extract was mainly rich in C-glycosylated, flavonoids, Triterpene glycosides, bryoniosides, glycosides, cabenoside D (Barros *et al.* 2011). The compound of bryoamarid was listed belong to the plant roots composition (Barreira *et al.* 2013). Many other phytochemical constituents were identified in the fruit of *B. dioica*, such as flavones, alkaloids, and polyphenols (García-Herrera *et al.* 2013).

The Antioxidant Power of plants is due to phytoconstituents including flavonoids and polyphenols (Kilani-Jaziri *et al.* 2011). In our study, the total content of phenolic compounds was evaluated using the Folin-Ciocalteu reagent. This method is widely used to study the phenolic antioxidants for its simplicity (Lopez-Velez *et al.* 2003). Our results showed that the roots extract of *B. dioica* possessed an important content of phenolic compounds ($430.643 \pm 23.578 \text{ mg GAEq/g}$ dry extract) and flavonoids ($7.216 \pm 0.329 \text{ mg Qeq/g}$ dry extract). By the time the IC₅₀

value of DPPH activity of the tested extract was estimated at 0.975 mg/mL. The antioxidant power of aqueous extract of Algerian *B. dioica* roots was used to perform for comparison (Barreira *et al.* 2013), in which it was reported that, The DPPH IC₅₀ value was determined at 0.031 mg/mL. The DPPH IC₅₀ determined in methanolic extract of *B. dioica* fruits was determined at 1.21 ± 0.02 mg/mL. On the other hand, the values of polyphenols and flavonoids content of aqueous extract of *B. dioica* roots were 226.87 mg GA eq/g dry extract and 53.67 mg QE/g dry extract respectively as reported in the literature (Chekroun *et al.* 2015).

Natural products play an important role in chemotherapy drugs and primarily target proliferating tumor cells. Thus, the search for natural anti-cancer agents that may reduce cancer cell proliferation and considered as an appropriate strategy for conceptualizing efficient anticancer drugs. In this study, we also investigated the ability of *B. dioica* roots extract to inhibit the cell proliferation of three human cancer cell lines T-24, HT-29, and Hep G-2. According to our results, the plant showed an important antiproliferative activity against T-24, HT-29, and HepG-2 cell lines, with values of IC₅₀ of 14 ± 3 µg/mL, 48 ± 4 µg/mL, and 18 ± 2 µg/mL respectively. We noted that the antiproliferative effect was more remarkable in bladder cancer cell lines than colon and liver cancer cell lines. The results of the antiproliferative effect of our plant extract are in accordance with other reported in previous literature. The aqueous extract of Algerian *B. dioica* showed a toxic effect on human Burkitt's lymphoma cell lines (BL41), with an IC₅₀ value of 15, 63 µg/mL (Benarba *et al.* 2012).

Many types of research highlighted the cytotoxic effect of *Bryonia* species, in which it was reported that the IC₅₀ of methanolic extract of *Bryonia laciniosa* leaves versus MCF-7 and human cervical carcinoma cell lines (SiHa) was estimated at 44.79 µg/mL and 74.77 µg/mL respectively (Moghe *et al.* 2011). It was also shown that *Bryonia aspera* roots exert a cytotoxic effect on MCF-7 cells lines with an IC₅₀ value of 14.2 µg/mL (Sahranavard *et al.* 2012).

The mitochondrial intrinsic pathway is one of the mechanisms described as being responsible for the antiproliferative effect induced by *B. dioica* extract (Benarba *et al.* 2012). This mechanism may be involved in our treated cell lines with methanolic extract of *B. dioica* roots. Isolated compounds from *B. aspera*, such as dihydrocucurbitacin D, dihydrocucurbitacin I, dihydrocucurbitacin B, epi-iso dihydrocucurbitacin B, cucurbitacin L and neocucurbitacin C showed excellent anticancer activities against MCF-7 and Hep G-2 cell lines (Sahranavard *et al.* 2012).

The GC-MS analysis of *B. dioica* roots extract showed the existence of six compounds; 1, 2,3-triméthylbenzène, Coumaran, 2-Formyl-9-[α -dribofuranosyl]hypoxanthine, Maaliol, 3-méthylglucose, Phthalic acid, di(2-propylpentyl) ester. Pharmacological and biological properties of *B. dioica* roots discussed in the current research work, are mainly

attributed to these detected bioactive compounds by GC-MS. The chemical compounds shown in plant extract could generate remarkable activities by the action of a single molecule or by a synergistic effect between them without excluding the potentiation effects also. Indeed, the revealed compound in plant extract, Phthalic acid, di(2-propylpentyl) ester in an earlier report exhibited a cytotoxic effect on brine shrimp nauplii *Artemia salina* (Habib and Karim 2009).

The results of the acute toxicity study showed that the tested dose of methanolic extract of *B. dioica* roots (2000 mg/kg) was very lethal with oral administration to mice, hence, the predicted LD₅₀ of this extract is lower than 2000 mg/kg. Our results were in accordance with other reports (Bnouham *et al.* 2006b), in which it was reported that the LD₅₀ of *B. dioica* roots was estimated at 340 mg/kg with oral administration to mice. The clinical signs of toxicity reported in the present work such as convulsion and reversal reflection may depend on a probable neurotoxic effect generated by the plant extract (Norrby 2000). Salivation and hypoactivity are frequently related to the properties of plant chemicals (Depierreux *et al.* 1994). 1, 2,3-triméthylbenzène reported in the GC-MS analysis of *B. dioica* roots could be responsible for the acute toxicity results reported in the current research work. Earlier reports showed a toxic effect occurred in animals exposed to 1,3,5-triméthylbenzene (Świercz *et al.* 2006).

Conclusion

Phytochemical screening of the organic of *B. dioica* roots was useful to provide information on the potential of this plant as a source of secondary metabolites. The outcome suggests that the *B. dioica* could be a promoting source of multiple therapeutic agents that lead to be effective against free radical-mediated diseases and cancer treatment.

Acknowledgments

The authors extend their appreciation to the Researchers Supporting Project Number (RSP-2019/110), King Saud University Riyadh Saudi Arabia for financial Support.

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