

Arbutus unedo L aqueous extract is associated with in vitro and in vivo antioxidant activity

H. Naceiri Mrabti^{1*}, I. Marmouzi¹, K. Sayah¹, L. Chemlal¹, Y. El Ouadi²,
H. Elmsellem^{2*}, Y. Cherrah¹, My A. Faouzi¹

¹University Mohammed V in Rabat, Faculté de Médecine et de Pharmacie, Laboratoire de Pharmacologie et Toxicologie, équipe de Pharmacocinétique, BP 6203, Rabat Instituts, Rabat, Morocco

²Laboratoire de chimie analytique appliquée, matériaux et environnement (LC2AME), Faculté des Sciences, B.P. 717, 60000 Oujda, Morocco

Received 20 Oct 2016,
Revised 10 Nov 2016,
Accepted 13 Nov 2016

Keywords

- ✓ *Arbutus unedo* L.;
- ✓ Phenolic compounds;
- ✓ Mineral;
- ✓ Antioxidant;
- ✓ MDA;
- ✓ SOD;

H. Naceiri Mrabti
h.elmsellem@gmail.com/
naceiri.mrabti.hanae@gmail.com

ABSTRACT

Arbutus unedo L. (*Ericaceae*) roots and leaves have been used traditionally for multiple uses, such as gastrointestinal disorders, skin diseases and urinary infections. Some studies showed an *in vitro* antioxidant activity of this plant. Aim of the study: to characterize the nutritional composition and to determine the *in vivo* antioxidant effect of *Arbutus unedo* L aqueous extract. We used an inductively coupled plasma atomic emission spectroscopy to determine the mineral composition of *Arbutus unedo* L aqueous extract. The *in vitro* antioxidant activity was measured by calculating the radical-scavenging activity (RSA) as a percentage of 2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH) discoloration. The *in vivo* antioxidant activity was determined using the dosage of malondialdehyde (MDA) and superoxide dismutase (SOD) stress markers in diabetic mice. Phytochemical screening revealed the presence of multiples chemical metabolites such as tannins, anthraquinones and flavonoids. These substances were present with High concentration in leaves and roots. While terpenoids were more present in leaves than in roots. Saponins and alkaloids are totally absent for both of them. Moreover, mineral analysis demonstrated a higher content of Na and Zn in roots, and interesting amounts of P, Mg, Ca, Mn, Fe and K in leaves. The aqueous extracts of both parts exhibited an antioxidant power with IC₅₀ of 4.52±0.69 and 7.24±0.73µg/mL in DPPH assay, in root and leaves respectively. There was no significant difference between *Arbutus unedo* L group and methformin group ($p > 0.05$) in all of the examined parameters (MDA and SOD) in liver and kidney tissues. In liver and kidney, the levels of MDA in *Arbutus unedo* L group ($p < 0.05$) and methformin group ($p < 0.05$) were significantly lesser than in control group. Inversely, the two study groups presented a high level of SOD compared to control group. Our results show that aqueous extract of *Arbutus unedo* L is associated with an *in vivo* antioxidant activity and a lack of certain toxic substances in its composition. Other investigations are necessary to determine the importance of this antioxidant effect in animals and in humans.

1. Introduction

The wild edible plants constitute an important contribution in nutritional cultures and health promotion of local communities [1]. The wild strawberry tree *Arbutus unedo* L. (*Ericaceae*) grows in wood margins and rocky slopes and is widely spread in the Mediterranean region and North Africa. In Morocco, it is known as "Sasnou" and it is one of the most frequent medicinal plants widely used in folk medicine in the cases of gastrointestinal disorders, skin diseases and urinary infections [2]. Much attention has been given to the *Arbutus unedo* L fruits and leaves [1,3]. However, few papers have described the health beneficial effects of its roots [4]. The fruit of the *Arbutus unedo* L. is generally used for preparing alcoholic beverages (wines, liqueurs and brandies), jams, jellies and marmalades, and less frequently eaten fresh [5]. It can also be added to yoghurts either in pieces or as flavors and be used, like other berries, in confectionery, such as for pie and pastry fillings and cereal products, among other applications [6]; nevertheless they are also used in folk medicine as antiseptics, diuretics and laxatives [7]. Previously, it has been shown that *Arbutus unedo* L. is endowed by promising activities such as antiplatelet, vasorelaxant, diuretic, anti-hypertensive, vasodilatory and anti-inflammatory [8,9]. Antioxidant activity of plant leaves and fruits have been already investigated [10,11]. Moreover, phytochemical analysis shows that *Arbutus unedo* L. contains flavonoids such as afzeline, juglamine, avicularine, quercitroside and hyperoside in the aerial part [12]. Phytochemical studies have shown that the leaf extract contains phenolic antioxidant compounds, acid derivatives, (+)-catechin and (+) catechingallate such as flavonoids (quercetin, isoquercetin, kaempferol, hyperoside and rutin) [13,14], tannins, phenolic glycosides, anthocyanins, gallic [15] (Fiorentino et al., 2007). Also several compounds have been isolated from the roots of *Arbutus unedo* L. such as (+)-catechin, (+) catechingallate and a number of phenolic compounds were also identified by GC-MS such benzeneacetic acid 4-hydroxy, caffeic acid, gallic acid, protocatechic acid and bis(2-ethylhexyl) phthalate [16]. Previous reports described preliminary anti-hyperglycemic effect of the strawberry tree roots [17]. However, no attempt has been

undertaken to investigate the antidiabetic properties of the strawberry tree. Due to the influence of geographical variation on nutritional quality [1], this study aims to characterize the nutritional composition, antioxidant effect of crude extract of leaves and roots in vitro by using DPPH and in vivo using dosage MDA and SOD of roots of the Moroccan strawberry tree.

2. Materials and methods

2.1 Plant material and extraction procedure

Roots and leaves of *Arbutus unedo* L. were collected from Beni Mellal region in Morocco (32°20'22"N 6°21'39"W, at 620 m of altitude, 493 mm of average annual rainfall). The whole plant was ground and 50 g of powder mixed with 500 ml of distilled water (10%). The mixture was heated and boiled under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at -20 °C, and then lyophilised (Free Zone® Dry 4.5, USA).

2.2 Phytochemical screening

The roots and leaves extracts of *Arbutus unedo* L. were screened for their qualitative chemical composition, using standard methods [18,19]. The identification of the following groups was considered: flavonoids, tannins, Alkaloids, free quinones, anthraquinones, terpenoids and saponosides.

2.3 Mineral composition

The mineral composition (Ca, Mg, Mn, Fe, Zn, K, Na and P) was determined using an inductively coupled plasma atomic emission spectroscopy (ICP AES, JobinYvonUltima 2). 150 mg of the roots and leaves powder was washed with 2 mL of HNO₃ acid (70%) in a teflon beaker, before being incinerated at 110°C. Then, 0.5 mL of hydrofluoric acid (HF) was added and the covered beaker was placed on a sand bath. The sample mixture was heated until a clear solution was obtained. After removing the cover, the mixture was evaporated until drying. Finally, 2 mL of HCl acid was added and the residue was extracted by 25 mL of HCl (2.0 M).

2.4 Phenolic content

The amount of total phenolic contents was determined according to Folin-Ciocalteu method as described by Spanos & Lister [20,21]. Briefly, 0.5 mL of sample solution was mixed with 2.5 mL of Folin-Ciocalteu reagent diluted with distilled water 1:10, followed by the addition of 4 mL of Na₂CO₃ (7.5 %, w/v). The mixture is then incubated in a water bath at 45°C for 30 min and the absorbance was measured at 765nm using a Shimadzu-2450 UV-Vis spectrophotometer, against blank sample. The standard curve of gallic acid is obtained under the same conditions as above using a range of concentrations (0-300 mg/L). The total phenolic content was measured as gallic acid equivalents (mg GAE/g edw).

2.5 Flavonoids content

The total flavonoids in the extracts were determined using a colorimetric method [22]. Indeed, 1 mL of dissolved sample was placed in a 10 mL volumetric flask. Distilled water was added to obtain a total volume of 5 mL and then 0.3 mL of NaNO₂ (5%) was added. About 0.3 mL of AlCl₃·H₂O (10%) was added after 5 min and the mixture was allowed to stand for another 6 min. About 2 mL of NaOH (1M) was added and the total volume was increased to 10 mL with distilled water. The solution was mixed well and allowed to stand for 30 min. The absorbance was recorded against a blank at 510 nm. The flavonoid content was determined as the rutin equivalent from the calibration curve of rutin standard solutions and expressed as rutin equivalent (mg RE/g edw).

2.6 Condensed tannins content

Condensed tannin contents were determined also using a colorimetric method [23]. An aliquot (50 µL) of sample or standard solution was mixed with 1.5 mL of vanillin 4% (prepared with methanol) and then 750 µL of concentrated HCl were added. The well-mixed solution was incubated at ambient temperature in the dark for 20 min. The absorbance against blank was read at 500 nm. (+)-Catechin was used to make the standard curve (0.05–1 mg/mL).

2.7 Antioxidant Activity

2.7.1 Free radical scavenging activity

The free radical scavenging activity of the strawberry extracts was measured by 2,2'-Diphenyl-1-picrylhydrazyl hydrate (DPPH) [24]. Briefly, the solution of DPPH (0.2 mM) was prepared in methanol and 0.5 mL of this

solution was added to 2.5 mL of plant extract and was allowed to stand at room temperature for 30 min. Then absorbance was then read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$RSA (\%) = \frac{AD - AE}{AD} \times 100$$

Where A_D is the absorbance value of the DPPH blank sample, and A_E is the absorbance value of the test solution. A_E was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

2.7.2 Animals

Healthy male Swiss mice (18–35 g) were used in the experiments. The animals were bred in the animal center at the Faculty of Medicine and Pharmacy, University Mohammed V in Rabat. All animals were housed in collective cages in temperature-controlled ($23\text{ }^\circ\text{C} \pm 2\text{ }^\circ\text{C}$) and artificially lighted rooms on a 12-h light/12-h dark cycle with free access to water and standard diet.

2.7.3 Ethics approval

The study was conducted in accordance with the accepted principles outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health and all efforts were made to minimize animal suffering and the number of animals used. Ethics approval was obtained from the University Mohammed V in Rabat.

2.7.4 Animal treatment

Twenty four mice were divided into four groups, each group with six males these latter are rendered diabetic by intraperitoneal injection of a freshly prepared solution of Streptozotocin in a dose of 200 mg / kg, after injection of nicotinamide in a dose of 210 mg / kg. After a week, diabetes is confirmed in mice by measuring fasting glucose with a glucometer Type One Call. Only mice having blood glucose levels higher than 126 mg / dl were considered diabetic and used to experimentation.

All the mice were divided into 4 groups of 6 mice each and kept under the same conditions:

- Group control I: receiving daily by oral route distilled water.
- Group II (6 mice) receiving daily by gastric gavage the aqueous extract of *Arbutus unedo* L(bni mellal) at a dose of 500 mg / kg.
- Group III (6 mice): receiving each day by gastric gavage metformin at the dose of 300 mg / kg.
- Group IV: (6 mice) diabetic control: receiving daily by oral route distilled water

Fasting blood glucose in mice is measured every week for a treatment period of one month. At the end the animal will be sacrificed for the study.

2.7.5 Preparation of homogenates

The first step is to cut the tissue into small pieces, prepare 10% (w / v) of it with the phosphate buffer (0.05M, pH 7.4) then pre-homogenize it with a potter.

The second step is to prepare the homogenate with a sonicator and centrifuge 3000g of it for 10 min at $4\text{ }^\circ\text{C}$.

Finally, the last step is to collect the supernatant and make aliquots that we keep at $-20\text{ }^\circ\text{C}$ for 6 months.

2.7.6 Superoxide dismutase activity

The measurement of SOD activity was determined in tissue by Beauchamp [25].

The assay mixture contained (per litre):50mM of phosphate buffer (pH 7.2), 0.25% triton x-100, 10 mM EDTA (pH 8), 120mM L-Méthionine, 0.75 mM NBT, 10 μM riboflavine was added the end of the reaction.

The reaction was carried out at $25\text{ }^\circ\text{C}$ and a 15W lamp for 10 min in an incubator. Absorbance is read at 560 nm, the SOD (superoxide dismutase) activity was measured according to the following equation (according to Sun et al:

$$\text{Inhibition\%} = (A_{\text{white}} - A_{\text{sample}} / A_{\text{white}}) \times 100$$

(SOD a unit of activity is defined as the amount of enzyme required to inhibit by 50% the reduction of NBT).

2.7.7 Lipid peroxidation

Lipid peroxidation in the liver and kidney were estimated colourimetrically by measuring TBARS according to the method of Ohkawa[26]. The reaction mixture contained (100 µL) of samples of liver or kidney or standard MDA (malondialdehyde) was added to test tubes containing (300 µL) of thioforbituric acid (TBA)0.6% and (700 µL)of phosphoric acid1% The reaction mixture was heated at 95 °C for 30 min.After cooling,2 ml of n-butanol was added to each tube, and the tubes were vortexed for 20 s and then centrifuged at3000 r/min for 10min, MDA was measured spectrophotometrically at535 nm.

3. Results and Discussions

3.1 Phytochemical screening

The phytochemical screening using qualitative analysis of *Arbutus unedo* L lead to the identification of the following constituents: tannins, flavonoids and anthraquinones with high concentrations in leaves and roots, for free quinones is present with simple concentration, or terpenoids is more present in leaves than roots, however alkaloids and saponins are not detected for both of them (Table1).The chemical compounds detected in our preliminary screening are involved in many therapeutic strategies against pathological disorders and diseases. However alkaloids, saponins and coumarins were not detected. Through phytochemical prospecting of the extracts, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial [27,28], antioxidant[29] antitumor and antiaphidic[30].

Table.1:Phytochemical screening of *Arbutus unedo* L.leaves and roots

	Roots	Leaves
Tannins	+++	+++
Anthraquinones	+++	+++
Terpenoids	+++	+
Flavonoids	+++	+++
Free Quinones	+	+
Saponins	-	-
Alkaloids	-	-

(-) Absent; (+) present; (++) present with moderate concentration;(+++) present with high concentration.

3.2 Mineral composition

Minerals content in *Arbutus unedo* L roots and leaves.expressed in mg/kg are shown in Table2. Eight mineral elements, including Calcium (Ca), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Fer (Fe), and Zinc (Zn) were quantified *Arbutus unedo* L. has a higher content of Ca (R:3454.64 ± 82.91 to L: 5738.31 ± 198.54 mg/kg), K (R:238.68 ± 11.19 to L:10534.86 ± 365.55 mg/kg), P (R:51.10 ± 0.79 to L:1819.66 ± 48.94 mg/kg), Mg (R:655.48 ± 6.29 to L: 1620.13 ± 59.78 mg/kg); Na (R:455.79 ± 14.22 to L:137.27 ± 2.89mg/kg),Fe (R:21.86 ± 0.53 to L:344.78 ± 16.44 mg/kg) and Mn (R :4.43± 0.54 to L:29.61 ± 0.33 mg/Kg)and Zn(R:15.66±0.75to L:12.05±0.40 mg/kg). Interesting differences has been registered between leaves and roots. For instance, roots have been shown to contain more Na and Zn. In the other hand, strawberry tree leaves exhibit higher amounts of P, Mg, Mn, Fe and K. Previous works have described the mineral content of *Arbutus unedo* L fruit [31]. However, to our knowledge this is the first reports of mineral contents of *Arbutus unedo* L roots and leaves. Mineral elements are endowed by interesting health promoting activity. For instance, their contribution in renal and cardiovascular systems [32].The rich content of *Arbutus unedo* L.leaves and roots and the interesting mineral complementarily (Table 2) can constitute an interesting add to human diet and therapy.

Table.2:Mineral composition of *Arbutus unedo* L. roots and leaves in mg/Kg

Mineral composition	Roots	Leaves
Ca	3454.64 ± 82.91	5738.31 ± 198.54
Na	455.79 ± 14.22	137.27 ± 2.89
P	51.10 ± 0.79	1819.66 ± 48.94
K	238.68 ± 11.19	10534.86 ± 365.55
Fe	21.86 ± 0.53	344.78 ± 16.44
Mg	655.48 ± 6.29	1620.13 ± 59.78
Zn	15.66 ± 0.75	12.05 ± 0.40
Mn	4.43 ± 0.54	29.61 ± 0.33

Data are reported to mean (n = 3) ± Standard Error.

3.3 Phenolic contents

Total phenolic, flavonoids and tannins contents are presented in table 3. The phenolic content in aqueous extracts with IC₅₀ of roots (47.55 ± 0.67 GAE/g edw). It is relatively higher than that given by the leaves (37.30 ± 0.19 GAE/g edw). Similarly, the high levels of flavonoids content have been recorded in roots with values ranged from 49.66 ± 2.13 RE/g edw. Condensed tannins were also more concentrated in the roots (33.38 ± 3.34 RE/g edw) than in the leaves (24.58 ± 0.63 CE/g edw). Significant differences (p < 0.05) in total Phenolic compounds are natural antioxidants occurring in green vegetables, fruits and oils. Some phenolic have been reported to possess anti-inflammatory, antiviral and anti-cancer activities [33]. The tannin components of epicatechin and catechin demonstrated strong antimicrobial activity against bacteria and fungi [34]. Flavonoids are synthesized by plants in response to microbial infection [35] are effective against a broad range of microorganisms.

Leaf extract of *Arbutus unedo* L. had lower amount of total phenol, determined by the Folin-Ciocalteu method, than the leaf extracts in the experiment carried out by other authors using ethanolic extraction [36,37] or boiling water extraction [38]. These compounds have gained attention because of the protective effect played by antioxidants in many pathological diseases in which oxidative stress is implicated.

Table.3: Phytochemical content of *Arbutus unedo* L.

	PC	FC	CTC
roots	47.55 ± 0.67	49.66 ± 2.13	33.38 ± 3.34
leaves	37.30 ± 0.19	25.73 ± 2.01	24.58 ± 0.63
Ascorbic acid	3.12 ± 0.67	-	-

Data are reported to mean (n = 3) ± Standard Error. Phenolic contents (PC), (Flavonoids content (FC), Condensed tannins content (CTC)

3.4 Antioxidant activity

In a preliminary investigation of the antioxidant potential of *Arbutus unedo* L. extracts, the radical scavenging activity by DPPH were evaluated (table 4). The remarkably stable property of DPPH• radical is mainly due to the steric crowding around the divalent nitrogen and, to a lesser extent, due to the effect “push-pull” of electron donor and acceptor (diphenylamino and picryl group respectively) onto the divalent N [39]. While reacting, the free radical converges from purple to the yellow by accepting a lone pair of electron or hydrogen atom. The scavenging effects of *Arbutus unedo* L. extracts and the ascorbic acid on DPPH radicals expressed by IC₅₀ are illustrated in Table 5. Roots extracts exhibited relatively better activity than its leaves equivalent. Interestingly, *Arbutus unedo* L. showed lower but comparable IC₅₀ with Ascorbic acid (3.12 ± 0.67 µg/mL), which is a Vitamin C equivalent. Previous studies have investigated the DPPH radical scavenging effect of the strawberry tree leaf and fruits [38,40,41]. Similarly, Marquez Garcia Have shown that *Arbutus unedo* L. has a correlation between the phenol content and its antioxidant activity [42], this could be related to the observations described by Kraus [43]. However few papers have described the roots bioactivity [44].

The reported antioxidant activity presented here can be attributed to the phenolic content in the plant extracts. It is also interesting to highlight the huge differences between roots and leaves clearly correlated to the chemical composition. Natural sources with antioxidants activities are of great interest in nutritional therapy and oxidative stress prevention. *Arbutus unedo* L. especially constitutes a valuable alternative and complement in therapies.

Table.4: DPPH Radical scavenging activity (% of inhibition) of *Arbutus unedo* L.

	Concentrations in µg/mL					
	1.66	3.33	6.66	10.00	13.33	16.66
roots	27.68 ± 4.70	41.35 ± 7.32	66.45 ± 3.55	82.42 ± 0.91	86.68 ± 0.98	89.88 ± 1.15
leaves	17.57 ± 1.08	42.15 ± 0.90	49.53 ± 2.43	64.32 ± 1.30	75.77 ± 0.45	83.08 ± 1.51
Ascorbic acid	45.33 ± 0.63	59.99 ± 1.12	72.26 ± 2.54	85.76 ± 1.78	90.79 ± 0.98	94.13 ± 1.08

Data are reported to mean (n = 3) ± Standard Error

Table.5:IC₅₀ values (µg/mL) of *Arbutus unedo* L.

	DPPH
roots	4.52 ± 0.69
leaves	7.24 ± 0.73
Ascorbic acid	3.12 ± 0.67

Values represent means ± SD (standard deviations) for triplicate experiments.

3.5 Superoxide dismutase activity

Table 6 shows the enzymatic activity of SOD on liver and kidney tissues. There was no significant difference between *Arbutus unedo* L group and methformin group ($p > 0.05$). The aqueous extract of *Arbutus unedo* L and Methformin could have similar anti-oxidative stress effect. The levels of SOD in liver and kidney for *Arbutus unedo* group and methformin group were significantly higher than in control group ($p < 0.05$). Similar results concerning SOD were described by [45]. The decreasing trend in SOD activity could also lead to an excess of availability of O₂ and H₂O₂, which in turn generates hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation [46-49]. In line with this, in our study, it was observed a decrease in SOD activity in liver and kidneys of diabetic mice associated with a concomitant increase in lipid peroxidation in these tissues (table 6,7). Corroborating with these results, other studies have demonstrate that the activity of the antioxidant enzymes, such as SOD is reduced in tissues of diabetic rats. This may result in a number of deleterious effects due to the accumulation of ROS [50]. One possible mechanism for this reduction in SOD activity may be due to the inactivation caused by the excess of free radicals and/or by non-enzymatic glycation due to the persistent hyperglycemia, which has been extensively reported to occur in diabetes [51,46]. The administration of SOD can prevent tissue damage due to the superoxide anion [52]. The SOD seems to reduce also the extent of myocardial infarction [52, 53]. These findings suggest that *Arbutus unedo* L. can strengthen the antioxidant enzymatic defence system, reduce free radicals and alleviate liver and kidney damage caused by oxidative stress in diabetic mice.

Table.6:Result of Superoxide dismutase activity on liver and kidneys from STZ-induced diabetic mice and those treated with *Arbutus unedo* (500 mg/kg) and methformin (300mg/kg)

SOD total (U/g of tissu)	Gr Arbutus unedo L	Gr control	P –value
liver	918.5±89.5	700.2±51.4	<0.05
kidneys	841.8±92.3	616.9±42.8	<0.05
SOD (U/g of tissu)	Gr Methformin	Gr control	p-value
liver	904.3±78.10	700.2±51.4	<0.05
kidneys	832.1±49.2	616.9±42.8	<0.05
SOD (U/g of tissu)	Gr Arbutus unedo L	Gr Methformin	p- value
liver	918.5±89.5	904.3±78.10	>0.05
kidneys	841.8±92.3	832.1±49.2	>0.05

Values are mean ± S.D. of duplicate determinations of SOD in six mice per group

3.6 Lipid peroxidation

Table 7 includes all results (MDA) in mice liver and kidney in all three groups. There was no significant difference between *arbutus unedo* group and methformin group ($p > 0.05$). The levels of MDA in *Arbutus unedo* group ($p < 0.05$) and methformin group ($p < 0.05$) were significantly lesser than in control group. MDA aldehyde is formed during the cleavage of the polyunsaturated fatty acids having at least three double bonds [54].

Extensive evidence has demonstrated that the increase of lipid peroxidation plays an important role in the progression of diabetes by altering the transbilayer fluidity gradient, which could hamper the activities of membrane-bound enzymes and receptors [55, 56]. Our findings are consistent with other studies where an increase

in lipid peroxidation in many tissues such as liver, kidney, pancreas and brain of rats submitted to an experimental model of diabetes was described [46, 57-59]. Lipid peroxidation promotes serious in membrane potential, increased permeability and eventual cell rupture[60]. In this respect, oxidation of lipids or lipid peroxidation has crucial importance in the pathogenesis and complications of diabetes.

Table.7: Result of serum malondialdehyde (MDA) levels

MDA (nmol/g of tissu)	Gr <i>Arbutus unedo</i> L	Gr control	P -value
liver	107.30±21.34	383.5±43.4	<0.05
kidneys	34.56±18.79	183.2±37.9	<0.05
MDA (nmol/g of tissu)	Gr Methformin	Gr control	p-value
liver	100.90±27.65	383.5±43.4	<0.05
kidneys	22.94±9.34	183.2±37.9	<0.05
MDA (nmol/g of tissu)	Gr <i>Arbutus unedo</i> L	Gr Methformin	p- value
liver	107.30±21.34	100.90±27.65	>0.05
kidneys	22.94±9.34	34.56±18.79	>0.05

Values are mean ± S.D. of duplicate determinations of MDA in six mice per group

4. Conclusions

Our results show that aqueous extract of *Arbutus unedo* L is associated with an in vivo antioxidant activity and a lack of certain toxic substances in its composition. Considering its high phenolic content and mineral profile, this plant may be interesting as a rich dietary source of a wide range of phenolic compounds, together with ascorbic acid and fat-soluble antioxidants. The results of this study suggest that the aqueous extract of *Arbutus unedo* L may be useful in the development of an anti-oxidant Phytomedicine. Other investigations are necessary to determine the importance of this antioxidant effect in animals and in humans. In conclusion, the results of this study showed that *Arbutus unedo* L. may provide effective protection against oxidative stress damage in the liver and kidney in mice with diabetes induced by Streptozotocin. *Arbutus unedo* L may reduce lipid peroxidation and increase enzymatic and non-enzymatic antioxidant defence in these tissues. These findings provide evidence that *Arbutus unedo* L may be useful for the treatment of hepatic and renal complications associated with Diabetes mellitus and raise the possibility of a new application as a complementary therapy associated with hypoglycaemic drugs.

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