

Protective role of *Limonium bonduelli* extract against non-enzymatic peroxidation in brain and testes induced by iron *in vitro*.

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Abstract

Infertility and Neurodegenerative diseases have been linked to oxidative stress arising from peroxidation of membrane biomolecules and high levels of iron have been reported to play an important role. The present study sought to determine the antioxidant activity and protective ability of *n*-butanol extract of *Limonium bonduelli* on lipid peroxidation induced by FeSO₄ in rat brain and testes homogenates *in vitro*. *n*-butanol extract of the aerial parts (leaves and flowers) was prepared, and the ability of the extract to inhibit FeSO₄ induced lipid peroxidation in isolated rat brain and testes was determined using spectrophotometric method. The study revealed that the extract inhibited malondialdehyde (MDA) production in FeSO₄ induced lipid peroxidation in the brain and testes in a dose-dependent manner and the highest percentage of the inhibition was 89.80% similar to vitamin C in the same concentration (100 µg/mL) in brain and 82.33% in testes (200 µg/mL). *Limonium bonduelli* extract demonstrated important anti-lipid peroxidative effect, which may be useful in preventing the progress of various oxidative stress related diseases. The higher inhibitory effect of the extract could be attributed to its phytochemical content.

Keywords: Iron Overload; Brain; Testes; *Limonium bonduelli*; Antioxidant.

Introduction

Iron is vital for almost all living organisms by participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport [1]. However, iron concentrations in body tissues must be tightly regulated because excessive iron leads to tissue damage, as a result of formation of free radicals [2, 3]. The potential of Fe(II) to catalyse hydroxyl radical formation via the Fenton reaction means that iron is potentially toxic [4]. The toxicity of iron in specific tissues and cell types (liver, macrophages and brain) is illustrated by studies with appropriate cellular and animal models [5]. The increased level of oxidative stress in neurodegenerative diseases brain [6] is reflected by the increased brain content of iron (Fe) and copper (Cu) both capable of stimulating free radical formation, increased protein and DNA oxidation in brain, enhanced lipid peroxidation and increased level malondialdehyde (MDA) [7]. Several studies have shown that nutritional antioxidants (especially vitamin E and polyphenols) can block neuronal death *in vitro*, and may have therapeutic properties in animal models of neurodegenerative diseases [8]. However, one practical way to prevent/or manage neurodegenerative diseases is through consumption of foods rich in antioxidants [9,10].

Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility [11]. Transition metals such as iron induce lipid peroxidation, protein carbonyl expression and

lipid soluble antioxidant depletion in testicular tissue with the consequent disruption of spermatogenesis [12]. Strategies to modulate the level of oxidative stress within the male reproductive tract include the use of oral antioxidant compounds to reinforce the body's defence against oxidative damage [13-15].

The genus *Limonium*, formerly known as *Statice*, is a member of the Plumbaginaceae family and involves 300 wild species. In Algeria, this genus is represented by 23 species [16]. *Limonium* species are traditionally used for the treatment of infections, fever, hemorrhage and other disorders. Different pharmacological activities have been reported such as: antiviral, antitumor, antipyretic, hemostatic, depurative, antifungal, antimicrobial [17], antioxidant [18] neuroprotective [19] and anti-inflammatory [20]. According to records, *Limonium* chemical composition is very complex, containing flavonoids [21], amino acids, vitamins, tannins, polysaccharides and alkaloids [22]. Many chemical compounds have been isolated and identified from *Limonium bonduelli* such as Aureusidin 4-glucoside (cernuoside) [23], eriodictyol, luteolin, apigenin and 4-hydroxy-3-methoxy benzoic acid [18].

The objective of this study is to investigate the inhibitory effect of *n*-butanol extract from the aerial parts (leaves and flowers) of *Limonium bonduelli* on FeSO₄ induced lipid peroxidation in rat brain and testes *in vitro*.

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Materials and Methods

Plant material

Aerial parts of *Limonium bonduelli* L. (*Plumbaginaceae*) were collected on April 2011 at Mogheul near Bechar in the South West of Algeria. The voucher specimen was identified by Prof. Mohamed Kaabeche from university of Setif and was deposited at the Research Unity VARENBIOMOL under the reference: LB/236/04-11.

Extraction procedure

The dried aerial parts of *Limonium bonduelli* (1500g) were powdered and macerated at room temperature with EtOH–H₂O (8:2 v/v) for 48 h three times. After filtration, the filtrates were combined, concentrated under vacuum (at 35 °C), diluted with 600 ml H₂O, and filtered to remove chlorophyll and successively extracted with (3x400ml), chloroform, ethyl acetate and *n*-butanol. The organic layers were dried with Na₂SO₄. Removal of solvents under reduced pressure. CHCl₃ (1.5g), EtOAc (13g), *n*-butanol (42 g) resulted in final extracts (Figure 1).

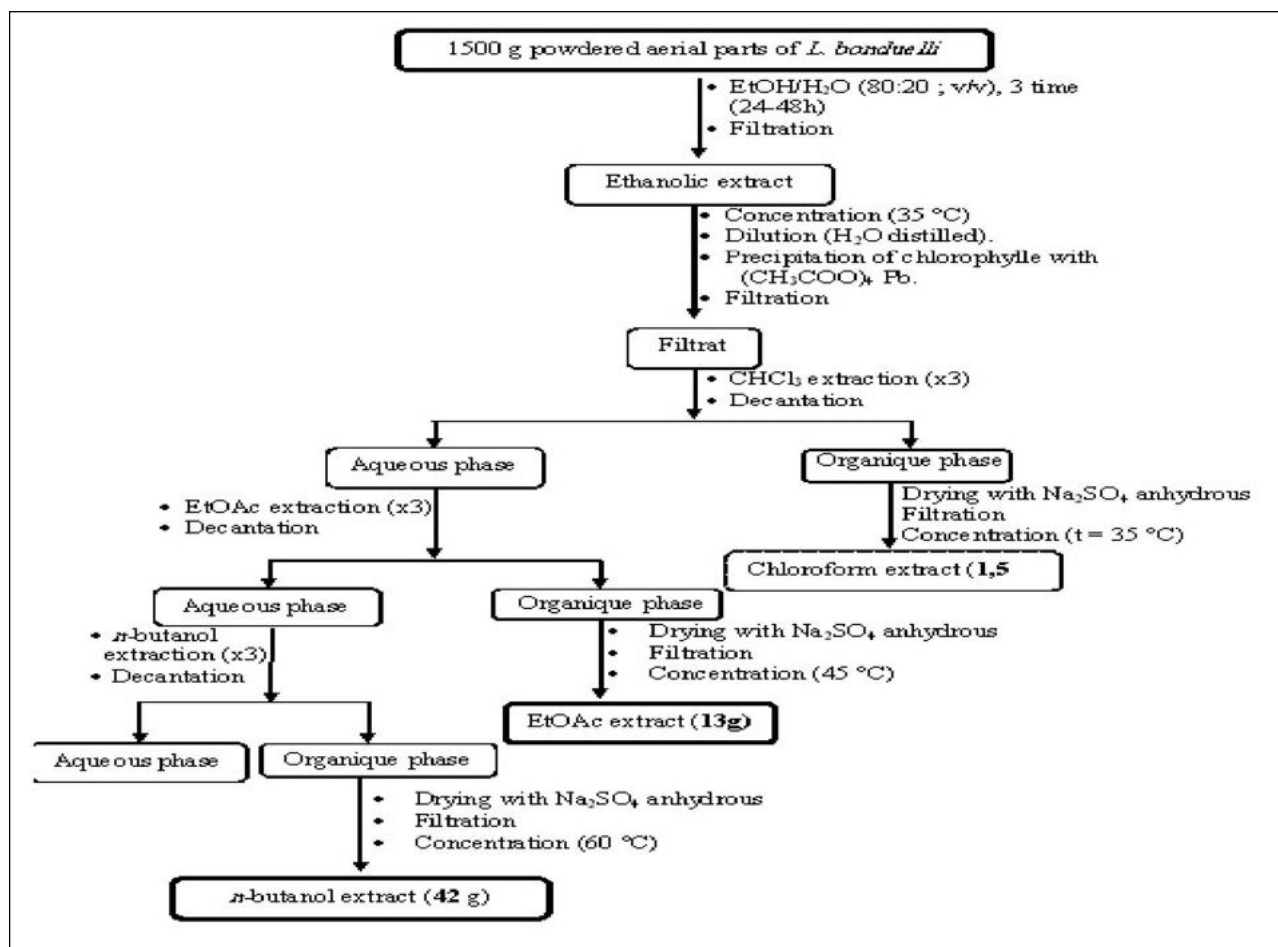


Figure 1. Scheme of bioguided extraction of *L. bonduelli* extracts (CHCl₃, EtOAc and BuOH).

Preliminary phytochemicals screening

The *n*-butanol extract (1%) was used for phytochemical screening following the methodology of Harborne, 1998 [24].

Lipid peroxidation assay

Experimental animals

Male Wistar albino rats (180-200 g) were used in experiments. Animals were exposed to 12 hours light-dark cycles at 25 ± 2 °C temperature and housed at animal house of the Department of

Animal Biology, Constantine 1University,Algeria. The animals had free access to water and standard diet.

Preparation of tissue homogenates

The rats were decapitated under chloroform anaesthesia and the brain and testes were rapidly dissected and placed on ice and weighed. The brain and testes were subsequently homogenized in cold KCl (1.15%) to produce a 10% homogenate and centrifuged at 40000 rpm for 20 minutes to remove precipitation.

Lipid peroxidation and thiobarbituric acid reactions

One of the major mechanisms of cell injury in aerobic organisms subjected to oxidative stress is lipid peroxidation of biological membranes. Malondialdehyde (MDA) is the end-product of lipid peroxidation and the production of this aldehyde is used as a biomarker to measure the level of oxidative stress in injury [25]. A modified thiobarbituric acid-reactive species (TBARS) assay described by Cao and Ikeda, 2009 [26] was used to measure the lipid peroxide formed, using brain and testes homogenates. Briefly 10% homogenate were incubated with different dose of *n*-butanol or vitamin C in the presence of 50 μ l FeSO₄ (0.07 M) at 37 °C for 1 h. reaction was stopped by addition of 1ml trichloroacetic acid (TCA 20%), and 1.5ml thiobarbituric acid (TBA 1%) in succession, and the solution was then heated at 100 °C for 15 min. After centrifugation at 4000rpm for 20min to remove precipitated protein the absorbance was detected at 532 nm.

Data Analysis

The results of the triplicates were pooled and expressed as mean \pm standard deviation. Analysis of variance and the t-test were carried out. Significance was accepted at $P < 0.05$.

Results

The results of analysis of phytochemicals in *n*-butanol extract of *Limonium bonduelli* are outlined in Table 1 which illustrated a positive test for flavonoids and phenols. The extract showed high phytochemical content.

Table 1: Analysis of phytochemicals in *n*-butanol extract of *Limonium bonduelli*

Phytochemicals	<i>Limoniumbonduelli</i>
Phenols	+++
Steroids	-
Alkaloids	-
Flavonoids	+++
Terpenoids	-

The results of the present study demonstrated clearly, that *n*-butanol extract and vitamin C (standard) inhibited lipid peroxidation in different tissues homogenate in a dose dependent manner as shown in Figure 2,3 and 4.

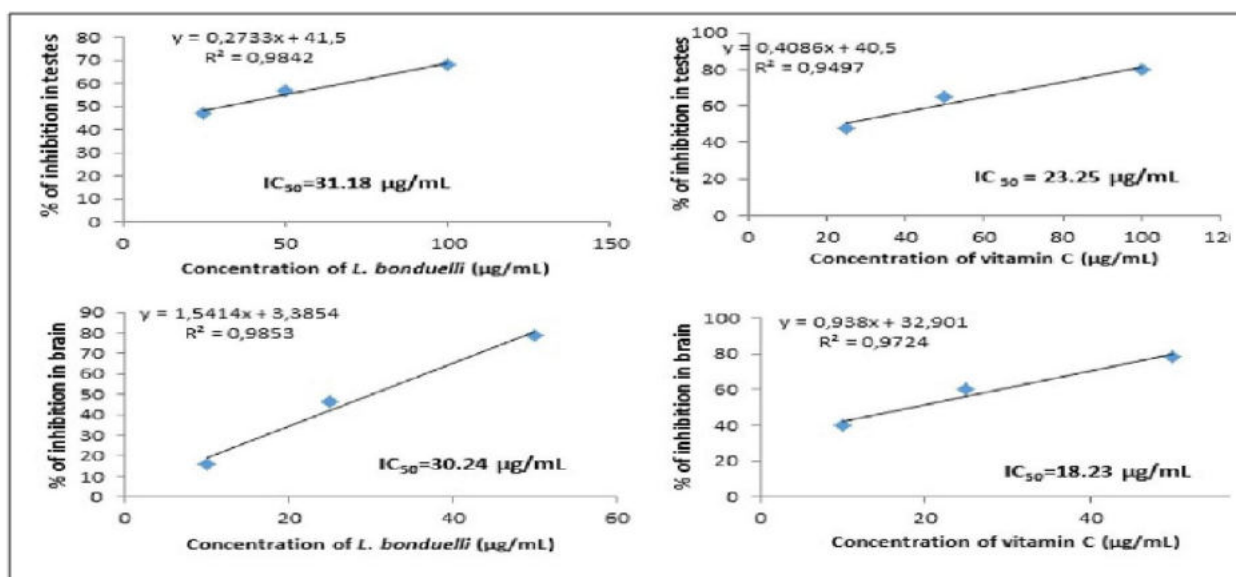


Figure 2. Lipid peroxidation half-inhibition values (IC₅₀) of plant extract and reference antioxidant. The Vitamin C produced greater inhibition (IC₅₀ = 23.25 \pm 0.41 μ g/mL, IC₅₀ = 18.23 \pm 0.25 μ g/mL) as compared to the extract (IC₅₀ = 31.18 \pm 0.55 μ g/mL, IC₅₀ = 30.24 \pm 0.35 μ g/mL) in testes and brain respectively (Fig 2) and the highest percentage of the inhibition was 89.80% similar to vitamin C in the same concentration (100 μ g/ml) in brain and 82.33% in testes (200 μ g/ml) (Figure 3 and 4).

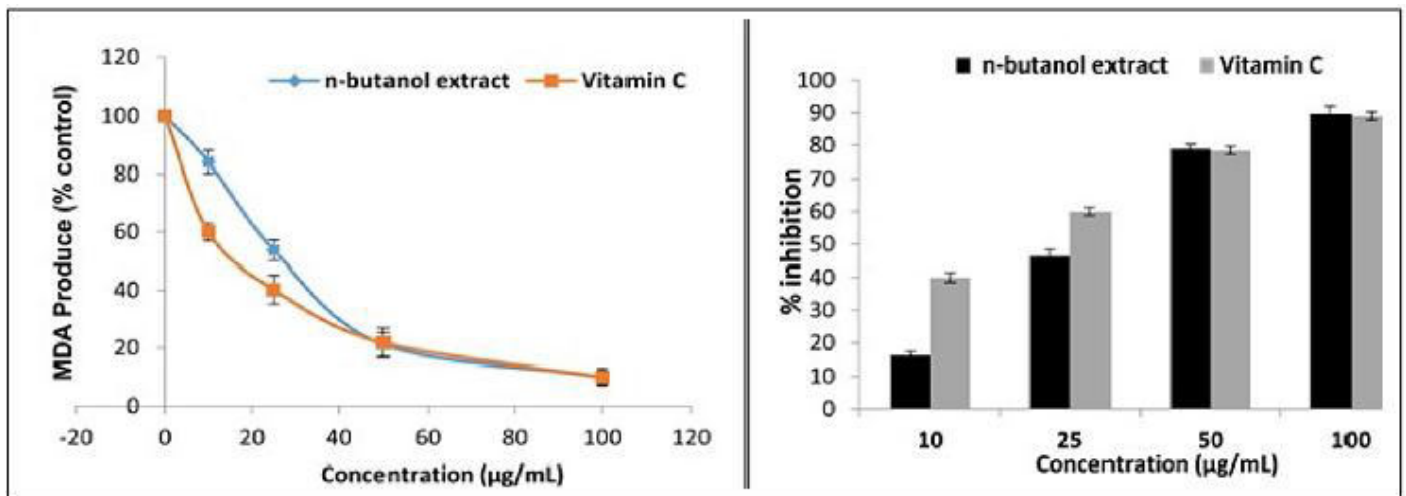


Figure 3. Inhibition of FeSO_4 induced lipid peroxidation in rat brain tissue homogenate by *Limonium bonduelli* butanolic extract and Vitamin C. Values represent mean \pm standard deviation of triplicate experiments.

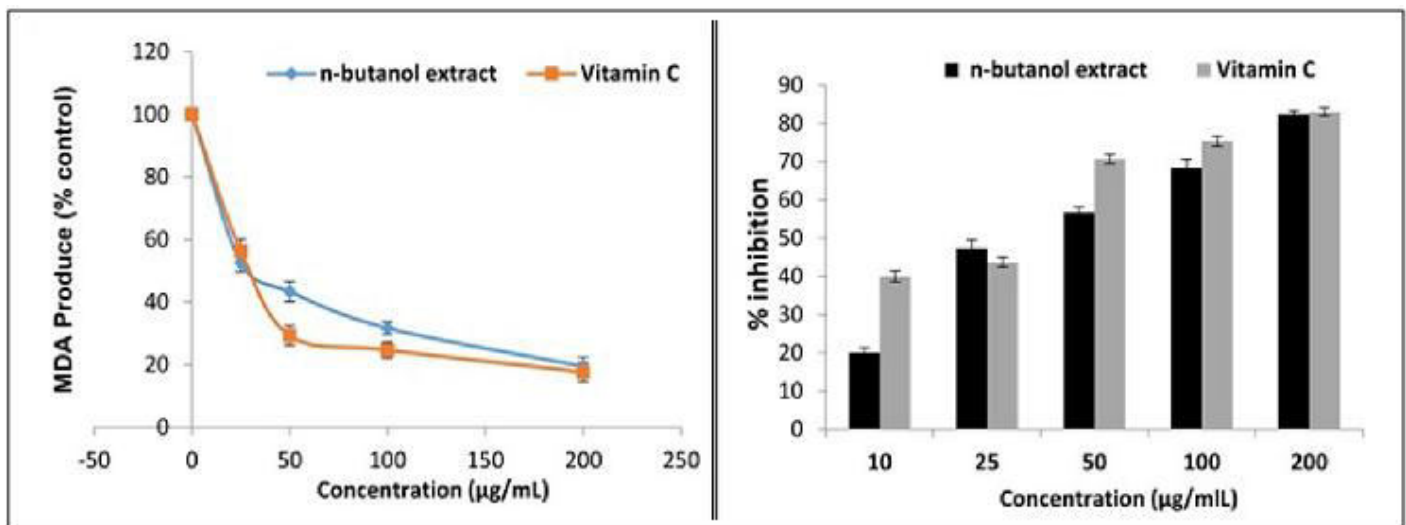


Figure 4. Inhibition of FeSO_4 induced lipid peroxidation in rat testes tissue homogenate by *Limonium bonduelli* butanolic extract and Vitamin C. Values represent mean \pm standard deviation of triplicate experiments.

Discussion

Iron is a two-edged sword for biological systems, essential for many cellular activities, but also able to cause damage to macromolecules or disrupt sensitive processes. In the brain this balance is even more delicate given the irreplaceable nature of neurons [27]. Iron accumulation or iron overload in brain is commonly associated with neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, and also plays a role in cellular damage following hemorrhagic stroke and traumatic brain injury. Despite the brain's highly regulated system for iron utilization

and metabolism, these disorders often present following disruptions within iron metabolic pathways [28]. Furthermore, the mammalian brain is particularly sensitive towards oxidative damage, a result of the high oxygen demand and the high content of unsaturated lipids in the central nervous system coupled with the reduced access to the antioxidant defense system and the high amount of redox-active transition metal ions [29-31]. Oxidative stress in the central nervous system is an underlying cause of neurodegeneration and neural dysfunction. This has resulted in considerable research efforts being put towards reducing the risks and event of oxidative stress with the use of antioxidants [32].

In this study, incubation of the rat brain homogenates in the presence of FeSO_4 caused a significant increase in the MDA

content of the brain (Figure. 3). These findings agree with other reports on the interaction of FeSO₄ with the brain, in which FeSO₄ was shown to be a very potent initiator of lipid peroxidation in the brain [31]. The increased lipid peroxidation in the presence of Fe²⁺ could be attributed to the fact that Fe²⁺ can catalyze one-electron transfer reactions that generate ROS such as reactive hydroxyl radical (*OH) which is formed from H₂O₂ through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxy and alkoxy radicals, which favors the propagation of lipid peroxidation [33, 34]

The natural phenolic compounds and flavonoids have received increasing interest in the last years. They may reduce the risk of development of several diseases caused by oxidative stress, including neurodegenerative disorders [35] and testes injury [36,37]. Their protective effects stem from the ability to inhibit lipid peroxidation, chelate redox-active metals (by binding iron), and attenuate other processes involving reactive oxygen species (such as through radical scavenging) [38, 39].

The effect of the *n*-butanol extract of *Limonium bonduelli* on FeSO₄ induced oxidative stress in isolated rat brain homogenates is presented in Figure 2 and 3. The result revealed that incubation of rat brain in the presence of FeSO₄(0.07 M) and *n*-butanol extract of *Limonium bonduelli* caused a significant (P<0.05) decrease in the MDA content of the brain in a dose-dependent manner (10-100µg/mL). These results are consistent with those previously presented by others[9, 40] in that *Zingiber officinale*, Caffeic and Chlorogenic Acids were found to be effective on lipid peroxidation induced by Fe²⁺ in rat brain *in vitro*.

Iron is essential trace nutrients playing important roles in fertility. Excess of this element may lead to defective spermatogenesis, reduced libido, and oxidative damage to the testicular tissue and spermatozoa, ultimately leading to fertility impairment [41]. Iron can affect male and female fertility by induction of reactive oxygen species (ROS) production. Therefore, antioxidant therapy that inhibits iron-induced toxicity is under active investigation. Flavonoids have antioxidant and metal chelating properties which make them suitable candidates for neutralizing adverse effects of metals on semen quality [42].

In like manner, effect of the *n*-butanol extract of *Limonium bonduelli* on FeSO₄ induced oxidative stress in isolated rat testes homogenates was also studied and the result is represented in Fig 2 and 4. The study revealed that, incubating rat testes tissue homogenates in the presence of FeSO₄ (0.07 M) resulted in a significant (P<0.05) increase in the MDA production in the testes; however, the presence of the extract caused significant (P<0.05) decrease in the MDA content of the testes in dose-dependent manner. This finding is in agreement with results provided by others, who demonstrated the inhibitory effect of aqueous extract of *Moringa oleifera* and *Newbouldia laevis* leaves on FeSO₄ and Sodium Nitroprusside induced lipid peroxidation in rat testes *in vitro* [43]. In same study the methanolic extract from the pumpkin seed was shown to modulate FeSO₄ induced TBARs production in albino rat's testicular tissue *in-vitro* [44].

Conclusion

n-butanol extract of *Limonium bonduelli* demonstrated significant anti-lipid peroxidative effects, which may be useful in preventing the progress of various oxidative stress related diseases such as neurodegenerative and infertility. Phenolic compounds are majorly responsible for the antioxidant activity of plant material. However, these findings warrant extensive studies on chemical profiles. *In vivo* studies are needed to confirm this pharmacological efficacy and further studies are underway in our laboratory.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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