



## ***In vitro* Activity of *Arbutus unedo* Aqueous Extract against *Leishmania infantum* Promastigote**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors IM and AM designed the study, performed and wrote the protocol. Authors GIA and AH collected the plant samples. Author NA provided the parasite strain. Authors CZ and OM managed the literature searches. Author KH wrote the first draft of the manuscript and led the work. All authors read and approved the final manuscript.*

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**Short Communication**

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### **ABSTRACT**

Considered as a major public health problem, leishmaniasis incidence continues to increase due to lack of vaccine. In addition, drugs routinely used for the treatment of this disease have associated side effects. This justifies a need to develop new drug treatments. It is in this perspective that our study is inscribed.

*In vitro* antileishmanial activity of the aqueous extract of *Arbutus unedo* leaves was evaluated

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against promastigotes of *L. infantum* (MON-1/DZ/01/LIPA1227/01) by *in vitro* promastigote cell assay. The extract of the studied leaves showed remarkable antileishmanial *in vitro* activity (74% mortality at 100 µg/ml) against the promastigotes of *Leishmania infantum*, in a concentration dependent manner.

**Keywords:** *Leishmania infantum*; *Arbutus unedo*; aqueous extract.

## 1. INTRODUCTION

Leishmaniasis constitutes a heterogeneous set of diseases all due to the infection of the host by a protozoan transmitted by a vector insect, the sandfly.

In Algeria, leishmaniasis has been marked by a considerable increase, particularly visceral leishmaniasis, due to *L. infantum* which infect particularly young children between one and four years of age [1,2].

The treatment, in addition to being very expensive, generates many side effects. Because of its numerous biological activities already proven [3] and for the sake of remediation we try in this study to evaluate the effect of the aqueous extract of *Arbutus unedo* against *L. infantum*.

Through the literature over 239 natural molecules that have been tested for their antileishmanial effect [4].

The active compounds, which have been isolated and identified, belong to the classes of alkaloids, triterpenes, sesquiterpenes, miscellaneous lactones, quinoids, flavonoids, diterpenes, steroids, lipids, iridoids, oxygen heterocycles, benzenoids, carbohydrates, depsides, a sulfur compound, and a monoterpene [4].

*Arbutus unedo* is known to contain many of these natural compounds [5]. This rich and varied composition is on the one hand at the origin of the numerous biological effects (antioxidant, anti-inflammatory, anti-diabetic and anti-cancer) of the plant extract and on the other hand could be potentially at the origin of an antileishmanial activity.

Furthermore, there is no scientific works dealing with the antileishmanial activity of the aqueous extract of *Arbutus unedo* have been carried out, which motivated us for the realization of this study.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection

*Arbutus unedo* L. (Ericaceae) leaves were collected in December 2014 from Tizi-Ouzou, Algeria. A voucher specimen was deposited in the herbarium of Mouloud Mammeri University of Tizi-Ouzou, Department of Vegetal Biology (FSBSA/MK/2105). The sample was dried and then ground to obtain a powder that was stored at room temperature and in the dark until extraction.

### 2.2 Extract Preparation

Twenty grams of powder of *A. unedo* leaves were dissolved in an erlenmeyer with 200 ml of distilled water. The flask was fully coated with aluminum foil and was placed on a magnetic stirrer. After 24 h of maceration at room temperature, and stirring at 40 rpm. Then, extract was filtered by using glass wool followed by Whatman № 1 filter paper. Finally the filtrate was lyophilized.

### 2.3 *In vitro* Antileishmanial Activity

*L. infantum* MON-1/DZ/01/LIPA1227/01 promastigote grown on NNN medium. The cultures were centrifuged for 10 min at 2.500 rpm and then undergo 3 washes with physiological water and transferred to RPMI 1640 medium supplemented with 10% fetal calf serum for mass cultivation [6].

The screening was performed in flat-bottomed 96-well plastic tissue-cultured plates maintained at 25°C.

Promastigote forms from a logarithmic phase culture were suspended to yield 1 million of cells/ml. The test was carried out on 96 well microplates, each well was filled with 100 µl of the parasites suspension, and the plates were incubated at 25°C for 1 h before drug addition.

Finally the extract to be tested was dissolved in DMSO and added to each well in order to obtain

the final concentrations of 12.5, 25, 50 and 100 µg / ml.

The incubation was carried out at 25°C for 72 h [7]. The viability of promastigotes was assed by propidium iodide colorimetric method and *A. unedo* activity was evaluated by comparing the mortality rates of the test wells with an untreated control.

### 3. RESULTS

The lyophilized extract was diluted in 1% DMSO and DMSO control was found to be inactive.

Results showed the antileishmal activity of the *A. unedo* leaves aqueous extract in a dose dependent manner at concentrations 12.5 – 100 µg/ml. The maximum activity of the extract was evaluated at 74% mortality of promastigotes for the concentration of 100 µg/ml.

The collected data (Fig. 1) clearly indicate that the inhibitory concentration for the extract is lower than 12.5 µg/ml.

### 4. DISCUSSION

In recent years, the interest in scientific research for plant extracts has grown and it is aimed at the search for compounds with a powerful leishmanicidal effect.

In a recent study conducted in Turkey, *in vitro* testing of antileishmania activity against

*Leishmania. tropica* promastigote was carried out. Ethanol, water and n-hexane extracts of *A. unedo* leaves have been tested and the ethanol extract was found to be more effective than the other extracts [8].

There are few studies in the literature that report on the activity of *A. unedo* aqueous extract against *L.infantum*, which does not allow us to compare our results.

But as a point of comparison we can cite the work of Khademvatan [9], on *L. infantum* promastigotes, which relate an IC<sub>50</sub> of 53.5 ± 2.5 µg/ml, obtained through the extract of *Holothuria leucospilota*.

In another study carried out by Mansour [10], on the anti-*Linfantum* activity of *Vitis vinifera* L. leaves aqueous extract, report an IC<sub>50</sub> of 12.53 µg / ml.

Many studies have been interested in the compounds of medicinal plants such as polyphenols, triterpenes, saponins [11,12,13,14], resveratrol [15], tannins [16], alkaloids [17], and quinines [18].

The antileishmanial action of these natural compounds has not yet been elucidated; never theless [19], report that the antileishmanial action of the flavonoids is done by inhibition of the parasitic topoisomerase II.

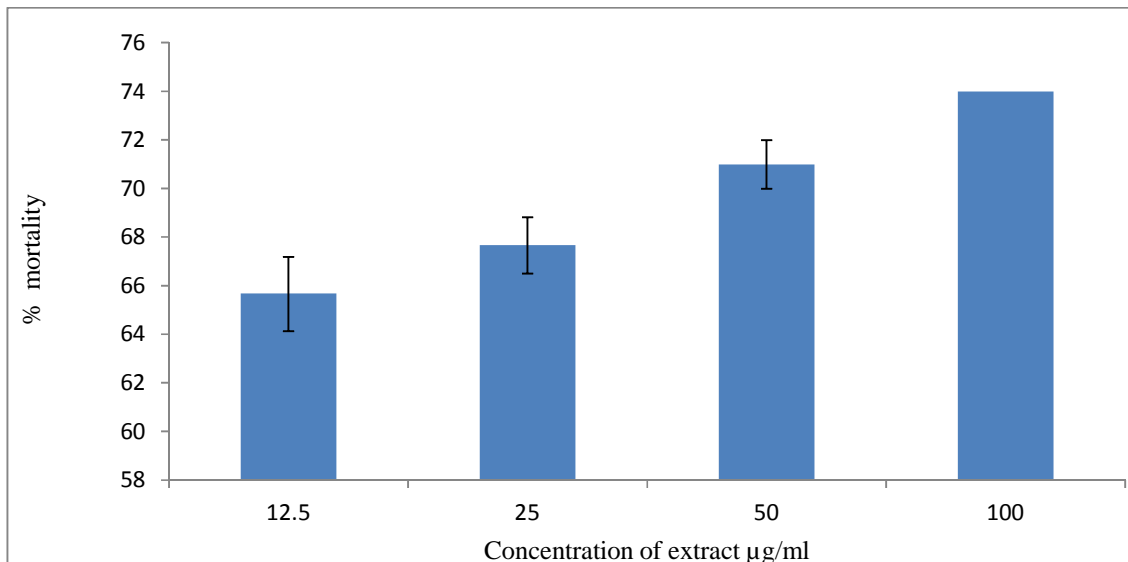


Fig. 1. Antileishmanial activity of *A. unedo* aqueous extract

## 5. CONCLUSION

In the search for novel antiprotozoal agents at a time when there is an urgent need for new innovative drug leads.

The results obtained suggest that the aqueous extract of *A. unedo* leaves can lead to the development of an effective treatment against leishmaniasis.

The antileishmanial activity of the studied extract may be due to its composition, rich in phenolic compounds.

The perspective of applying this extract to therapeutics involves the determination of its active fraction and its evaluation by *in vivo* tests.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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