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ANTIBACTERIAL EFFECT OF *ARBUTUS UNEDO L.* FRUIT AND ITS ESSENTIAL OILS ON *SALMONELLA TYPHI* (ATCC 14028) AND *PSEUDOMONAS AERUGINOSA* (ATCC 27853)

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ABSTRACT

In the recent years, there is considerable use of plants, especially fruits. These fruits are attracting more and more interest growing among both consumers than among dietiticians and nutritionists. They are important for human health because of richness in vitamin C, antioxidants (polyphenols), minerals and enzymes that fight against certain diseases.

Our choice is focused on the study of *Arbutus unedo L*, which is a wild fruit which is located in the north of Africa mainly in Algeria, and characterized by its very higher nutritional value and antioxidant and antimicrobial properties.

The objective of our research is to study the antibacterial effect of this fruit and its essential oils on selected bacterial pathogens; *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853).

The obtained results show that *Arbutus unedo* L. fruit and its essential oils have no inhibitory effect on the selected bacteria. This resistance is due to the composition of the cell wall of Gram-negative bacteria, which is rich in lipopolysaccharides.

KEYWORDS: *Arbutus unedo L.*, Essential Oils, Antibacterial Effect, Gram Negative

INTRODUCTION

Arbutus unedo L., the strawberry tree is an evergreen shrub leaf which belongs to the *Ericaceae* family and native of the Mediterranean regions. It is located mainly in North Africa; especially in the Algerian tell **(Alarcão-e-Silva and *al.*, 2001**). In Algeria, it is known as vernacular Arabic name "Lendj ".

Lendj has a very important medicinal and nutritional value; various studies have demonstrated the association of consumption of these fruits with a reduced risk of developing certain diseases such as hypertension, diabete, atherosclerosis and thrombosis and they are used in traditional medicine as antiseptics, diuretics and laxatives **(Ozcan et Haciseferogullari, 2007, Oliveira *et al.*, 2011).**

Many studies have been made on *Arbutus unedo* L. fruit and especially on essential oils with particular attention to characterize their different compounds **(Alarçao-e-Silva *et al.*, 2001, Kivack *et al.*, 2001, Barros *et al.*, 2010).** Others are intended to determine the nutritional value and research of antioxidant and antimicrobial effects (**Pallauf *et al.*, 2008, Fortalezas *et al.*, 2010, Benzeggouta, 2005, Dib *et al.*, 2010).**

Currently, no study was devoted to the antimicrobial effect of *Arbutus unedo* L fruit juice. All studies highlighted the antimicrobial effects of roots and leaves only. However, very little antimicrobial effects studies have been reported on its essential oils **(Miguel *et al.*, 2014).**

In Algeria, this fruit remain unexploited and very few studies have been devoted to its antimicrobial properties; for this reason, we are interested to study the inhibitory effect of *Arbutus unedo* L. fruit and its essential oils on pathogenic Gram negative strains; *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853).

MATERIALS AND METHODS

Material

Plant Material (*Arbutus unedo* L.)

The ripened fruit of *Arbutus unedo* L. (**Figure 1**) was collected from Guezoul forest locally know "Oued el Lendj", which is located on the western of the city of Tiaret (in the southern of Algeria). Indeed, harvesting was at an altitude of about 1040 m. These fruits are washed, wiped, sorted, and stored frozen at – 20 °C for analysis purposes. They have been used to obtain fruit juice and essential oils extract.

Figure 1: *Arbutus unedo L*. Fruit

Test Organism

Gram negative bacterial strains used for the experiment were collected as pure cultures from ATCC (American Type Culture Collection); *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853). They were obtained from Pasteur Institute (Algiers-Algeria).

METHODS

Microbiological Examination of *Arbutus unedo* L. Fruit

These analyzes allow to determine the presence or absence of microorganisms in the product.

According to **JORA (1998),** the stock solution was prepared by adding 9 ml of saline to 1 g of the fruit (10^{-1} dilution). A series of test tubes containing 9 ml of physiological saline was prepared and then 1 ml of the stock solution was added to the first tube (10^{-2} dilution), after 1 ml of 10^{-2} dilution was taken and added to the second tube (10^{-3} dilution). The same operation was repeated until 10^{-6}.

The searched germs are: fungal flora (yeasts and molds), and *Escherichia. Coli* which were determined according to **Guiraud (1998).**

- *Escherichia coli* is a good indicator of fecal contamination. This bacterium is capable of causing severe diarrhea, especially in young children with cholera-like syndromes **(Mehlman *et al.,* 1997).**
- Yeasts and molds can be found in the normal flora of a food product. They can produce toxic metabolites, and cause odors and flavors of food. Their presence is an indicator of environmental contamination **(Spencer and Ragout de Spencer, 2001, Branger *et al.,* 2007)**.

Antibacterial Effect of *Arbutus unedo* L.

a. Extraction of Juice and Essential Oils from *Arbutus unedo* L. Fruit

- The essential oils of *Arbutus unedo* L. were obtained by steam distillation **(Sukhdev *et al.,* 2008).** This operation is achieved by extraction of 50 g of plant material with 400 ml distilled water.These oils are stored in opaque glass bottles closed with a well-sealed cap, and kept at room temperature.
- The extraction of juice is done by crushing 100 g of fruits in laboratory blender following by centrifugation at 4500 rpm for 10 min.The supernatant was stored at 4 °C until use.

b. Antagonistic Effect of *Arbutus unedo* L. and its Essential Oils on Studied Bacteria

The bacterial inoculates were made up from overnight broth cultures. For antimicrobial assay, bacterial strains were grown on Mueller-Hinton Agar (MHA). Bacterial inoculates were adjusted to 0.5 McFarland standard turbidity; reaching microbial densities in the range 10^6–10^7 CFU / ml.

The antagonistic effect is carried out by wells diffusion method. The wells were filled with 1 ml of each extract (juice or essential oils).Then, Petri dishes were incubated at 37°C for 24 hrs.The diameters of the inhibition zones appearing around wells are measured **(Ela *et al.,* 1996).**

RESULTS AND DISCUSSION

Results

A. Results of Microbiological Control of the Fruit of *Arbutus unedo* L.

The results of the microbiological control of our fruit are present in Table 1.

Table 1: Results of Microbiological Control of *Arbutus unedo* L. Fruit

Bacterium	Dilution	Results
Escherichia coli	10^{-2}	-
	10^{-5}	-
	10^{-6}	-
Molds and yeasts	10^{-1}	-
	10^{-3}	-
	10^{-6}	-

- Absence

The results of microbiological control of *Arbutus unedo* L. show the absence of microorganisms that can affect its organoleptic properties. According to **JORA (1998)**, our fruit is of satisfactory quality and subsequently it is proper to human consumption.

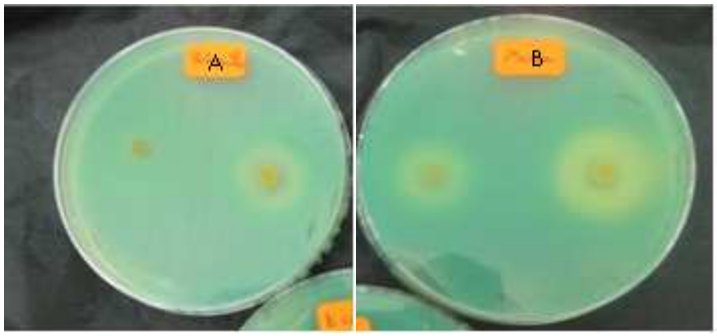
Microbiological control of our fruit is important in order to avoid possible interaction between the microorganisms during antagonistic test.

b. Results of Antagonism

There was no inhibitory effect of the extracts (fruit and its essential oils) on our selected strains as shown in the following figures.

(A) Salmonella typhi ***(B) Pseudomonas aeruginosa***

Figure 2: Results of the Inhibitory Effect of Fruit Essential Oils against Selected Strains



(A) Salmonella typhi ***B) Pseudomonas aeruginosa***

Figure 3: Results of the Inhibitory Effect of Fruit Juice against Selected Strains

GENERAL DISCUSSIONS

Using the well diffusion method to test the effect of *Arbutus unedo* L. and its essential oils on *Salmonella typhi* (ATCC 14028) and *Pseudomonas aeruginosa* (ATCC 27853), the results reveal a total absence of inhibition zones for these selected strains.

These results are similar to some studies using essential oils of several medicinal plants; Aniseed, Calamus, Camphor, Cedarwood, Cinnamon, Citronella, Clove, Eucalyptus, Geranium, Lavender, Limon, Lemongrass, Lime, Nutmeg, Orange, Palmarosa, Peppermint, Rosemary, Basil, Vetiver, and Sagebrush which revealed the absence or the lack of the inhibitory effect of Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Pseusomonas vulgaris*, *Bacillus.subtilis*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Listeria monocytogenes and Bacillus cereus* (Prabuseenivasan *et al.*, 2006, Bencheqroun *et al.*, 2012 and Elharas *et al.*, 2013). However, Kahriman *et al* (2010) has found that essential oil of *A. unedo* had a moderate antibacterial activity against *Listeria monocytogenes.*

Orak *et al* (2011) evaluated the antimicrobial potential of *Arbutus unedo* L. leaves. No inhibitory effect against *Escherichia coli* and *Salmonella enteritidis* has been found. Also, Dib *et al* (2013) studied the antimicrobial activities of water and methanol extract of the roots of *Arbutus unedo* L against two gram negative strains; *Escherichia coli* and *Pseudomonas aeruginosa*.Poor antibacterial activity against *Pseudomonas aeruginosa* was shown with water and methanol extract. However, moderate antibacterial activity was shown by water extract against *Escherichia coli*. In recent study,

antimicrobial activity of ethanolic extracts of five species of *Ericaceae* family native to the Balkan Peninsula: *Arbutus unedo*, *Bruckenthalia spiculifolia*, *Calluna vulgaris*, *Erica arborea* and *Erica carnea* was tested against three Gram negative bacteria; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*.The results showed no inhibitory effect of *Arbutus unedo* L.leaves extracts on studied strains **(Dragana *et al.*, 2014).**

In conclusion, the absence of the inhibitory effect of *Arbutus unedo* L. fruit and its essential oils against selected bacterial pathogens may be due to the resistance of gram negative bacteria cell wall. This resistance towards antibacterial substances may be due to outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes it impermeable to lipophilic solutes and porins constitute of a selective barrier to the hydrophilic solutes **(Nikaido and Vaara, 1985).** It would be interesting to continue this work with the study of other pathogenic bacteria effect on different extracts of *Arbutus unedo* L. Moreover, further antimicrobial studies on fruit juice are needed.

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