Antifungal activity of Myrtus communis extracts against plant pathogenic fungi Antifungal activity of Myrtus communis extracts

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Abstract: This study aims to determine the antifungal activity of M. communis fruit extract against plant pathogenic fungi. Myrtle berries were harvested during the month of December in three different sites in northern Tunisia: Bizerte (jebal Toro), Béja (Bellif Forest), and Ain Drahem (Majen essef). Juice was extracted by pressing. The antioxidant activity was conducted using 2.2-diphényl 1-pycrilhydrazyle (DPPH).
The antifungal activity of myrtle juice was tested against four fungal strains: Bipolaris sorokiniana, Fusarium graminearum, Fusarium nygamai, Rhizoctonia solani.
The Ain Drahem site showed the highest percentage of DPPH inhibition (37.5%).
Statistical analysis showed that the antifungal activity varied depending on the geographic origin as well as the fungal strain used. The juice from Ain Drahem was the most effective for the inhibition of the Bipolaris strain. Myrtle juice exhibited an important antioxidant and antifungal activities.

IndexTerms— Myrtus communis, juice, antioxidants, antifungal activity

I. INTRODUCTION

Myrtus communis, the common myrtle is a charming evergreen shrub belonging to the *Myrtaceae* family. It is native to the Mediterranean region, and its distribution extends to Western Asia. It is an aromatic and medicinal plant which shows multiple ornamental qualities, in particular its white flowering and its purple berries. Myrtle is also used, for example, as a leaf infusion to treat sinusitis, bronchitis, urinary tract infections, and various digestive problems, such as aerophagia or intestinal colic [1, 2]. *M. communis* is known for its essential oil used locally to soothe acne or de-inflame small wounds. Several scientific studies highlighted the biochemical and biological properties of this oil. It showed anti-inflamatory, antioxidative, analgesic, antimutagenic, neuroprotective and antidiabetic effects [3,4,5,6,7].

Touaibia [8] demonstrated that the essential oils of *M. communis* have an important anti-fungal activity on phytophathogenic strains. Contrarily to essential oil, few studies focused on antifungal activity of extract of this plant.

This study aims to determine the antifungal activity of *M. communis* fruit extract against plant pathogenic fungi.

II. MATERIAL AND METHODS

Plant material and juice extraction

Myrtle berries were harvested during the month of December in three different sites in northern Tunisia: Bizerte (jebal Toro), Béja (Bellif Forest), and Ain Drahem (Majen essef).

The fruits were washed and then crushed using an electric grinder. The paste obtained is then subjected to pressing which allowed the separation of the juice from the cakes.

Antioxidant activity

The juice samples were dissolved in ethanol at the rate of 50 µl of juice for 950 µl of ethanol; 50 µl of each dissolved sample was added to 1 ml of DPPH in the ethanol solution (60 µM: 2.4 mg of DPPH/100 ml of ethanol). After incubation at 27°C for 60 min, the absorbance of each solution was determined at 517 nm using a spectrophotometer [9].

The percentage inhibition of DPPH (IR) was calculated according to the following equation: IR = [(DOc – DOe) /DOc] x 100

Where DOc is the absorbance of the control (containing 50 µl of ethanol and 1 ml of DPPH) and DOe is the absorbance of the DPPH containing the juice samples.

The median inhibitory concentration IC50 was then determined for different juice concentrations: 2.5, 5, 10, 20 µl.

Antifungal activity

The antifungal activity of myrtle juice was tested against four fungal strains: *Bipolaris sorokiniana*, *Fusarium graminearum*, *Fusarium nygamai*, *Rhizoctonia solani*.

The preparation of the PDA medium was performed by adjusting with distilled water the aqueous extract of the broth of 200 g of potato to 1 liter and adding 20 g of agar and 20 g of glucose.

The culture was made on a PDA medium at the rate of 20 ml per Petri dish. 2 ml of juice were introduced into the 20 ml of PDA after having been mixed and homogenized with tween 0.1%. After cooling the medium, a 5 mm diameter disk of each fungal strain was placed in the center of the petri dish while placing the mycelial surface down. The dishes were incubated at 22°C for six days. The fungicidal effect was determined by calculating the growth diameter of the strain in question and comparing it to that of a negative control, i.e. a PDA medium without juice [10].

The results were calculated according to the method of Singh et al. [11] while calculating the percentage inhibition I according to the following formula:

$$I(\%) = [(dC-dE) /dC] \times 100$$

Where: dC: witness diameter (mm)
dE: diameter in the presence of oil tested (mm)

Statistical analysis

The statistical processing of the data was carried out using the SAS GLM (General Linear Models) procedure. An analysis of variance relative to the parameters studied was carried out.
Results are presented as the mean of three replicates ± standard deviation.

III. RESULTS AND DISCUSSION

Antioxidant activity

The percentages of DPPH inhibition of the three juices studied are presented in table 1.

Table1. Results of measurement of the percentage inhibition of DPPH

Site	Percentage inhibition of DPPH(%)
Ain Drahem	37.5 ^{a} ± 4.04
Bizerte	36.84 ^{a} ± 2.64
Beja	20.68 ^{b} ± 2.58

The different letters denote significant differences by the Student-Newman-Keuls test ($\alpha < 0.05$).
The Ain Drahem site showed the highest percentage of DPPH inhibition (37.5%), followed by the Bizerte site (36.84%) then Beja (20.68%).
These differences can be linked to the differences between the climatic and edaphic conditions between the three sites studied.
The values of the antioxidant activity of the juices, expressed in IC50, are presented in Table 2. The value of each IC50 expresses the concentration of the juice required to reduce 50% of DPPH in solution.

Table2. IC50 values of *M. communis* juices

site	IC_{50}
Ain Drahem	11.66 ^{a} ± 3.72
Bizerte	13.7 ^{a} ± 1.47
Beja	8^{b} ± 4.35

The different letters denote significant differences by the Student-Newman-Keuls test ($\alpha < 0.05$).
The differences between the IC50 values obtained for the three collection sites are significant.
All the IC50s obtained are lower than the IC50 of BHT (Butylated hydroxytoluen), considered as a positive control and which has a value equal to 25 ± 0.02 µg/ml. Myrtle juice thus has a higher antioxidant activity than BHT.
Work done by Wannes et al. [1] on the essential oil of myrtle leaves showed antioxidant activity resulting in an IC50 equal to 600 µg/mL. This activity is clearly lower than that determined for the juice studied.
The antioxidant activity of plants is mainly provided by active compounds and phenolic fractions, the amount of these compounds deposited in each part of the plant is generally different [12].

Antifungal activity

The percentages of inhibition of the fungi strains are summarized table 3.

Table3. Antifungal activity of *M. communis* juices

Strains	Ain Drahem	Bizerte	Beja	Negative control
B. sorokiniana	62.26^{b}±8.11	35.39^{a}±0.86	22.69^{a}±7.5	2.4±0.14
F. graminearum	14.96^{a}±15.5	29.11^{a}±9.74	26.66^{a}±9.42	2.6±0.14
F. nygamai	30.9^{ab}±0.49	54.51^{b}±2.45	40.79^{a}±9.08	3.4±0.28
R. solani	49.72^{b}±7.46	42.22^{ab}±3.14	44.72^{a}±0.39	1.9±0.14

The different letters denote significant differences by the Student-Newman-Keuls test ($\alpha = 0.05$).

Statistical analysis showed that the antifungal activity varied depending on the geographic origin as well as the fungal strain used.
The juice from Ain Drahem was the most effective for the inhibition of the *Bipolaris* strain. The other two vary from moderately weak (Bizerte) to weak (Beja). For *F. graminearum* strain the differences were not significant.
The juice from Beja location was the most effective against the strain *F. nygamai*.
The juice from Bizerte was the most effective for the inhibition of this strain. Juice from Ain Drahem site constitutes the inhibitoriest site of the growth rate of *R. solani* strain.
Other works on the antifungal activity of myrtle extracts with other fungal strains (such as *Aspergillus niger*) showed a very high sensitivity to myrtle extract by a percentage of inhibition that reached 78% [13]. These results therefore indicate that each strain has a different sensitivity to myrtle extract.
Myrtle juices showed an important antioxidant activity. This could explain its important antifungal activity. In addition Ain Drahem location, which showed the highest antioxidant activity, exhibited the most important antifungal activity.
Several studies reported the high antifungal properties of antioxidant agents. Among antioxidants phenols were mentioned as powerful antifungal compounds [14, 15, 16].

IV. CONCLUSIONS

Myrtle juice exhibited an important antioxidant and antifungal activities. This is likely to better valorize this plant. These properties recorded for myrtle make it possible to use it in programs to fight against phytopathogenic strains.

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