



Acute Toxicity and Antioxidant Activity of Algerian *Citrus deliciosa*

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ABSTRACT

Our phytochemical study focused on the leaves of *Citrus deliciosa* of Algerian origin, in order to evaluate its antioxidant activity and its acute toxicity, using scavenger activity of DPPH radicals, ABTS and acute toxicity. The ethanol extract of *Citrus deliciosa* contains 85.62 ± 2.24 $\mu\text{g}/\text{mg}$ dried matter and 3.02 ± 0.17 $\mu\text{g}/\text{mg}$ dried matter. The antioxidant activity was confirmed against the DPPH radical ($\text{IC}_{50} = 252.07 \pm 0.02$ $\mu\text{g}/\text{mL}$) and ABTS ($\text{IC}_{50} = 398.26 \pm 0.01$ $\mu\text{g}/\text{mL}$). No mortality or abnormal behavior was observed in mice at oral doses less of 1600 mg/kg. According to the results obtained, *Citrus deliciosa* leaves exhibit significant antioxidant activity and are safe at levels lower than 1600 mg/kg B.W., which supports their application in phytotherapy, pharmaceutical biotechnology, and the food sector for therapeutic or preventive purposes.

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Introduction

Traditionally, humans have used their environment and in particular plants to take care of themselves, it is estimated that the most of the current medicines have a natural origin, only a few portion of the medicines marketed therefore have a purely synthetic origin, plants in particular represent an immense source of chemodiversity, and continue to offer the best possibilities for discovering novel agents or active templates, which, when investigated in collaboration with synthetic chemists and biologists, offer the possibility of discovering novel structures that can result in agents that are efficient in a variety of human diseases (Sevindik et al., 2017; Newman et al., 2020; Mohammed et al., 2022).

The progress of the pharmaceutical sciences has made it possible to demonstrate the properties of called medicinal plants, whatever therapeutic or toxic. These properties are due to the richness of active compounds such as phenols which are currently the subject of numerous studies for their multiple biological activities that are very beneficial

to the human organism (Aouachria et al. 2017; Mohammed et al., 2023; Uysal et al., 2023).

The flavonoids in citrus have been the focus of years of research, beginning with the first description of hesperidin by Lebreton nearly two centuries ago (Manthey et al., 2011). Citrus flavonoids play an important role in the prevention of degenerative and infective diseases. Flavonoids are a widely distributed group of phenols compounds, called "nutraceutical substances", with anticancer, antiatherogenic, antimicrobial and anti-inflammatory properties (Tripoli et al., 2007).

Citrus deliciosa is a plant widely used in traditional pharmacopoeia, for its therapeutic virtues. Despite its biological and medicinal importance, leaves of this species have been studied very little (Şahin et al., 2019). Therefore, an experiment was conducted to determine phytochemical analysis of *Citrus deliciosa* leaves, including phenols and flavonoids contents, antioxidant activity and their acute toxicity that is not reported, to the best of our knowledge.

Materials and Methods

Chemicals

All reagents were purchased from Sigma Chemicals (Germany), Fluka and Prolab.

Animals

Adult female Swiss albino mice (19-30 g) were supplied by the animal center of Pasteur's Institute (Algiers, Algeria). The mice were kept under standard conditions with normal light cycle (12 h), with free access to food and water. Before any treatment, these animals were given a week of acclimation time. These experiment procedures were carried out in accordance with the internationally accepted guidelines for evaluating the safety and efficacy of plant medicines (OECD, 2008).

Plant Material and preparation of extracts

Leaves of *C. deliciosa* were collected from Blida (Algeria) in april 2022, dried under shadow at room temperature and powdered. The powder (0.5 g) was weighed and placed into a 100 mL conical flask, and treated with 80% (v/v) ethanol (40 mL). It was followed by an addition of 6 M HCl (10 mL). The mixture was refluxed for 2 h at 90°C and filtered by using Whatman No. 1 filter paper continued by evaporation of filtrate (Karimi et al., 2012).

Determination of Total Phenols

The total phenolics content was determined using the Folin Ciocalteu reagent and gallic acid as standard, briefly, 200 µL of ethanolic extract were added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µL of saturated sodium carbonate solution (75 g/L) was added. After incubation for 1h at room temperature, the absorbance at 765 nm was measured in triplicate. Gallic acid (0-160 µg/mL) was used for calibration of the standard curve. The results were expressed as microgram gallic acid equivalent (µg GAE)/mg of extract (Boussoulim et al., 2015).

Determination of Flavonoids

Flavonoids were quantified using aluminium chloride reagent AlCl₃ (Abdou Bouba et al., 2010), 2 mL of ethanolic extract was mixed with 1 mL of AlCl₃ (2% in MeOH), after 10 min of incubation at room temperature in the dark, the absorbance was measured at 430 nm, Quercetin (1-40 mg/L) were used as standards for calibration curve. The results were expressed in micrograms of quercetin equivalent per milligram of dried matter

DPPH Scavenging Assay

To test the electron and hydrogen atom donation ability of *Citrus deliciosa*, 1.5 mL of ethanolic extract were added to 0.5 ml of a MeOH solution of DPPH (0.1 mM). Ascorbic acid was used as standard. Absorbance at 517 nm was determined after 30 min, and the percent of activity was calculated (Hemalatha et al., 2010).

ABTS Radical-Scavenging Activity

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical-scavenging activity was measured by direct absorbance measurement of the radical (ABTS^{•+})

that was generated by reacting 5 mL of 7 mM aqueous ABTS solution with 88 µL of a 140 mM potassium persulfate solution (final concentration of potassium persulfate equals 2.45 mM). The solution was stored in the dark for 16 h and then diluted with ethanol to an absorbance of 0.70 ± 0.05 at 734 nm. 10 µL of ethanol extract or trolox was added to 1990 µL of the ABTS^{•+} solution. The mixture, protected from light, was incubated at room temperature; the decrease in absorbance was recorded at 0 and after 6 min at 734 nm (Nenadis et al., 2004).

Acute Toxicity

In order to study acute toxicity or any changes in normal behavior, 06 groups of 07 mice in each, were used in the present experimentation. The control group received normal saline solution (0.9% NaCl), and the treated groups received a single dose of 100, 200 or 400, 800 or 1600 mg/kg body weight (B.W.) of ethanol Extract by oral gavage. No food or water was given up to 4 h after drug treatment. Mice were closely observed during the first 4 hours after the administration of the treatment, and then once daily during the following 14 days (Yacine et al., 2013).

Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical evaluation of differences was carried out by student test using GraphPad Prism version 5.00. Differences were considered significant at P<0.05.

Results

Determination of Total Phenols and Flavonoids Content

The total phenols content is obtained by the Folin-Ciocalteu method (85.62 ± 2.24 µg gallic acid equivalent /mg dried matter). The dosage of flavonoids is formed according to the method of aluminum trichloride (3.02 ± 0.17 µg equivalent quercetin /mg dried matter).

Evaluation of Antioxidant Activity

DPPH assay

The DPPH assay has the great advantage of being easy to implement and not requiring special equipment (just a simple spectrophotometer). The scavenging effects of the *C. deliciosa* leaves on DPPH were examined at different concentrations. It showed IC₅₀ of (252.07 ± 0.02 µg/mL) (Table 1) that is lower than that of ascorbic Acid (1.63 ± 0.03 µg/ml).

ABTS radical-scavenging activity

The free radical scavenging activity of *C. deliciosa* leaves was also determined using ABTS radical, (trolox equivalent antioxidant capacity (TEAC)). It showed IC₅₀ of (398.26 ± 0.01 µg/mL) (Table 2) that is lower than that of Trolox (50.72 ± 0.04 µg/mL).

Acute Toxicity

Extract of *C. deliciosa* didn't demonstrate any behavior changes or mortality in mice at doses of 100, 200, 400, 800 and 1600 mg/kg (B.W.) during the 14 days of the experiment (Table 3).

Table 1. DPPH scavenging activity of *Citrus deliciosa* leaves.

	IC ₅₀ (µg/mL)
Ethanol extract	252.07 ± 0.02***
Ascorbic acid	1.63 ± 0.03

*** P<0.001, the comparison was realized against ascorbic acid.

Table 2. ABTS scavenging activity of *Citrus deliciosa* leaves.

	IC ₅₀ (µg/mL)
Ethanol extract	398.26 ± 0.01***
Trolox	50.72 ± 0.04

*** P<0.001, the comparison was realized against trolox

Table 3. Dose, mortality and abnormal behavior in oral administration of *Citrus deliciosa* extracts to mice.

Dose mg/kg	Death	Abnormal behavior
0	0/7	None
100	0/7	None
200	0/7	None
400	0/7	None
800	0/7	None
1600	0/7	None

Discussion

The total phenols content of *C. deliciosa* leaves is bigger than that found in peel with 92.08 ± 2.50 mg annic acid equivalent/g of the extract's dried weight (Siahpoosh & Javedani, 2012), *Citrus aurantium* L. Bloom (4.55 ± 0.005 mg gallic acid equivalent/g DW) (Karimi et al., 2012), our flavonoids results is similar to that of Thomson Navel leaves (2.67 mg ± 0.05 QE/g DM) (Lagha-Benamrouche et al., 2013).

Flavonoids and phenols compounds are the main antioxidant compounds of fruits and vegetables (Huang et al. 1998; Mohammed et al., 2021; Sevindik et al., 2023).

Concerning DPPH radical-scavenging activity of methanol extract, our result showed that the scavenging activity of *C. deliciosa* leaves is better than that of *C. medica* cv Diamante leaves (IC₅₀ = 502.0 ± 3.01 µg/1108yceu) (Menichini et al., 2011). *Citrus deliciosa* var. *ponkan* peels showed a lower activity (IC₅₀ = 0.6 mg 1108yceu⁻¹) than our extract (Ghasemi et al., 2009), phenols and flavonoids exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron-donating agents (Rice-Evans et al., 1996).

ABTS radical scavenging activity of *C. deliciosa* leaves was also determined, our showed a good scavenging effect against ABTS radical. High correlation was found between total phenols content and antioxidant activity in selected fruits, vegetables and grain products (Velioglu et al., 1998).

Even at a level of 1600 mg/kg B.W. of methanol extract, no death or negative effects were seen in the acute toxicity study, which support the safety of the leaves of *citrus deliciosa*.

Conclusion

C. deliciosa leaves are safe at doses lower than 1600 mg/kg B.W. and have considerable antioxidant activity, which encourages the use of these substances in

phytotherapy, pharmaceutical biotechnology and the food industry for curative or preventive purposes.

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