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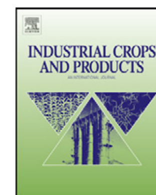

Antioxidant, antibacterial, and antiviral effects of *Lactuca sativa* extracts

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ABSTRACT

Antioxidant, antibacterial and antiviral effects of aqueous and methanol extracts of *Lactuca sativa var longifolia* leaves were investigated. The antioxidant activity was evaluated using the DPPH assay. The effect of the extracts against 5 Gram-positive and 6 Gram-negative bacteria was tested. The antiviral activity was determined against human cytomegalovirus (HCMV) strain AD-169 (ATCC Ref. VR 538) and coxsackie B virus type 3 (CoxB-3) using a cytopathic effect (CPE) reduction assay. The methanol extract had the highest total phenolic contents (235.31 mg CE/g extract). It exhibited a significantly ($p < 0.05$) greater hydroxyl radical-scavenging activity ($IC_{50} = 3.5\ \mu g/ml$) than the aqueous extract (4.1 μg/ml). It was also the most effective extract with the lowest MIC (2.5 mg/ml) against all Gram negative and Gram positive bacteria. Methanol and aqueous extracts exhibited antiviral activity against HCMV and Cox-B3 viruses with IC_{50} of 200 μg/ml.

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1. Introduction

Antioxidants are substances or nutriments in our food that may protect cells from the damage caused by unstable molecules known as free radicals. They delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Gutteridge, 2007). The effects of reactive oxygen species (ROS) on cell metabolism have been well documented in a variety of species. These include not only roles in apoptosis (programmed cell death) but also positive effects such as the induction of host defense genes and mobilization of ion transport systems (Rada and Leto, 2008; Conner et al., 2002).

ROS are produced as a product of normal cellular functioning; excessive amounts can cause deleterious effects (Wondrak, 2009). Memory capabilities decline with age, evident in human degenerative diseases such as Alzheimer's disease, which is accompanied by an accumulation of oxidative damage. So natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases (Lu and Foo, 2002; Tepe, 2008).

Lactuca sativa var longifolia called romaine lettuce or cos lettus, belongs to the botanical family of Asteraceae. It is a variety of lettuce which grows in a tall head of sturdy leaves with a firm rib down the center. Richly flavoured romaine lettuce is firm and crisp enough to be heated and served in warm salads. It is an essential ingredient in Tunisia salad and in Mexican and American caesard salad. Anticonvulsant and sedative-hypnotic effects have been mentioned for the leaves of this plant (Chu et al., 2002). Sayyah et al. (2004) investigated that seeds extract had analgesic and antiinflammatory activity in rats. The aim of the present study was to identify and characterize antioxidant, antibacterial and antiviral activities in *L. sativa var longifolia* leaves extracted by methanol and water solvents.

2. Materials and methods

2.1. Plant material

The herb was purchased from local market from Sousse (Tunisia) in Mars and the plant leaves were authenticated and a voucher specimen was deposited in our laboratory of Faculty of Pharmacy.

2.2. Preparation of extracts

2.2.1. Methanol extract

The leaves herbs were extracted with absolute methanol, in a 1:10 (w/v) ratio of herb to solvent, for 4 h under a continuous reflux set-up in a Soxhlet extractor. After the extraction, the methanol extracts were clarified by filtering through Whatman # 1 filter paper, followed by centrifugation at 14,000 × g for 5 min. All clarified methanol extracts were stored at −20 °C prior to experimentation.

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2.2.2. Aqueous extract

Samples of leaf of lettuce were extracted with water at 80 °C, in a 1:10 (w/v) ratio, for 4 h under continuous shaking. After extraction, the water extracts were clarified by filtering through Whatman # 1 filter paper, followed by centrifugation at 14,000 × g for 5 min. All clarified water extracts were stored at −20 °C prior to experimentation.

2.2.3. Total phenolic contents

The polyphenol content of the extracts was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Zovko et al., 2010), calibrating against catechin standards and expressing the results as mg catechin equivalents per gram of extract (CE)/g extract. Data presented are average of three measurements.

2.2.4. Antioxidant property by DPPH assay

Radical-scavenging activity of the methanol and aqueous extracts of leaf extract were determined according to Yen and Chen (1995). The capacity of herb extracts to scavenge the lipid-soluble DPPH radical, which results in the bleaching of the purple color exhibited by the stable DPPH radical, is monitored at an absorbance of 517 nm. Background interferences from absolute methanol and water were deducted from the activities of the corresponding extracts prior to calculating radical-scavenging activity, on an equivalent phenolic content basis, as follows:

$$\text{Radical scavenging activity}\,(\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

IC_{50} value (mg extract/ml) is the inhibitory concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. Rutin was used for comparison as positive control.

2.3. Antibacterial activity

2.3.1. Microrganisms

The microorganism strains employed in the biological assays are listed in Table 2. Different American Type Culture Collection (ATCC) reference bacteria were used as well as clinical isolates strains.

2.3.2. Determination of the antibacterial activity

The antimicrobial activity of the extracts was evaluated through the determination of the minimal inhibitory concentration (MIC) by the microdilution method (Smania et al., 2006). All extract stock solutions were, prepared by dissolution in 10% dimethyl sulfoxide (DMSO). The plant extract concentrations tested ranged from 1 to 10 mg/ml. The MIC of each extract was defined as the lowest concentration which inhibited bacterial growth, after incubation at 37 °C between 18 and 24 h. The minimal bactericidal concentration (MBC) was determined by subculture on blood agar at 37 °C between 18 and 24 h.

2.4. Antiviral activity

2.4.1. Cell toxicity assay

The cytotoxic activity was tested against human diploid embryonic lung fibroblasts cells (MRC-5) (Bio-223 merieux, France) using the MTT assay (Polydoro et al., 2004). Cells were seeded in 96-well plates at a concentration of 5×10^4 cells/well and incubated for 24 h at 37 °C in a 5% CO_2 enriched atmosphere. After treatment with various concentrations of each extract (100, 200, 400 and 800 μg/ml), the cells were incubated for an additional 48 h at 37 °C. After that, the medium was removed and cells in each well were incubated with 50 μl of MTT solution (5 mg/ml) for 4 h at 37 °C. MTT solution was then discarded and 50 μl dimethyl sulfoxide (DMSO) were added to dissolve insoluble formazan crystal. Optical density was measured at 540 nm. Data were obtained from triplicate wells. CC_{50} parameter is defined as the concentration (μg/ml) of substrate that causes 50% death of cells.

Table 1
Total phenolic content and DPPH radical-scavenging activity of *Lactuca sativa* extracts.

Extracts	Total phenolic content (mg CE/g)	IC_{50} (μg/ml)
Methanolic extract	235.31 ± 2.1	3.5 ± 0.1
Aqueous extract	95.42 ± 3.2	4.1 ± 0.3
Rutin	–	78.43 ± 0.1

Values are given as means ± SD; total phenolic content (mg CE/g) is given in mg catechin equivalent/g extract; IC_{50} (mg/ml) concentration scavenging 50% of DPPH free radicals.

2.4.2. Titration of viruses

Human cytomegalovirus (HCMV) strain AD-169 (ATCC Ref. VR 538) and coxsackie virus type B3 were used for the antiviral activity. Serial 10-fold dilutions (10^{-1}–10^{-5}) were prepared in MEM containing 2% FCS, were inoculated into confluent cells in quadruplicate wells of 96-well plates and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 3–5 days. When a cytopathic effect (CPE) in the virus-infected cells was observed microscopically, virus titers were expressed as 50% Tissue Culture-Infective Dose ($TCID_{50}$) and were determined by the method of Reed and Muench (1938).

2.4.3. Antiviral activity assay

We have adopted a cytopathic effect (CPE) reduction assay for screening the antiviral activities of the plant extracts (Kujumgiev et al., 1999). In brief to confluent cell monolayers in a 96-well plates, 100 $TCID_{50}$ (50% Tissue Culture-Infective Dose) virus suspension and serial twofold dilutions of crude extracts were added simultaneously. As positive control, cells were infected with the same concentration of virus but without the addition of extract, and as a negative or cell control, only MEM-D was added to the cells. The plates were incubated at 37 °C in a humidified CO_2 atmosphere for 3–5 days. The concentration that reduced 50% of CPE in respect to the virus control was estimated from the plots of the data and was defined as the 50% inhibitory concentration (IC_{50}). The selective index (SI) was calculated from the ratio CC_{50}/IC_{50} (Kujumgiev et al., 1999).

3. Results and discussion

3.1. Total phenolic contents

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant and antibacterial activities. The TPC was expressed in mg catechin equivalent per gram of extract (mg CE/g of extract). The results of the total phenolic content of *L. sativa* extracts were given in Table 1. The methanol and aqueous extracts contained great quantity of phenolic substances. The results indicate that methanol extract had the highest total phenolic contents (235.31 mg CE/g extract). This behavior is probably due to the methanol capacity to solubilize flavonoid components from the *L. sativa* substances detected by the Folin–Denis method. The results presented in Table 1 indicate the efficiency of methanol for the extraction of total phenolic compounds. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups (Peter and Wong, 2006).

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