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Research paper

Chemical composition, antioxidant and evidence antimicrobial synergistic effects of *Periploca laevigata* essential oil with conventional antibiotics

Loubna Ait Dra[a], Malika Ait Sidi Brahim[a], Brahim Boualy[b], Abdellah Aghraz[a], Mustapha Barakate[c], Saadia Oubaassine[d], Mohamed Markouk[a], Mustapha Larhsini[a,*]

[a] Laboratory of Biotechnology, Protection and Valorization of Plant Resources; Phytochemistry and Pharmacology of Medicinal Plants Unit (URAC35 Association unit), Faculty of Sciences Semlalia, Cadi Ayyad University, POB 2390, 40000 Marrakech, Morocco
[b] Laboratory of Chemistry, Polydisciplinary Faculty, Hassan 1er University, POB 145, 25000 Khouribga, Morocco
[c] Laboratory of Biology and Biotechnology of Microorganisms, Faculty of Science Semlalia, Cadi Ayyad University, POB 2390, 40000 Marrakech, Morocco
[d] Laboratory of Coordination Chemistry and Catalysis, Faculty of Science Semlalia, Cadi Ayyad University, POB 2390, 40000 Marrakech, Morocco

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ABSTRACT

Medicinal plants constitute a potential reservoir of several effective antioxidant and antimicrobial agents especially in developing countries were resources are lacking. Compounds of natural origin are more effective, safer and have less side effects. For continuing research on biological properties of Moroccan medicinal plants and identification of new natural antioxidant and antimicrobial agents, the present study was conducted to investigate the chemical composition, antioxidant and antimicrobial effect of the association between some conventional antibiotics and *Periploca laevigata* essential oil obtained by hydro-distillation. The chemical composition was analyzed by a gas chromatography/mass spectrometry (GC/MS) system and a total of 22 compounds were identified. *n*-hexadecanoic acid (14.6%) and 4,4,7a-Trimethyl-5,6,7,7a-tetrahydro-4H-benzofuran-2-one (11.8%) were found to be the main constituents. A moderate antioxidant activity was found as evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method, reducing power and ß-carotene-linoleic acid assays with IC_{50} values ranging from 0.69 to 2.86 mg/ml. The antimicrobial investigation showed an inhibitory effect against seven and four respectively tested bacterial and fungal species. In general, Gram positive bacteria (*S. aureus*, *M. luteus*, *B. cereus* and *B. subtilis*) were found to be more sensitive than Gram-negative ones. The antifungal activities showed that *C. albicans* was the most susceptible. The association between conventional antibiotics (cefexime, ciprofloxacin, gentamicin and fluconazole) and the essential oil showed a high synergistic effect. Combination with gentamicin exhibited interesting synergistic effect against both Gram-positive and Gram-negative bacteria with lowest FIC index values (0.28-0.50), followed by essential oil-ciprofloxacin which is especially active against Gram-positive bacteria (FICi = 0.31-0.38).Whereas, the association essential oil-fluconazole showed a total synergistic effect (FICi = 0.25-0.37) for yeasts except *C. albicans*. The results showed that the essential oil possesses compounds with antimicrobial and antioxidant properties and a good synergistic effect in association with antibiotics. The present study suggests that the essential oil can be considered as new and potential source of natural antioxidant and antimicrobial agents.

1. Introduction

The toxicity of synthetic antioxidants used for stabilization of food products (Ito et al., 1986) and the resistance of many microbial strains to the antibiotics causes a really problem (Goossens, 2009). To overcome these problems, the use of medicinal plants with antioxidant and antimicrobial properties is one of the most interesting paths to explore. In recent years, essential oils (EOs) and plant extracts have been increasingly enticing the attraction due to the beneficial therapeutic values and to the potential of these extracts as a source of natural antioxidants and biologically active compounds, such as antibacterial, antifungal and insecticidal substances (Celiktas et al., 2007).

Indeed many studies have shown significant antibacterial activities of EOs of some medicinal plants against many resistant microbial strains (Bozin et al., 2006; Bounatirou et al., 2007; Vahdani et al., 2011). Also EOs and the components of essential oils were reported to have antioxidant activity (Alitonou et al., 2012).

Periploca laevigata Aiton (Asclepiadaceae), locally known as “el

* Corresponding author at: Cadi Ayyad University, Faculty of Sciences Semlalia, Department of Biology, POB 2390, 40000 Marrakesh Morocco.
E-mail address: larhsini@uca.ma (M. Larhsini).

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hallaba" is a Mediterranean medicinal shrub widely distributed in the Sahara area and found in North Africa (from Morocco to Egypt) (Ghrabi, 2005). In traditional Moroccan medicine, particularly in the Western Sahara, the decoction of the seeds is used as local analgesic frictions in rheumatism and for the treatment of arthrosis (Bellakhdar, 1997). In the literature, the plant was reported to treat headaches, diabetes, rheumatism and gastric ulcer (Ghrabi, 2005; Hajji et al., 2010). In Tunisia, this shrub is used as a food ingredient (tea) and as in herbal preparation (Hajji et al., 2010).

To overcome the resistance to antibiotics and to enhance the efficacy of natural antimicrobial products, the combination of conventional antibiotics with natural antimicrobial agents is one of the promising strategies (Hemaiswarya et al., 2008) and constitutes an alternative against infectious diseases caused by resistant microorganisms. Several studies indicated a synergistic interaction between EOs and antibiotics (Nazer et al., 2005; Rosato et al., 2009; Fadli et al., 2012).

Previous studies reported the chemical composition and antioxidant activities of different solvent extracts from *P. laevigata* (Hajji et al., 2009). But, to the best of our knowledge, there are no available reports on biological activities of the essential oil of the species and the synergistic interaction between antibiotics and EO obtained from leaves. Therefore, the aim of the present work was to determine the chemical composition of essential oil extracted from leaves of *P. laevigata* by GC–MS. In vitro antioxidant, antibacterial and antifungal synergistic effects between classical antibiotics and *P. laevigata* EO were also investigated.

2. Materials and methods

2.1. Plant material and isolation procedure

P. laevigata was collected during the vegetative stage, in April 2014, from Essaouira region (South-West of Morocco, N 31.55341°/ W009.62484). The identification of the plant material was done in the laboratory of Biotechnology, Protection and Valorization of the Plant Resources by Prof. Abbad. A voucher specimen (PEL 064) was deposited in the Herbarium of the laboratory BIOTEC-VRV (Faculty of Sciences Semlalia, Marrakech).

Leaves were separated from branches, and then air-dried in the shade at room temperature and subjected to the hydro-distillation for 4 h using a Clevenger-type apparatus. The extraction of the essential oil was repeated many times (150 g). The essential oil was stored in darkness at 4 °C until use.

2.2. Gas chromatography/mass spectrometry (GC/MS) analysis

The essential oil was analyzed using GC/MS method (Shimadzu GC/MS-16A gas chromatograph instrument), equipped with a quadruple detector and DB5 capillary column (25 m × 3 mm). The injector and detector temperatures were set at 250 °C. The column temperature was programmed from 40 to 200 °C at 10 °C/min. 1 µl of oil was injected into GC–MS instrument for analysis. Helium gas was used as carrier gas at flow rate of 1 ml/min. The chemical components of essential oil were identified by comparing the retention indices (RI) and mass fragmentation patterns with those on the stored NIST library (National Institute of Standards and Technology).

2.3. Antioxidant activity

2.3.1. DPPH free radical-scavenging activity

The stable free radical 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used to evaluate the free radical-scavenging activity of *P. laevigata* EO, according to the protocol described by Burits and Bucar (2000) and Sahin et al. (2004). Fifty micro-liters of various concentrations of the essential oil in methanol were added to 2 ml of a 60 µM methanol solution of DPPH. After a 20 min incubation period in the dark at room temperature the absorbance of the samples was measured using a spectrophotometer at 517 nm. A blank containing the same amount of methanol and DPPH was used as negative control. Butylated hydroxytoluene (BHT) and quercetin were used as positive controls. The inhibition of the DPPH in percent (%) was calculated in the following way:

$$I\ \% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentages against sample concentrations. The test was carried out in triplicate and the results were expressed as mean ± standard deviation (SD).

2.3.2. Reducing power assay

The reductive potential was determined according to the method of Oyaizu (1986). Briefly, 1 ml of different concentrations of samples were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe\ (CN)_6$] (1%). The mixture was then incubated at 50 °C for 20 min. The reaction was stopped by adding 2.5 ml of trichloroacetic acid 10% (TCA) and the mixture was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Butylated hydroxytoluene and quercetin were used as positive controls.

The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph by plotting the absorbance at 700 nm against the corresponding sample concentration. The experiment was performed in triplicate and statistical analysis was done in term of mean ± SD.

2.3.3. ß-Carotene/linoleic acid bleaching test

The ß-carotene bleaching test was performed as described by Miraliakbari and Shahidi (2008) with slight modification. A stock solution of ß-carotene and linoleic acid was prepared with 0.5 mg of ß-carotene in 1 ml of chloroform, 25 µl of linoleic acid and 200 mg of tween 40. The chloroform was completely evaporated under vacuum at 50 °C and 100 ml of distilled water were then added to the flask with vigorous shaking to form a clear yellowish emulsion. Then, 2.5 ml of this emulsion were transferred into a series of test tubes containing 350 µl of various concentrations of the samples. Immediately after the addition of the emulsion, the test tubes were incubated in a hot water bath at 50 °C for 2 h. A blank containing all reagents except the test compound was used as negative control. After incubation, the absorbance values were measured at 470 nm. Antioxidant activities (inhibition percentage I%) of the samples were calculated using the following equation:

$$I\ \% = \frac{A_{sample2h} - A_{blank2h}}{A_{initial\ blank} - A_{blank2h}} \times 100$$

Where $A_{sample2h}$, $A_{\ blank2h}$ are the absorbance of the test compound and control respectively after 2 h assay and $A_{initialblank}$ is the absorbance of control at the beginning of the experiment.

The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against the sample concentrations. Quercetin and BHT were used as reference compounds. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

2.4. Antimicrobial activity

2.4.1. Microbial strains

Microorganisms used to study the antimicrobial activity of *P. laevigata* EO are seven bacteria; *Staphylococcus aureus* (209 PCIP 53156),