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Chemical analysis and biological activities of *Populus nigra*, flower buds extracts as source of propolis in Algeria

Nadjet Debbache, Dina Atmani, Djebbar Atmani*

Laboratoire de Biochimie Appliquée, Universté Abderrahmane Mira, Bejaia 06000, Algeria

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ABSTRACT

Seven fractions obtained by a selective extraction procedure from *Populus nigra* flower buds were examined for their antimicrobial and antioxidant properties, *in vitro*, using radical scavenging and inhibition of lipid peroxidation assays. The aqueous chloroform fraction that exhibited the best antioxidant activity was investigated for anti-inflammatory activity *in vivo*. Quantification of total phenolic compounds in these fractions was equally carried out.

The results obtained with the aqueous fraction of chloroform were particularly interesting, being the most effective on DPPH (IC_{50} = 24.61 μg/mL), ABTS (IC_{50} = 17.09 μg/mL), NO (IC_{50} = 9.52 μg/mL), HOCl (IC_{50} = 187.90 μg/mL) and OH^- (IC_{50} = 113.79 μg/mL) radicals in addition to exerting a high inhibition on both xanthine oxidase (XO) activity and lipoperoxydation (IC_{50} = 60.7 and 24.93 ± 1.22 μg/mL, respectively). Moreover, the same fraction (200 mg/kg) has equally demonstrated a potent anti-inflammatory potential (62.36%) in carrageenan-induced mice paw edema model. On the other hand, it is rather the organic extracts that exhibited the highest antimicrobial activity against tested microorganisms. Hence, these results suggest that *P. nigra* is a promising source of bioactive compounds that can be exploited as antioxidants and bactericidal in food products as well as in pharmaceutical therapeutic use.

1. Introduction

In the wake of the propagation of diseases caused by oxidative stress such as cancer, Alzheimer and inflammatory related pathologies, it has become urgent to find natural non-toxic antioxidants devoid of adverse side effects. That is why researchers are accelerating the pace in the field of ethnopharmacology.

Free radicals which induce oxidative stress are generated by several enzymes like xanthine oxidase involved in various inflammatory disorders (Granger et al., 1986). Moreover, their excessive formation by activated neutrophils implicates them in acute inflammation (Halliwell et al., 1988). Thus, antioxidants which prevent their formation are expected to suppress or at least attenuate the inflammatory reaction (Hazra et al., 2010). Anti-inflammatory drugs are reputed for their undesirable side effects. In this context, the quest for sources of novel antioxidants as substitutes is being pursued. Moreover, as the etiology of some inflammatory conditions is microbial, combating microorganisms is one way of eradicating the inflammatory response. The problem of microbial resistance to usual medications is growing which makes its use in the future uncertain, fueling the search for new, appropriate and efficient antimicrobial drugs of natural origin.

It is well documented that in the temperate zone all over the world, the main source of bee glue is the resinous exudate of *Populus nigra* buds or propolis (Bankova et al., 1995). The biological activities of the latter, which have attracted both commercial and scientific interests, are attributed to the same plant-derived substances found in the original source (*P. nigra*) (Bankova, 2005). In addition, the use of these substances in food and food supplements is considered as safe by the United States Food and Drug Administration (FDA) and the Scientific Committee (SC) of European Food Safety Authority (EFSA) (EFSA Journal, 2012), meaning that they are devoid of toxic side effects characteristic of synthetic antioxidants.

Algeria harbors a large variety of medicinal and aromatic plants which were proved valuable pertaining to their antioxidant properties (Djeridane et al., 2006; Atmani et al., 2009; Berboucha et al., 2010). Traditional uses of *P. nigra* flower buds in the treatment of many inflammatory-related conditions such as arthritis, bronchitis and respiratory tract diseases are wide.

Previous investigations indicated that radical scavenging activity of plant extracts was dose- and radical/method specific (Hazra et al., 2010). Also, because of the complex nature of phytochemicals present in extracts and owing to the complexity of oxidation–antioxidation processes, antioxidant activities of plant extracts cannot be evaluated by a single method. To provide a comprehensive picture of the antioxidant nature of a given extract, commonly accepted assays, including enzymatic and non-enzymatic methods, were used to evaluate the total antioxidant

* Corresponding author. Tel.: +213 34214762.
E-mail address: djatmani@yahoo.com (D. Atmani).

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effects of plant extract (Prabhakar et al., 2006; Parmar and Kar, 2009).

Few data have been published on the biological activities of the buds extracts of *P. nigra* and the corresponding propolis. In this study, the antioxidant capacity of several extracts of *P. nigra* flower buds was investigated using several radicals, including hydrogen peroxide (H_2O_2), hydroxyl (OH^-), nitric oxide ($NO^\bullet$), hypochlorous acid (HOCl), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) and (2,2-diphenyl-1-picrylhydrazyl) DPPH. The aqueous fraction derived from chloroform which exhibited the best antioxidant potential was further investigated for anti-inflammatory activity *in vivo*. Anti-bacterial and anti-fungal activities of plant extracts were also assessed. Furthermore, total phenols, flavonoids and tannins in plant extracts were quantified.

2. Materials and methods

2.1. Chemicals

All the reagents and chemicals were purchased from Sigma, represented by Prochima Sigma, Tlemcen, Algeria.

2.2. Plant material

Fresh flower buds of *P. nigra* were collected in Spring in pollution-free areas and far from towns and plantations in the forest of Tizi Neftah, Province of Amizour, Department of Bejaia (Algeria). The plant was identified in the Laboratory of Botany, University of Bejaia (Algeria).

2.3. Bacterial and fungal strains

Gram-negative strains *Escherichia coli* (O111B4), *Pseudomonas aeruginosa* (165RIS) and *Klebsiella pneumoniaea* (TE47), Gram-positive strains *Staphylococcus aureus* (209P) and *Bacilus subtilis* (ATCC635335) and pathogenic fungi *Aspergilus niger* (939N) and *Fusarium polyferatum* were tested. Except for *P. aeruginosa* (165RIS) and *S. aureus* (209P) which were donated by Pasteur Institute (Algiers, Algeria), all strains were generously provided by Professor Aziz TOUATI (University of Bejaia).

2.4. Laboratory animals

Albino mice of either sex weighing around 23 g were purchased from Pasteur Institute (Algiers, Algeria). Animals were provided with standard food and water *ad libitum* and were maintained at a constant temperature of $23 \pm 1\,^\circ C$, relative humidity of $65 \pm 5\%$ and 12/12 h light/dark cycle. They were weighed, randomized into groups ($n = 8$), and kept for 2 weeks to acclimatize to laboratory conditions. Experiments were conducted in strict compliance with internationally accepted principles for laboratory animals (Directive of the European Council 86/609/EC).

2.5. Plant material and extraction procedure

The fresh flower buds were air-dried in the shade and ground to a fine powder (diameter 63 μm). Phenolic compounds were extracted using previously described procedure (Atmani et al., 2009). Ground powder was macerated in ethanol (1:3; w/v) to obtain a crude extract which was dried and subjected to a partition in ethyl acetate and water (1:3:1; w/v/v) to yield two separate fractions (the organic fraction and the aqueous fraction of ethyl acetate). Two equal amounts of the organic fraction of ethyl acetate were further fractionated using hexane and water (1:3:1; w/v/v) on one hand and chloroform and water (1:3:1; w/v/v) on the other hand. Obtained organic and aqueous fractions were dried to be used for different experiments.

2.6. Chemical analysis

Total phenolic quantification of plant extracts was carried out using the Folin–Ciocalteu reagent and catechin as standard (Lowman and Box, 1983).

The $AlCl_3$ method described by Maksimovîc et al. (2004) was employed for the determination of flavonoid content of the sample extract. Concentrations of flavonoids were deduced from a standard curve and expressed as mg of quercetin equivalent/gram of extract.

Tannins were determined by precipitation using the bovine serum albumin (BSA) method developed by Hagerman and Butler (1978). Concentrations of tannins were expressed as mg of tannic acid equivalent per gram of extract.

2.7. Antioxidant activity in vitro

In all the tests, radical scavenging activity was calculated as follows: % radical scavenging activity = $A_0 - A_1/A_0 \times 100$, where A_0 is the absorbance of control solution and A_1 is the absorbance in the presence of plant extract. The most active extracts (% inhibition ≥ 50%) were assayed for half-inhibitory activity (IC_{50}) and assayed for anti-inflammatory activity *in vitro* and *in vivo*. IC_{50} was determined from a graph in which scavenging activity was plotted against varying concentrations (25–125 μg/mL) of extract using a linear regression curve.

2.7.1. Diphenylpicrylhydrazil (DPPH) radical scavenging activity

Radical scavenging activity of *P. nigra* flower buds extracts against the stable synthetic radical, diphenylpicrylhydrazil (DPPH), was determined spectrophotometrically using the method of Masuda et al. (1999). Fifty microliters of a solution of DPPH (5 mM) dissolved in methanol were added to 4.9 mL of test sample (100 μg/mL) or reference compound. After 30 min of incubation at room temperature, the absorbance was recorded at 517 nm.

2.7.2. ABTS scavenging activity

Antioxidant capacity was measured based on the scavenging of $ABTS^{\bullet+}$ radical cation (Re et al., 1999). Solutions of ABTS (7 mM) and potassium persulfate (2.45 mM) were mixed and incubated in the dark at room temperature for 12–16 h. The product was diluted in ethanol for optimal absorption ±0.7 at 734 nm. The reduction between $ABTS^{\bullet+}$ and test sample (100 μg/mL) was monitored by a decrease in absorption at 734 nm during 30 min. Caffeic acid, quercetin and BHA were used as standards.

2.7.3. Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide (H_2O_2) was determined using the method of Ruch et al. (1989). Briefly, 2 mL of the test sample or reference compound (100 μg/mL) dissolved in methanol were added to 2 mL of a H_2O_2 solution (40 mM in phosphate buffer, pH 7.4). After an incubation of 10 min, the absorbance was read at 230 nm.

2.7.4. Hydroxyl radical scavenging activity

Hydroxyl radical (OH^-) scavenging activity was assayed as described by Halliwell and Gutteridge (1985). Hydroxyl radical was generated in the presence of Fe^{3+}, EDTA, ascorbate and H_2O_2 and monitored by evaluating hydroxyl radical-induced deoxyribose degradation. The reaction mixture contained, in a final volume of 2 mL, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4–KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 μM); EDTA (100 μM); H_2O_2 (1.0 mM); ascorbic acid (100 μM) and (100 μg/mL) of the test sample. After incubation for 1 h at 37 °C, 0.5 mL of the reaction mixture was added to