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Antioxidant and antimicrobial activities of *Lycium shawii* fruits extract

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ABSTRACT

Crude extracts from the fruit of *Lycium shawii*, a plant collected from the south of Tunisia, were screened for their *in vitro* antioxidant, antimicrobial and antifungal activities. The dried fruits were extracted with EtOH and an aqueous suspension of the obtained EtOH extract was partitioned successively with $CHCl_3$, CH_2Cl_2, EtOAc and n-BuOH, leaving residual water extract. Total phenolic content of extracts from these fruits were also determined. β-Carotene bleaching assay and Folin–Ciocalteu reagent were used to determine total antioxidant activity and total phenols of fruit extracts. Total phenolic and flavonoid contents varied from 100 to 377 mg GAE/g DW and 3.3–110.6 mg quercetin/g DW, respectively. Several extracts showed high antioxidant capacity and an antimicrobial activity against different strains. This is, to our knowledge, the first report on the detail chemical composition, antioxidant and antimicrobial activities of *L. shawii* extracts. The results provided evidence that the studied fruit might indeed be potential sources of natural antioxidant and antimicrobial agents.

1. Introduction

The interest in many traditional herbs and plant food supplements, as a source of nutritional antioxidants, is due to our increasing knowledge of the role of antioxidants and free radicals in human heath and disease [1–3]. Plants are rich in a wide variety of secondary metabolites and their antioxidant, anti-inflammatory, antimicrobial or cytotoxic properties are used to developed drugs, dietary supplements and cosmaceuticals [4,5]. The phytochemical constituents of the plant extracts are the major basis of pharmacological activities of medicinal plants [6] whereas flavonoids are antioxidants [7] and minerals play significant roles in many processes taking place in living systems.

The flavonoid class is the most prominent and the most important plant antioxidant. Therefore, it is of great interest to carry out a biochemical screening of these plants in order to validate their use in folk medicine and to reveal their biologically active principles by isolation and characterization of their constituents.

Lycium shawii (Awsaj) is a thorny perennial shrub that belongs to the Solanaceae family [8]. It grows along sandy stone ridges. The flowers are produced during March–April in its natural environment and throughout the year in irrigated soil. In Tunisia, this plant is found in arid regions. It is usually present in depression areas and floodplains, and has been the centre of the country (Kairouan) to the far south (and Remada Dehibet). It also exists in the islands (Kerkennah, Djerba, Kneiss) [9]. The aerial parts shoots and flowers of the plant *L. shawii* is used in the form of dry powder by traditional healers as anti-diabetic [10] and hypotensive agent [11]. In fact, recent experiments have indeed demonstrated that *L. shawii* extract possesses hypoglycemic activity *in vivo* [12]. *L. shawii* extract has also been reported to possess antiplasmodial antitrypanosomal activity [13]. The roots of *L. shawii* are boiled and the decoction is used to treat sores in the mouth, coughs, backache and administered internally to cure tick fever in livestock. Leaves are used to treat constipation and stomach ache. It provides honey for wild bees and food and shelter for wild birds and animals [14]. Previous studies investigated beneficial effects of different parts of *L. shawii* but, to our knowledge, the first report on the detail chemical composition, antioxidant antimicrobial and antifungal activities of *L. shawii* extracts. The results provided evidence that the studied fruit might indeed be potential sources of natural antioxidant and antimicrobial agents.

2. Materials and methods

2.1. Plant materials

Fruits of *L. shawii* were collected in September 2009 from a region of south of Tunisia. These endemic species were

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authenticated by Professor Mohamed Chaïb [9]. The plant grows as a spreading shrub, with tiny branches with violet purple flowers and produces orange red fruits called Sakkoum or Awsag.

2.2. Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), β-carotene, linoleic acid, ascorbic acid, Folin–Ciocalteu reagent, Tween-20 and sodium carbonate were purchased from Sigma–Aldrich, USA). All solutions were freshly prepared in distilled water. The solvents used for extraction and partition were from Sigma–Aldrich.

2.3. Basic composition of L. shawii

The basic components in *L. shawii* fruits, including moisture, fat and ash were determined according to the official method AOAC [15]. For mineral composition, the *L. shawii* fruits were incinerated into ash, dissolved in 1 ml of 2 M HCl and diluted to 100 ml with deionised water. The resulting solution was used for the determination of K, Na, Mg, Ca, Fe, Zn and Mn using atomic absorption spectrophotometer (Zenit series).

2.4. Extraction procedures

2.4.1. Infusion

10 g of fresh fruit of *L. shawii* is being infused in boiled distilled water for 15–20 min. The whole is filtered. The infusion is repeated 3 times. The filtrate is then freeze-dried: the fraction denoted infusion: Inf.

2.4.2. Maceration with ethanol

100 g of fresh fruit and 200 ml of 80% ethanol are introduced into an Erlenmeyer flask. The whole is macerated for 48 h at 40 °C with stirring before filtration. The filtrate was recovered while the residue is subjected to two further extractions with the same solvent. The residue is exhausted and the filtrate obtained subject to evaporation of ethanol and then freeze-drying: the aqueous phase denoted (phase V).

2.4.3. Fractional extraction with solvents of increasing polarity

L. shawii fruits were dried and finely ground using a homogenizer and extracted three times with 80% of ethanol, for 24 h. Mixture was filtered through Whatman filter paper. The extract was subjected to successive appropriate solvent with increased polarity (hexane, dichlomethane, ethyl acetate and n-butanol) for 48 h at a temperature not exceeding the boiling point of the solvent under a continuous reflux setup in a Soxhlet extractor.

The butanoic extract IV has two phases:

- A solid phase was rinsed with ethanol (we obtained a precipitate which is soluble in water). This fraction is denoted VI.
- A yellowish liquid phase was evaporated to remove all traces of organic solvent, and then lyophilized. We noted that this phase does not freeze-dried and have an oily appearance: this fraction is denoted VII.

The combined extracts for each solvent were filtered through a Whatman filter paper and dried at around 40 °C under reduced pressure. Residues of *L. shawii* fruits extracts were dissolved in 50 mg/ml ethanol/water (20/80, v/v) before use in antimicrobial assay.

2.5. Determination of total sugar

The total sugar of *L. shawii* samples was determined using a phenol–sulphuric acid method as described by Dubois et al. [16]. A 0.2 ml of crude solution was mixed with 0.2 ml of 5% phenol solution and 1 ml of concentrated sulphuric acid was added, after which the mixture was shaken for 30 min and the absorbance was measured at 490 nm using a spectrophotometer (Shimadzu UV-Visible). The total sugar was calculated based on the standard curve of glucose.

2.6. Determination of protein

The protein content in *L. shawii* sample was determined using a Bradford method [17]. The protein content was calculated based on the BSA standard curve and was expressed as μg/ml equivalent.

2.7. Determination of total phenolic content

Total phenolic contents were evaluated with Folin–Ciocalteu's phenol reagent [18]. 5 ml of the extract solution was mixed with 5 ml Folin–Ciocalteu reagent previously diluted with water (1:9, v/v). After 5 min, 4 ml of 7% Na_2CO_3 solution was added with mixing. The tubes were vortexed for 5 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm using the Hewlett Packard UV-vis spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where y is the absorbance and x is the concentration.

2.8. Total flavonoids

The content of flavonols was determined as described by Miliauskas et al. [19]. The quercetin calibration curve was prepared by mixing 2 ml of (0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml) quercetin ethanolic solution with 2 ml of (20 g/l) aluminium trichloride solution and 6 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20 °C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of quercetin solution. All determinations were carried out in three replications. The content of flavonols, as quercetin equivalents (QuE) was calculated by the following formula: $X = (CV)/m$; where X: flavonols content (mg QuE/g plant extract); C: the concentration of quercetin solution (mg/ml), established from the calibration curve; V and m: the volume and the mass of plant extract in (ml) and (g) respectively.

2.9. Antioxidant activity

2.9.1. Assay of DPPH scavenging activity

The DPPH radical-scavenging activity of the test extracts was examined [20]. Different concentrations (0.025–0.5 μg/ml) of each extract were added, at an equal volume, to methanolic solution of DPPH (100 μM). The mixture was allowed to react at room temperature in the dark for 30 min. Vitamin C and rutin were used as standard controls. Three replicates were made for each test sample. After 30 min, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates.