First Phytochemical Characterization and Essential Oil Analysis of the Traditional Catalan Wild Salad: “Coscoll” (Molopospermum peloponnesiacum (L.) Koch)

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Abstract

“Coscoll” (Molopospermum peloponnesiacum (L.) Koch) whose stems are traditionally consumed raw in salads in Catalonia is associated in oral tradition with many virtues as digestive, purifying, exciting, antioxidant and hematocathartic activities. However, stem composition and biological activity had never been studied. Nutritive values of plant material were determined by official methods and constituents from essential oil of Molopospermum peloponnesiacum stems were characterized for the first time in this study. The main constituents were dialpiol (60.1%) and 3-carene (15.2%). Moreover, total polyphenol content using Folin-Ciocalteu method, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity were determined. Molopospermum peloponnesiacum stem total polyphenol content was 101.0 ± 10.0 mg/100 g fresh weight (Gallic Acid Equivalent) and DPPH radical scavenging activity, relatively high, was close from spinach one. Thus, these first results reveal the potential beneficial properties of this plant, in relation with its traditional use in Catalonia.

Keywords: Molopospermum peloponnesiacum; stems; Nutritive values; Essential oil analysis; Total polyphenol content; DPPH radical scavenging activity

Introduction

Molopospermum peloponnesiacum (L.) Koch is a perennial plant endemic to mountainous area of Southern Alps and Pyrenees. Molopospermum is a monotypic genus belonging to the Apiaceae family and Apioideae subfamily. Its phylogenetic classification is not clearly defined [1-3] but most recent phylogenetic study based on chloroplast genome trnQ-trnK region analysis seems to integrate Molopospermum into the clade of Annesorhizeae [4].

In Pyrenees, where the plant is also name “Coscoll” in Catalan language, M. peloponnesiacum young shoots are traditionally eaten raw in salads. Oral tradition extols many virtues of coscoll, as digestive, purifying, and exciting activities. It has been collected throughout generations in this country and cures are traditionally made from coscoll for its hematocathartic properties [5].

M. peloponnesiacum contains a large amount of volatile compounds and gives off a strong odor. Root and fruit essential oil have been identified in literature, containing mainly 3-carene, trimethylbenzoic acids, and dialpiol as major compounds [6-7]. However, to our knowledge, stem composition or biological activity had never been characterized. This paper thus deals with the chemical characterization of stems. We described nutritive characterization and essential oil analysis. In addition, we determined stem polyphenol content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity in order to identify potential beneficial properties in this plant material traditionally consumed.

Materials and Methods

Plant material

Plant material was collected in May 2011 in Mantet Col (Pyrénées-Orientales, France) during inflorescence emergence stage, as stems are traditionally consumed. All plants specimen were 3-4 years old and were identified by Cédric Bertrand based on morphological description.

A voucher specimen was deposited at the Herbarium of the University Claude Bernard Lyon1, city of Villeurbanne, France, under the name “Collection Piola” and collector number 4.

An aliquot was dried and conserved for phytochemical analysis. Dry mass stems was 15% w/w.

Global characterization of plant material

Protein dosage was determined according to the AOAC Official Method 984.13 [8]. Lipid amount was determined using the ISO 1443 method [9] and lipid profile was analyzed according to the AOCS Ce 1h-05 method [10]. Soluble sugar content was determined after oximation and silylation then analyzed by gas chromatography with a 30 m SILSCB column coupled with FID detection, using xyitol as internal standard. Mineral amount was evaluated according to the AOAC Official Method 923.03 [11]. Fiber dosage was determined according to the AOAC Official Method 985.29 [12]. Energetic values were calculated according European regulation 1169/2011 [13]. All essays were done on a mix of stems from six different plants.

Essential oil distillation

100 g fresh stem parts from six Molopospermum peloponnesiacum specimen from the same mountain area were directly subjected to hydrodistillation for 2 h. Volatile oil was collected by extraction using hydrodistillation method.
heptane and aqueous phase was separated with anhydrous sodium sulfate. Extracted oils were stored in glass vessels at -20°C and protected from light. Essential oil extraction yield was 0.047% w/w fresh stems.

**GC-FID and GC-MS analysis of the essential oil**

Essential oil components were identified using gas chromatography with Thermo Scientific Focus GC system coupled with DSIQ II system mass detector. Analysis was realized with a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 μm, 5%-phenyl-arylene-95%-dimethylpolysiloxian, Phenomenex, Torrance, California, US). Column head flow was set at 1 ml/min, using helium as carrier gas. Column temperature was programmed as follows: 80°C for 3 min, 80°C to 110°C (2°C/ min), 110°C to 240°C (5°C/ min), 240°C to 290°C (10°C/ min) and 290°C for 3 minutes. Total run time was 52 min.

Injector temperature was maintained at 280°C, and injection volume was 1.0 µl in split mode with a 10 ml/min split flow. Transfer line temperature was 300°C. Electron multiplier voltage was set to 1330 V by automatic tuning. Mass spectra were recorded at 70 eV from 45-325 with Thermo Scientific Focus GC system coupled with DSQ II system mass detector. Analysis was realized with a HP-5MS column (30 m × 0.25 mm i.d. x 0.25 μm, 5%-phenyl-arylene-95%-dimethylpolysiloxian, Phenomenex, Torrance, California, US). Column head flow was set at 1 ml/min, using helium as carrier gas. Column temperature was programmed as follows: 80°C for 3 min, 80°C to 110°C (2°C/ min), 110°C to 240°C (5°C/ min), 240°C to 290°C (10°C/ min) and 290°C for 3 minutes. Total run time was 52 min.

**Preparation of alcoholic extract**

50 g of dry stems were extracted three times with 500 ml of ethanol for 15 minutes in an ultrasonic bath, then filtered and dried under vacuum. Extraction yield was 11.4% (w/w dry stems).

**Determination of total phenol contents**

Total polyphenol content was determined by Folin-Ciocalteu phenol method as described by Singleton et al. [19] with some modifications. 175 µl of appropriately diluted sample or gallic acid standard were added to 25 µl of Folin-Ciocalteu phenol reagent and 50 µl of 20% (w/v) Na 2CO3 solution and mixed. After incubation for 30 minutes at room temperature, absorbance was measured at 752 nm versus a prepared blank containing solvent instead of gallic acid or sample. The calibration equation for gallic acid was y=0.0395 x +0.0008 (R²=0.9993).

Total phenolic content was expressed as mg gallic acid equivalent (GAE)/100 g of fresh plant. Mean and standard deviation (n=3) were calculated.

Folin reagent and gallic acid were purchased from Sigma-Aldrich (Saint-Louis, Missouri, USA).

**Determination of DPPH radical scavenging activity**

*M. peloponnesiacum* stems ethanolic extract free radical scavenging activity was determined in 96-well microplates using the DPPH method [20] with some modifications. 20 µl ethanol solution containing a different concentration of *M. peloponnesiacum* stems extract was added to 200 µl of freshly prepared 2,2-diphenyl-1-picrylhydrazyl methanol solution (0.2 mM) (Sigma-Aldrich, Saint-Louis, Missouri, United States). Ethanol was used as the control. After 60 minutes of incubation at room temperature in the dark, absorbance was measured at 515 nm using a microplate reader Aviso, Sirrus HT (Ebersberg, Germany). Vitamin-C was used as standard (Supelco, Bellefonte, Pennsylvania, US).

Standard curves for assay was obtained by measuring the DPPH scavenging activity of 1, 5, 10, 25, 50 mg vitamin C/L. DPPH scavenging activity was expressed as mg vitamin C equivalent (VCE)/100 g of fresh plant. Mean and standard deviation (n=3) were calculated.

**Results and Discussions**

**Global characterization of Molopospermum peloponnesiacum stems**

Content of soluble sugars, fibers, protein, ashes and lipids in fresh and dried stems are presented in Table 1. Soluble sugars, fibers and protein values were particularly high in *Molopospermum peloponnesiacum* stems whereas lipid amount was very low.

**Essential oil analysis**

The essential oil composition is listed in Table 2. Main compounds identified in stem essential oil were dillapio (60% relative content) and 3-carene (15% relative content). Stem essential oil composition was very close to the root essential oil composition determined in literature for dillapio chemotype [7]. Dillapio is a well-known phenylpropenoid, extracted from essential oils of several plants as matico (*Piper aduncum*), parsley (*Petroselinum crispum*), pepper elder (*Piperomia pellucida*) or dill (*Anethum graveolens*) [21-24]. This compound presents in vitro antiemishmanial, gastropotective and anti-inflammatories activities [21-23]. Its structure, close to phenylisopropylamine structures could suggest a possible psychotropic activity as tonic or exciting [25].

**Determination of total polyphenol content and DPPH radical scavenging activity**

Total polyphenol content and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of *M. peloponnesiacum* stems are shown in Table 3. Total polyphenol content determined for the *peloponnesiacum* stems expressed in Gallic Acid Equivalent (GAE) was 101.0 ± 10.0 mg GAE/100 g fresh weight. DPPH radical scavenging capacity expressed in vitamin C equivalent (VCE) was 77.7 ± 5.59 mg VCE/100 FW. *M. peloponnesiacum* stem has relatively strong DPPH radical scavenging activity.

| Analyse     | Unit          | Value*     |
|-------------|---------------|------------|
| Water       | g/100g FW     | 85         |
| Soluble sugar| g/100g FW     | 5.56 (37.0)|
| including simple sugar | g/100g FW | 3.46 (23.1)|
| Fiber       | g/100g FW     | 4.66 (31.1)|
| Protein     | g/100g FW     | 3.28 (21.9)|
| Ash         | g/100g FW     | 1.46 (9.7) |
| including sodium | mg/100g FW | 3.9 (26)   |
| Lipid       | g/100g FW     | 0.04 (0.3) |
| FA saturated| g/100g FW     | 0.02 (0.1) |
| FA monosaturated | g/100g FW | 0.00 (0.0) |
| FA polysaturated | g/100g FW | 0.03 (0.2) |
| Energetic value | Kcal/100g FW | 45 (300)188 |

FA : Fatty acids ; FW : fresh weight ; DW : Dry weight.

* Values were obtained according official dosage methods; essays were done on a mix of stem from six different plants

**Table 1**: Nutritive composition of *Molopospermum peloponnesiacum* stems.
Retention indices were calculated using n-alkanes. Percentage are calculated from characterizing this part of the plant that is traditionally consumed and to characterize this part of the plant that is traditionally consumed and to determine retention indices from literature. FID data from literature for spinach (71 mg VCE/100 g FW) and nearly twice that of "Catalan Wild Salad: "Coscoll". For comparison, this result is close to that obtained in experiments have been performed using the "Biodiversité et Biotechnologies Marines" (Bio2Mar) facilities at the University of Perpignan.

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Conclusion

In this study, Molopospermum peloponnesiacum stems composition was characterized for the first time. We looked at its nutritive values and antioxidant capacity. Our results indicate a large amount of soluble carbohydrates, fibers and protein in the stems and an essential oil containing dialloxiol and 3-carene as major compounds, similar to that described previously for root essential oil. This first study permitted to chemically characterize this part of the plant that is traditionally consumed and to get a better idea of its potential beneficial properties.

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