Journal of Pharmaceutical and Biomedical Analysis 123 (2016) 53–62

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

An insight on the alkaloid content of *Capparis spinosa* L. root by HPLC-DAD-MS, MS/MS and ^{1}H qNMR

Mohamad Khatib[a], Giuseppe Pieraccini[b], Marzia Innocenti[a], Fabrizio Melani[a], Nadia Mulinacci[a,*]

[a] Department of Neurofarba, Pharmaceutical and Nutraceutical Division, University of Florence, Via Ugo Schiff 6, Sesto Fiorentino, Firenze, Italy
[b] Mass Spectrometry Center (CISM) of University of Florence, Viale G. Pieraccini 6, Firenze, Italy

ARTICLE INFO

Article history:
Received 29 January 2016
Accepted 30 January 2016
Available online 2 February 2016

Keywords:
Caper
Spermidine alkaloids
Stachydrine
Quantitative determination
Alkaloids distribution

ABSTRACT

The *Capparis spinosa* L. has a wide distribution in the Old World from South Europe, North and East Africa, Madagascar, Southwest and Central Asia to Australia and Oceania. The consolidated traditional use of *C. spinosa* root as remedy against different pains in human is well known since the antiquity. Various secondary metabolites have been found in caper plant, nevertheless, few studies have been focused to the analysis of root constituents. To date, several free and glycosilated spermidine alkaloids and a more polar alkaloid, the stachydrine, have been isolated from the root of *C. spinosa*.

Aim of this work was to improve the knowledge on the alkaloid content of the root of a Syrian sample of *C. spinosa* by HPLC-DAD-MS^{n} and to propose methods to quantify these molecules in different raw extracts. A decoction, an hydroalcoholic extraction and a fractionation process to selectively recover the spermidine alkaloids were applied. To our knowledge, this is the first HPLC-DAD-MS^{n} profile that pointed out the co-presence of stachydrine, several isobaric forms of capparispine and/or capparisine in free and glycosylated forms and some isobars of isocodonocarpine or codonocarpine as monoglycosides in extracts of *C. spinosa* root. The determination by HPLC/DAD for the spermidine alkaloids expressed as *p*-OH-coumaric acid gave values up to 3.5 mg/g dried root and the stachydrine evaluated by ^{1}H NMR was close to 12.5 mg/g dried root. Overall, the total alkaloids were almost doubled in hydroalcoholic extract with respect to the decoction, and the stachydrine in the cortex was almost double than in the whole root.

1. Introduction

The *Capparis spinosa* L. has a wide distribution in the Old World from South Europe, North and East Africa, Madagascar, Southwest and Central Asia to Australia and Oceania [1]. The caper is a perennial spiny shrub, which can be found growing wild everywhere in Syria around dry and rocky areas. It is particularly abundant in the harsh environments of Euphrates valley, Aleppo province, Idlib, Salamie area of the Hama province, and in Al Jazira (northeastern Syria). *C. spinosa* L. is adapted to dry heat and intense sunlight and it is a valuable commodity for the nomadic communities living in the Syrian desert. While caper is widely cultivated in other Mediterranean countries, wild species are mainly found in Syria. Unlike to other countries, the caper buds collected before flowering are sell to foreign traders of the neighboring countries, mainly Turkey [2]. Foreign factories process capers, and sell them to European countries. Overall the estimated yearly harvest is close to 4000 tons [3].

The relationship between capers and human beings can be traced back to the Stone Age. Rests of *C. spinosa* were unearthed in archaeological sites in Syria as early as the lower Mesolithic on the area of Tell Abu Hureyra, 9500–9000 B.C., [4]. The first recorded use of the caper bush for medicinal purposes was by the Sumerians in 2000 B.C.The root cortex of caper was used as analgesic and carminative agent [5]. A high economic and medicinal value in different traditional medicines like Iranian, Unani, Chinese, Ayurvedic and Greco-Arabian contexts has been recognized for this plant where it is widely known with the common name caper [6]. Several authors reported that teas made with caper root and young shoots are considered to be beneficial for the treatment of rheumatism and stomach problems, against colicky pains, dyspepsia, dropsy, anemia and gout in traditional medicines [5,7] as hypoglycaemic agent [9,10]. Cortex root, which has a bitter taste, is used as appetizer, astringent, tonic, antidiarrheic and to treat hemorrhoids, spleen

* Corresponding author.
E-mail address: nadia.mulinacci@unifi.it (N. Mulinacci).

http://dx.doi.org/10.1016/j.jpba.2016.01.063
0731-7085/

disease and rheumatism [11,12]. The moist pastes prepared from the root cortex are used for topic applications to treat swollen joints, skin rashes, burns, wounds and dry skin [9] and for its antibacterial activity [13].

A recent review highlights the traditional use of *C. spinosa* root as well known remedy against different pains in human [14]. The powdered root is widely used for oral administration in Syrian tradition, alone or associated to local applications on the painful part of "dough" prepared mixing the powder with water. In Jordanian traditional medicine, the root cortex are macerated and placed between gauze on aching area for 15–25 min in order to relieve inflammation and muscle pain [15]. This ability to reduce pain in humans is particularly interesting because few natural compounds have been recognized as efficacious to date.

Some pharmacological evidences on the activities of the alkaloids of caper can be found in literature. The more polar alkaloid, stachydrine was able to increase the blood coagulation, thus shortening bleeding time and blood loss [16]. The assumption of generous quantities of alkaloids, e.g., spermidine alkaloids and stachydrine, induced a depressant activity on central nervous system in rats [17]. Since the antiquity, the stachydrine containing species are widely used against rheumatism and other diseases in traditional medicines [18]. Stachydrine has also been recognized as *in vitro* anticancer agent in solid tumor of prostate and able to markedly inhibit the invasive capacity of malignant cancer cells [19].

Various secondary metabolites have been found in caper plant, among them alkaloids, some phenols, sterols in free and glycosylated forms, but few studies have been focused to the analysis of root constituents of *Caparis spinosa*. To the group of spermidine alkaloids belong three main molecules, capparispine, capparispine 26-*O*-β-D-glucoside and cadabicine 26-*O*-β-D-glucoside isolated for the first time in 2008 from the root of *C. spinosa* [20]. Other similar spermidine alkaloids, isocodonocarpine, capparidisine and capparisinine, have been isolated from dried root of *C. decidua*, and their structures elucidated by mass spectrometry, UV, IR and NMR spectroscopy [21–23]. Successively other alkaloids as capparisine, codonocarpine, cadabacine, and stachydrine [25,26] as well as, two new *N*-acetylated spermidine alkaloids, 14-*N*-acetylisocodonocarpine and 15-*N*-acetylcapparisine [24] have been isolated from the same roots. The stachydrine was isolated from the root of *C. spinosa* for the first time in 1969 [27] but also leaves and fruit have been reported to contain stachydrine and 3-hydroxy stachydrine [12,28]. To date the majority of the reports on *C. spinosa* are related to the aerial parts of the plant, such as flowers and fruits but less attention has been devoted to investigate on the roots of *C. spinosa* L. Even a recent review, Pharmacological properties of *C. spinosa* Linn [29], cited very few data on roots composition and effects.

Aim of this work was to improve the knowledge on the alkaloid content of the root of a Syrian sample of *C. spinosa* by HPLC-DAD-MS^n and to propose a method to compare the content of these molecules in different extracts. Decoction, hydroalcholic extraction and a fractionation process to selectively recover the different alkaloids were applied on this root. A quantitative evaluation was carried out by HPLC/DAD for the spermidine alkaloids and by ^{1}H NMR for stachydrine.

2. Materials and methods

2.1. Root sample

The roots of *C. spinosa* L. (*Capparaceae*) were collected from Kafr Nabl, province of Idlib, in the north of Syria in 2011. The sample was authenticated by General Commission for Scientific Agricultural Research, Damascus, Syria. The fresh roots were freeze-dried as whole within two week from harvest and maintained at room temperature, in dark until the analyses. Working on dried root was easy to remove the external part (cortex) and the internal part separately (see root picture in highlights).

2.2. Ethanol and water extraction

All the extractions have been done using a fine powdered material from whole root, cortex and internal part separately. The decoction was done in distilled water under stirring for 1 h at boiling. After cooling and filtration the supernatant was collected, treated with 2 volumes of ethanol and kept for 3 h at 0 °C for precipitation of crude polysaccharides. The final solution was centrifuged at 4500 rpm for 12 min to remove the precipitate, then dried under vacuum and dissolved in a mixture of ethanol/H_2O (7:3 *v/v*) obtaining the sample **S1** The ethanol/water extraction was performed applying a drug/solvent ratio of 1:30 *w/v*. The ethanol/water mixture (7:3 *v/v)* was used in two successive steps, each of 12 h under magnetic stirring at room temperature obtaining the **S2** sample from whole root. The same procedure was applied adding a sonication step of 30 min obtaining the **S3** sample from whole root, the **S4** from the inner part and the **S5** from the external cortex (35 MHz) after each extractive step. All the hydroalcoholic extracts were then filtered through Whatman filter paper to remove solid residues before the HPLC-DAD and ^{1}H NMR analyses.

2.3. Fractionation process for alkaloids

Briefly, an aliquot exactly measured of the hydroalcoholic extract was dried until water residue (below 30 °C), added with NH_3 to pH 10, then an equivalent volume of CH_2Cl_2 was used for a liquid–liquid extraction (repeated two times). The organic phases were combined, dried and then dissolved in a mixture of ethanol/water/formic acid 70:30:1 *v/v/v* obtaining the CH_2Cl_2 sample (**S6**). The same procedure was applied to treat the water residue to prepare the sample for the instrumental analysis (**S7**).

2.4. HPLC-DAD analyses

The analyses were carried out using a HP 1200L liquid chromatograph equipped with a DAD detector (Agilent Technologies, Palo Alto, CA, USA). A 150 mm × 2 mm i.d., 4 μm, 80 Å, Fusion column (Phenomenex, Torrance, CA,USA) with pre-column was used. The mobile phases were (A) 0.1% formic acid/water and (B) CH_3CN. The multi-step linear solvent gradient used was: 0–2 min 2% B; 2–20 min, 2–25% B; 20–25 min 25–35% B; 25–28 min 35–95% B; 28–32 min 95–95% with a final plateau of 2 min at 2%B; equilibration time 10 min; flow rate 0.4 mL/min and oven temperature 26 °C; injection volume 5 μL. The UV–vis spectra were recorded in the range 220–500 nm and chromatograms were also acquired at 280 and 350 nm. All solvents used were HPLC grade; CH_3CN was from E. Merck (Darmstadt, Germany).

2.5. HPLC–MS and MS^n experiments

HPLC–MS and MS^n experiments were done in positive and negative mode using the same column and applying the elution method previously described for the HPLC-DAD analyses. The UHPLC was a Platin Blue (Knauer, Berlin, Germany), operating in the same condition as described for HPLC-DAD analyses. The UHPLC was directly connected to a LTQ linear quadrupole ion trap mass spectrometer (Thermo Scientific, Bremen, Germany) via an ESI interface. The analyses were performed in scan mode in the range 134–800 *m/z*, alternating the two polarities. The following MS parameters were