Determination of Dicaffeoylquinic Acid in Selected Medicinal Plants using HPLC-PDA and LC-ESI-MS/MS

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Summary: Medicinal plants play a key role in serving the ailing humanity and are a rich source of a number of chemicals of pharmacological importance. Dicaffeoylquinic acids (DCQAs) are naturally occurring polyphenolic compounds with a broad spectrum of pharmacological activity. Dicaffeoylquinic acid was qualitatively analyzed using HPLC-PDA and HPLC-MS/MS in selected medicinal plant named as *Achillea millefolium*, *Equisetum arvense* and *Matricaria chamomilla*. Confirmations of the detected peaks were performed on the basis of MS/MS fragmentation pattern and the UV-Visible spectra obtained from the analyses.

Introduction

Medicinal plants play a vital role in making the life of human easy and their use can be traced back to the pre-historic time. According to the World Health Organization (WHO), 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare. More than 80,000 species of plants are in use through out the world. They are a source of various pharmacologically active compounds used against different diseases like antioxidants, anti inflammatory, antibacterial anticancer, anti-diabetic, anti-depressant, cardiovascular diseases etc and also for different other ailments such as carminative, laxative, demulcent, antitussive, expectorant, sedative, antiseptic, astringent etc.

Dicaffeoylquinic acids (DCQAs) are naturally occurring polyphenolic compounds with a broad spectrum of pharmacological activity. They are widely distributed in plants [1-5]. Fig. 1 show the structure of dicaffeoylquinic acid which consists of two caffeic acids attached to one quinic acid through ester bonds. They have been investigated as a potential class of compounds against HIV by selectively inhibiting HIV-1 integrase and preventing replication of HIV-1 in tissue culture at nontoxic concentration. HIV-1 integrase is an essential enzyme that mediates integration of the HIV genome into the host chromosomes [1]. Therefore, DCQAs have gained more attention and are considered as leading compounds in the discovery of medicines for the treatment of HIV infection [6]. Further antioxidative and anti-apoptic activities have been investigated for these compounds [7, 8]. It has been shown that these compounds have neuroprotective effects [9]. Because of these different pharmacological properties analysis of dicaffeoylquinic in medicinal plants is important. Liquid chromatography coupled with mass spectrometry has been used for the determination of dicaffeoylquinic acid in herbs [8, 10, 11] . The present work is focused on the determination and characterization of dicaffeoylquinic acid in three plants *Achillea millefolium*, *Equisetum arvense* and *Matricaria chamomilla*.

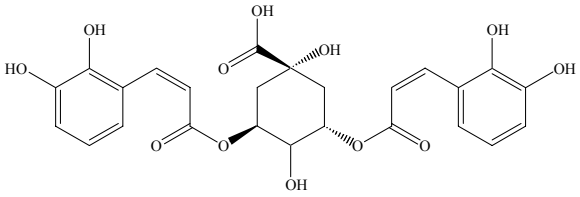

Fig. 1: Molecular structure of dicaffeoylquinic acid.

Results and Discussion

Chromatographic conditions were optimized for the analysis of these plant extracts in order to achieve maximum separation of peaks. A long chromatographic run of about 67 minutes was used to achieve the maximum possible base line separation of the obtained peaks from the extracts

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besides target analyte peaks. Photodiode array detector was used which is capable of scanning the sample within the selected range of wavelengths. In these analyses wavelength range from 200 to 600 nm was used. Identification of the analytes was performed on the basis of UV spectra obtained. PLC-PDA chromatograms and UV Spectra of the identified peaks have been shown in Fig. 2.

Table-1 shows the results obtained from the HPLC-PD and LC-ESI-MS/MS analyses. The UV spectra and the fragmentation pattern of the analytes of interest were compared to those of the standards published in literature [11, 12] and it was determined that these peaks belong to different isomers of dicaffeoylquinic acid. The fragmentation pattern of the dicaffeoylquinic acid (Fig. 3) in the positive ionization mode using LC-MS/MS consists of the molecular ion at m/z 517 and the precursor ion at m/z 355. Signals at m/z 499 was due to loss of water molecule from the protonated molecular ion ($M+H^+$-OH), at 355 ($M+H^+$-(3-(2,3-Dihydroxy-phenyl)-2-propenyl)), at 339 ($M+H^+$-(3-(2,3-Dihydroxyphenyl) -2-propenoate)) and at m/z 163 (Caffeic-OH).

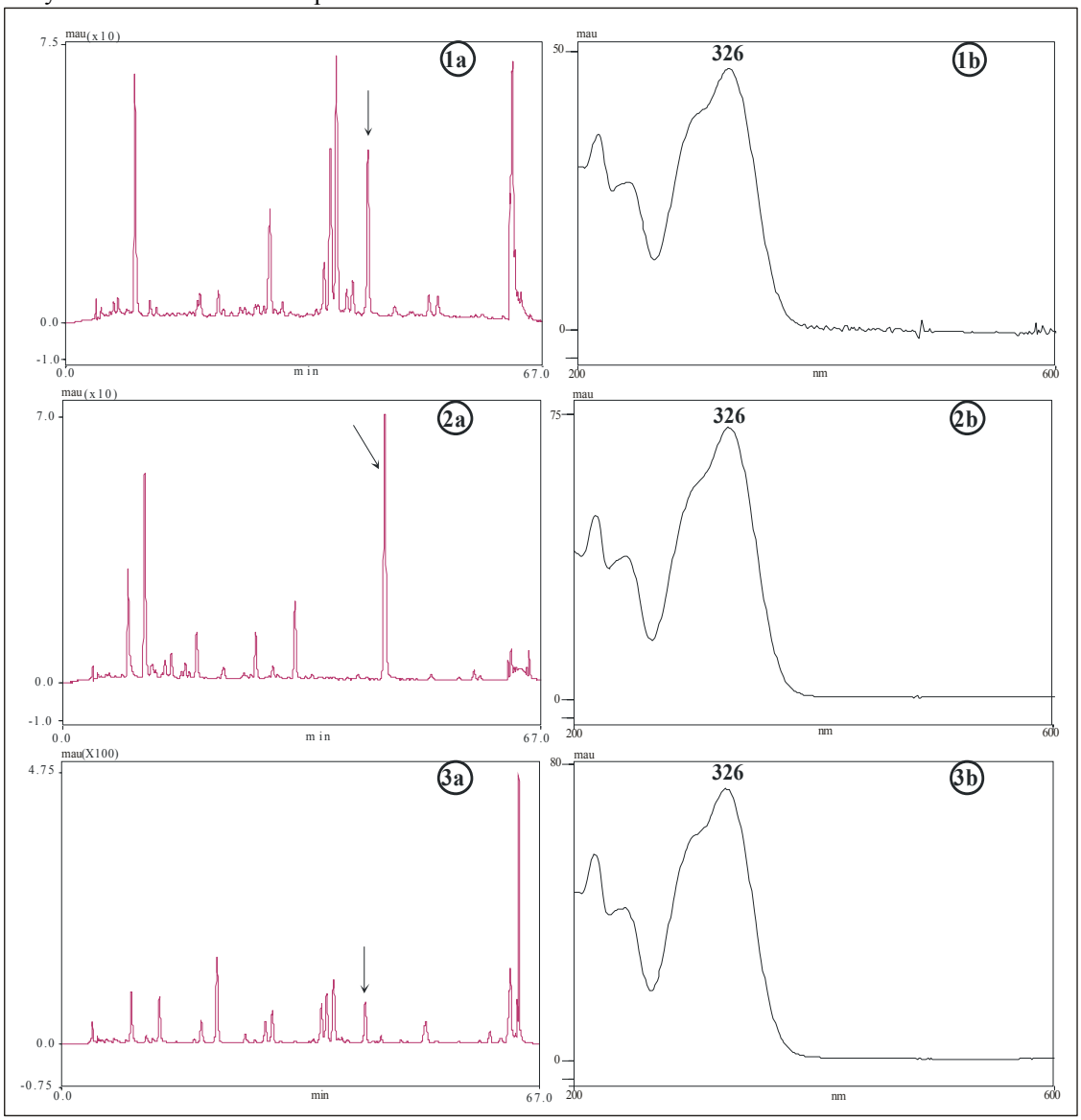

Fig. 2: HPLC-PDA chromatograms with UV spectra of the identified peak of (1a&b) *Achillea millefolium*, (2a&b) *Equisetum arvense* and (3a&b) *Matricaria chamomilla*. Chromatographic conditions: column: Varian Pursuit diphenyl, 250 x 4.6 mm, particle size: 5 µm, mobile phase: A: 0.085% phosphoric acid and B: acetonitrile, column temperature: 30 °C and flow rate: 0.8 ml per minute.

Table-1: Result from HPLC-PDA and LC-ESI-MS/MS.

Herb name	Ret. Time (min)	λ_{max} (nm)	ESI (+) MS/MS	Structure
Achillea millefolium	41.88	326	516.73,498.93,355.07, 339.13,162.93	Dicaffeoylquinic acid
Equisetum arvense	43.95	326	516.60,499.0,355.0, 318.07,163.0	Dicaffeoylquinic acid
Matricaria chamomilla	40.94	326	516.70,499.10,355.10, 339.20,163.0	Dicaffeoylquinic acid

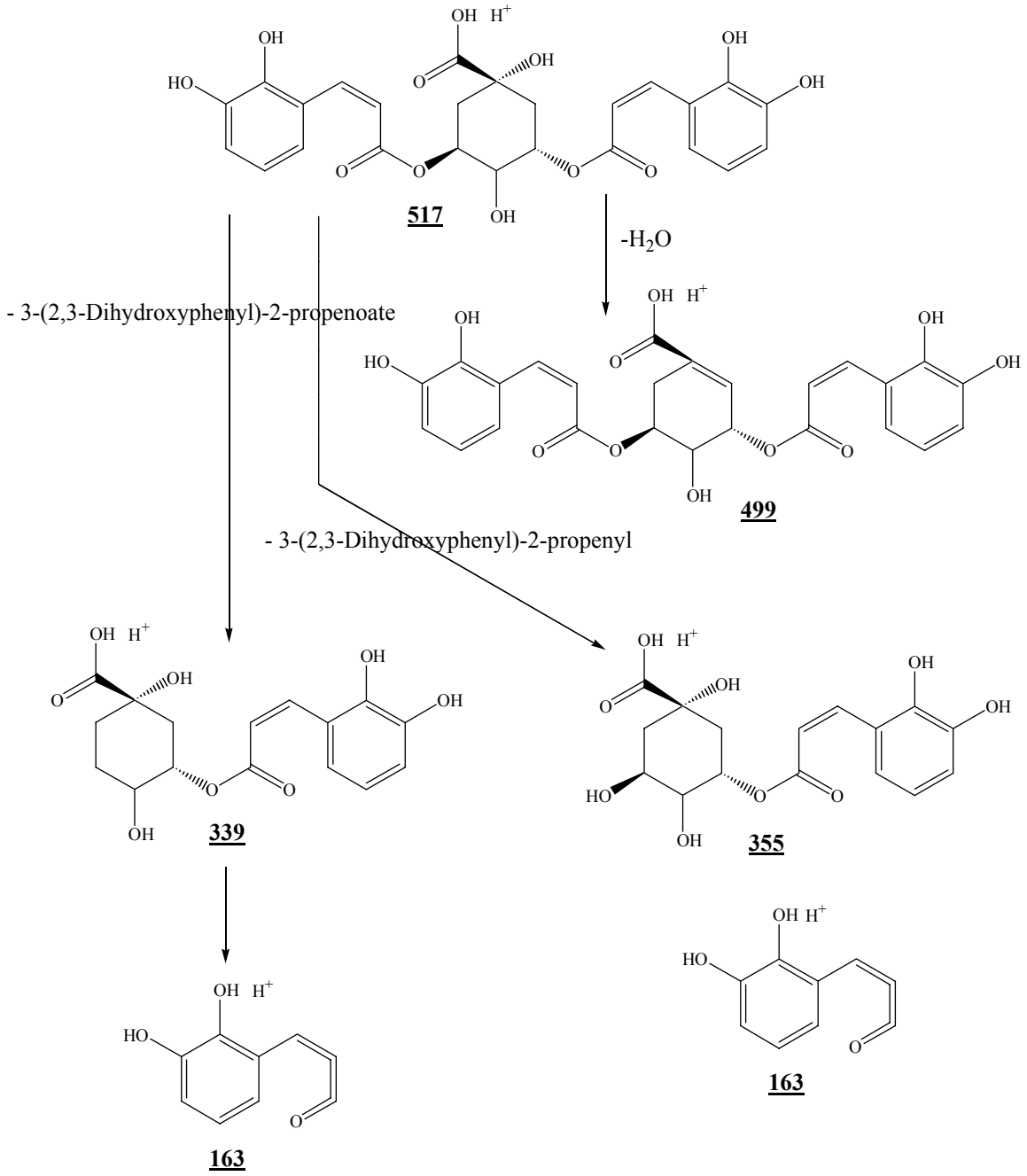

Fig. 3: Structural representation of fragmentation pattern of dicaffeoylquinic acid in LC-ESI-MS/MS.

The method applied is a sensitive and reliable LC-MS/MS procedure. Further, precision and accuracy of the results obtained were acceptable with good recoverability and reproducibility.

Experimental

Chemicals and Reagents

MeOH, acetonitrile, formic acid were purchased from Merck KGaA (Darmstadt,

Germany). Phosphoric acid (85%) was obtained from Sigma Aldrich (Steinheim, Germany). Nitrogen gas was purchased from Messer Austria GmbH. All these chemicals and reagents were of analytical grade and used without further purification. Water purified by a Nano Pure-unit (Barnstead, Boston, MA, USA) was used.

Extraction

About one gram of each powdered plant material was extracted in 20 ml of 50% MeOH by refluxing for 20 minutes with continuous stirring using six place heating carousel reaction station. Extracts were allowed to cool at room temperature and then centrifuged for 10 minutes at 14 x 1000 g using eppendorf centrifuge (Eppendorf 5415 D, Hamburg, Germany). They were preserved at -20 °C temperature for further work.

HPLC-PDA Analysis

Instrumentation

Shimadzu HPLC was used for the analysis of dicaffeoylquinic acid. The HPLC system comprised of an online degasser unit (DGU-14A), two solvent delivery pumps (LC-10Advp), an autoinjector (SIL-10ADvp), a column oven (CTO-10Avp) and a system controller (SCL-10Avp). Detection of the analytes was performed using a photo diode array detector PDA (SPD-M10 Avp). The system control and data analysis were performed using the manufacturer's software packages (LCMS-Solution, version 3 and LCMS-Post run, version 3-H2) [13, 14]

Chromatographic Parameters

The chromatographic separation was performed on a reversed stationary phase column (Pursuit diphenyl, 250 x 4.6 mm, 5 µm particle size from Varian (Darmstadt)). Gradient elution was carried out using mobile phase A: 0.085% phosphoric acid and B: acetonitrile. Column temperature was set to 30 °C and a flow rate of 0.8 ml per minute was used. Zero time condition was 12% B and a linear gradient to 24% B was applied up to 50 minutes. Then elution was performed isocratic at 24% B for 5 minutes and after that a linear gradient to 100% B up to 56 minutes was applied. The column was equilibrated at 100% B for 4 minutes and then changed to zero time conditions in 2 minutes. 50% methanol was used as washing solution for the auto injector and the sample injection loop. The whole analysis took 67 minutes. PDA detector was operated in the wave length range from 200 to 600 nm.

HPLC-MS/MS Analysis

Instrumentation

LC system coupled to an ion trap mass spectrometer (LCQ, Thermo Finnigan) was used. LC system consisted of a degasser (Flux Instruments, Basel Switzerland), an injection port (VICI, E36-230), a solvent delivery pump (Rheos 2000 Flux Instruments) and an auto-sampler (CTC Analytics, Switzerland). Solvent flow was controlled by Janeiro II-2.1 (version 2.0). XCalibur (version 1.3) was used to control LC-ESI-MS.

Chromatographic and Mass Spectrometric Conditions

Chromatographic conditions remain the same as in section 2.3.2 except 0.085% formic acid was used as mobile phase A. Further PDA was not used in this experiment and the column was used at ambient temperature. The column was directly connected to MS detector through ESI interface.

MS was used in positive ionization mode and the scanning was performed in the mass range m/z values from 100 to 200. Spray voltage of 4.30 kV, capillary temperature of 230 °C and a capillary voltage of 26 V were applied. 10 µL of sample was injected at a flow rate of 0.8 mL/min and a split ratio of 1:3.

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