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Research Article

Qualitative and Quantitative Analysis of Polar Extract from *Centaurea fragilis* Dur. Using HPLC-TOF/MS

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ABSTRACT
In recent decade, liquid chromatography coupled with mass spectrometry (LC–MS) has become the most selective techniques for rapid screening and characterization of known and unknown constituents from the extracts of medicinal plants. *Centaurea* species are known for their multiple biological activities. With the aim of estimating the chemical composition of *n*-BuOH extract of the aerial parts of *Centaurea fragilis*, a rapid qualitative and quantitative screening was carried out using HPLC-TOF/MS. The use of HPLC-TOF/MS technique resulted in the qualitative and quantitative determination of nineteen (19) compounds from which 9 phenolic acids and 10 flavonoids knowing for their pharmacological properties.

Keywords: *Centaurea Fragilis*, Phenolic Acids, Flavonoids, Chlorogenic Acid, Hplc-Tof/Ms.

INTRODUCTION

The genus *Centaurea* which belonging to the Asteraceae family contains about 700 species essentially centered in the Mediterranean region[1]. In Algeria, the genus *Centaurea*, is represented by 45 species, including 7 in the Sahara[2,3]. Moreover, many species of this genus are used as medicinal herbs[4-6]. Previous phytochemical studies on this genus showed the variety of their chemical composition and their richness, especially, in flavonoids and sesquiterpene lactones[7-12] which are considered as chemotaxonomic markers of the genus *Centaurea*. *Centaurea fragilis* Dur. is an endemic species and in the best of our knowledge, no phytochemical or pharmacological studies are reported for this species. As a part of our continuing investigation of Algerian *Centaurea* species[13-20], the present study was aimed to screen and identify the main components of *n*-BuOH extract of *Centaurea fragilis* using HPLC-TOF/MS.

EXPERIMENTAL

Plant Material

The plant was collected from El kala region, Algeria, in May 2014. The plant was identified by Dr. D. Sarri. (Biology Department, University of M'Sila). A voucher specimen (CFA 05/14) has been deposited in the Herbarium of the VARENBIOMOL Unit Research, Frères Mentouri University, Constantine, Algeria.

Extraction Procedure

A quantity (482 g) of ~~leaves~~ aerial parts of *Centaurea fragilis* was dried at room temperature and cut into small pieces then macerated three times (24h for each time) with methanol/H_2O (80 %). After filtration and evaporation, the obtained extract was partitioned with solvents in increasing polarity: chloroform, ethyl acetate and *n*-butanol. Each extract was dried with Na_2SO_4, then filtered and evaporated under reduced pressure to give $CHCl_3$ extract (7g), ~~of~~ EtOAc extract (7.5g) and *n*-BuOH extract (29g). The present study focuses on qualitative and quantitative determination of components of the *n*-BuOH extract.

HPLC-TOF/MS Analysis

The HPLC analysis was performed with an Agilent Technology of 1260 Infinity HPLC System equipped with 6210 Time of Flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100mm, 3.5µm) column. Mobile phases A and B were ultra-pure water with 0.1% formic acid and acetonitrile, respectively. Flow rate was 0.6 mL min^{-1} and column temperature was 35°C. Injection volume was 10 µL. The solvent program was as follow: 0-1 min 10% B; 1-20 min 50% B; 20-23 min 80% B; 23-30 min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas temperature of 325 °C, nitrogen gas flow of 10.0 L min-1, nebulizer of 40 psi, capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered

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Table 1: HPLC-TOF/MS analysis of *n*-BuOH extract of *Centaurea fragilis*.

S. No	Compound identified	Rt (min)	*m/z*	Molecular formula	Contents (mg/kg plant)
1.	Formic acid	3.19	115.013	CH_2O_2	114.43
2.	Gentisic acid	4.51	153.0297	$C_7H_6O_4$	13.29
3.	Chlorogenic acid	5.47	353.1060	$C_{16}H_{18}O_9$	2064.50
4.	4-hydroxybenzoic acid	6.96	137.0344	$C_7H_6O_3$	12.74
5.	Protocatechuic acid	7.09	153.0296	$C_7H_6O_4$	7.95
6.	Vanillic acid	7.88	167.0467	$C_8H_8O_4$	4.64
7.	Syringic acid	8.08	197.0580	$C_9H_{10}O_5$	8.79
8.	Rutin	9.24	609.1718	$C_{27}H_{30}O_{16}$	96.92
9.	Scutellarin	9.74	461.0937	$C_{21}H_{18}O_{12}$	274.92
10.	Quercetin 3-*O*-*β*-glucoside	9.77	463.1092	$C_{21}H_{20}O_{12}$	34.92
11.	Sinapic acid	10.51	223.0746	$C_{11}H_{12}O_5$	19.86
12.	Diosmin	10.62	607.1461	$C_{28}H_{32}O_{15}$	205.09
13.	Hesperidin	10.76	609.2079	$C_{28}H_{34}O_{15}$	6.19
14.	Apigetrin	10.88	431.1189	$C_{21}H_{20}O_{10}$	1.00
15.	Neohesperidin	11.09	609.2083	$C_{28}H_{34}O_{15}$	3.59
16.	Baicalin	12.05	445.0982	$C_{21}H_{18}O_{11}$	81.71
17.	Morin	13.01	301.0516	$C_{15}H_{10}O_7$	5.52
18.	Cinnamic acid	15.16	147.0552	$C_9H_8O_2$	4.26
19.	Naringenin	15.69	271.0796	$C_{15}H_{12}O_5$	5.95

passing through a PTFE (0.45µm) filter by an injector to remove particulates.

RESULTS AND DISCUSSION

Identification and quantification of compounds by HPLC-TOF/MS analysis.

The *n*-BuOH extract was obtained from the aerial parts of *C. fragilis* and analyzed by HPLC-TOF/MS. The identification of individual compounds were performed on the basis of their retention times and mass spectrometry by comparison with those of different standards. Nineteen compounds were identified and listed in table 1. As it can be seen, the analyzed extract comprises a complex mixture of plant secondary metabolites. These constituents belong to two important chemical classes, phenolic acids and flavonoids, knowing for their pharmacological activities. These phenolic acids were identified as formic acid, gentisic acid, chlorogenic acid, 4-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, sinapic acid and cinnamic acid with chlorogenic acid having the highest concentration (2064.50 mg/kg plant) followed by formic acid 114.43 mg/kg plant. While, flavonoids were identified as rutin, scutellarin, quercetin-3-*O*-*β*-glucoside, diosmin, hesperidin, apigetrin, neohesperidin, baicalin, morin and naringenin with scutellarin and diosmin having the highest concentrations (274.92 and 205.09 mg/kg plant) followed by rutin and baicalin (96.92 and 81.71 mg/kg plant) respectively. Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants[21]. Chlorogenic acid, which exhibits many biological properties, including antibacterial[22], antioxidant[23], anti-inflammatory[24] and anticarcinogenic activities, particularly hypoglycemic and hypolipidemic effects[25], was found as the major phenolic acid in the *n*-BuOH extract of *C. fragilis*. Whereas flavonoids such as scutellarin, diosmin, rutin and others are well known to have special activities such as antioxidant[26-28], antimicrobial[29], anti-inflammatory[30], antiviral[31], cardiovascular protection and antitumor effects[32].

CONCLUSION

The present study helps to predict the formula and structure of 19 phenolic acids and flavonoids detected by HPLC/MS analysis from the *n*-butanolic extract of *Centaurea fragilis*. Further investigations may lead to isolation of bio-active compounds and their structural elucidation and screening of pharmacological activity will be helpful for further drug development. From these results, it could be concluded that *C. fragilis* contains various bioactive compounds and could be recommended as a plant of phytopharmaceutical importance.

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