The quality assessment of bioactive flavonoids in the *Pinus brutia Ten.* bark extract and Pycnogenol® by HPLC method

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INTRODUCTION

Barks of different pine species have been used as food and medicine for more than 2000 years. In ancient times, pine bark was used to treat inflammatory conditions. European herbals of the fifteenth and sixteenth century mention the efficacy of pine bark in skin disorders, mainly wounds and ulcers. In North America, indigenous peoples used pine bark to prevent and treat scurvy.

French maritime bark extract (Pycnogenol) and Pinus brutia Ten. bark extract have nutritional and traditional medicinal uses. In traditional medicine, its bark is used to treat a variety of health disorders. In this study, a simple, accurate and reproducible HPLC method was developed for determination of catechin hydrate, epicatechin, taxifolin and catechin gallate from the extract of two different sample.

APPARATUS AND PROCEDURES

The LC analysis was carried out on an Agilent 1260 series HPLC system with ternary solvent pump, online degasser, automatic injection system, column heater and multi wavelength detector was used. UV detection was performed at 280 nm. Analyses were run at a flow rate of 0.8 mL./min. YMC Pack ODS-AM (5 µm, 150 mm x 4.6 mm ID) column was used as stationary phase at 25°C. 0.1 % TFA and methanol in the ratio of 80:30 v/v was used as a mobile phase.

All chemicals were used without further purification. The certified standard of compounds and TFA were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). HPLC grade methanol was purchased from Merck.Ultrapure water, with conductivity lower than 0.05 m/Scm was obtained with a Milli-Q system (Millipore, Bedford, MA, USA).

Stock solutions of compounds were prepared by dissolving enough of the studied compounds in MeOH (50 mL) to give a 100 mg/L solution. All reagents were protected from light. Prepared solutions were stored at 4° C for several days and at -20° C for long-term storage.

As an example representative HPLC chromatograms of the standard mixture was given in Fig 1. Good linearity was observed over the investigated concentration range 1-20 ppm with correlation coefficient values greater than 0.99. This method was successfully applied in the quality assessment of bioactive flavonoids in the Pinus brutia Ten. bark extract and Pycnogenol.

Extraction

An amount of 0.03 m g of bark powder was extracted with methanol (v/v) at ambient temperature. All extracts were stored at –20°C for further studies.

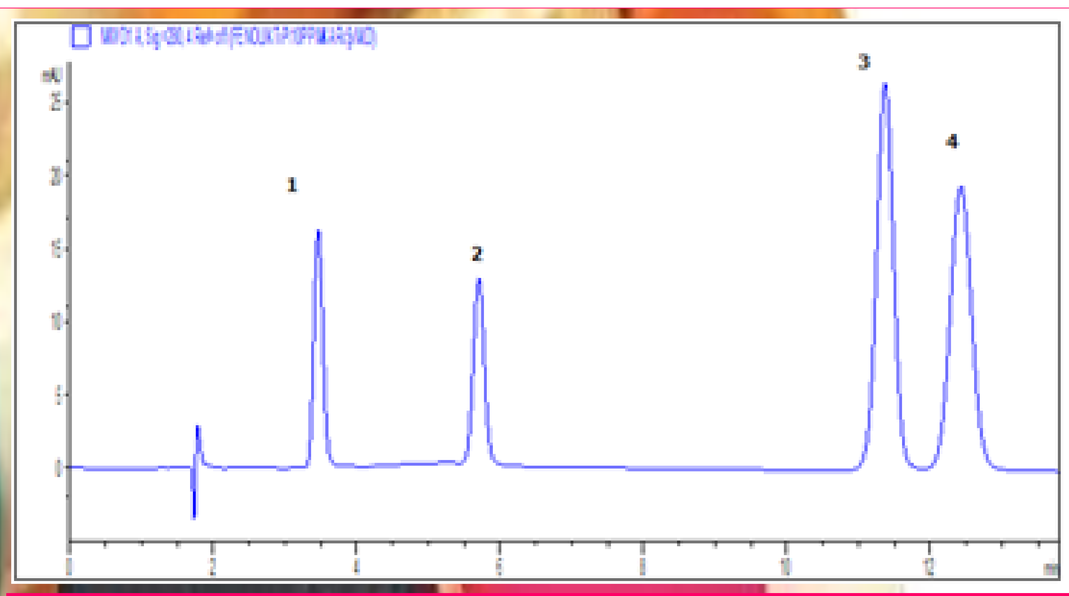

Fig 1. Standart mixture of four compounds (1: catechin hydrate (10 µg/mL) 2: epicatechin (10 µg/mL) 3: taxifolin (10 µg/mL) 4: catechin gallate (10 µg/mL).

RESULT AND DISCUSSION

Two different samples were used in this study.The chromatogram of extraction of Pinus brutia bark was given in Fig 2.

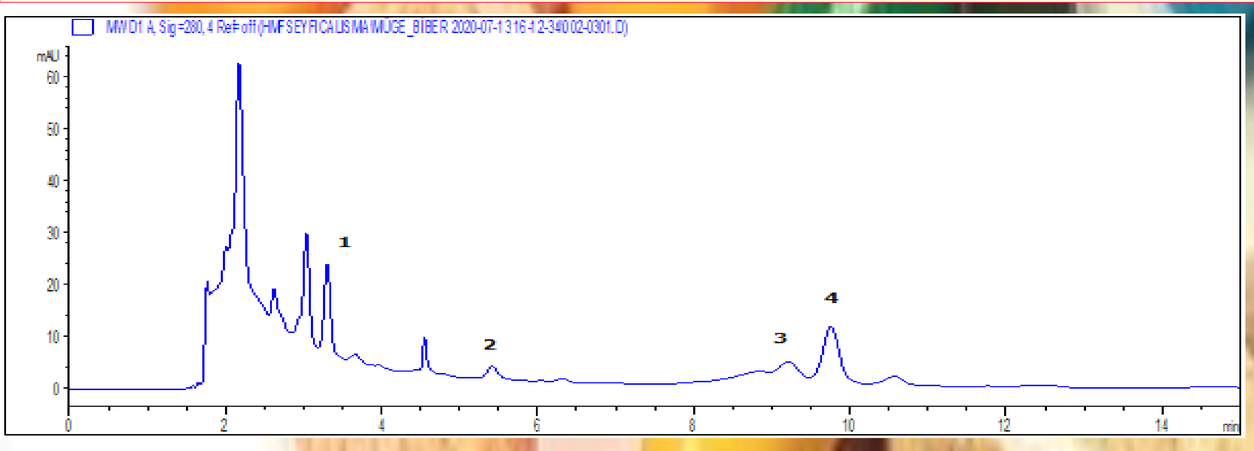

Fig 2. Chromatogram of extraction of Pinus brutia bark (1: catechin hydrate 2: epicatechin 3: taxifolin 4: catechin gallate.

Calibration data of four compouns were given in Table 1.

Calibration Data	Epicatechin	Catechin hydrate	Taxifolin	Catechin gallate
Linearity range (µg mL-1)	1.00 – 20.00 (n=5)	1.00 – 20.00 (n=5)	1.00 – 20.00 (n=5)	1.00 – 20.00 (n=5)
Slope	15.265	15.429	51.374	47.461
Intercept	-7.938	-9.457	-35.882	-52.696
Correlation coffecient(r)	0.999	0.999	0.999	0.999

Table 1. Calibration Data

The obtained extraction results of two sample with methanol were given inTable 2 with standart deviations.

Table 2 Extraction Results with Methanol (Results are the values obtained from 10 mg / 1mL sample concentration)

Compounds	Pycnogenol kapsül	Bark Powder
Epicatechin	5.675(0.18)	Not Detected
Catechin hydrate	52.074(0.18)	2.170(0.03)
Taxifolin	376.29(0.261)	54.12(0.083)
Catechin gallate	10.494(0.297)	Not Detected

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