Comparison of Extraction Techniques for Extraction of Bioactive Molecules from *Hypericum perforatum* L. Plant

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Abstract

Three methods commonly used for the extraction of bioactive molecules from natural plant material are compared. Dried *Hypericum perforatum* L. plant material is subjected to Soxhlet extraction, extraction by ultrasonication, and accelerated solvent extraction. The percentage of two bioactive compounds, hyperforin and hypericin, in the extracts is used as a parameter for comparison of the extraction procedure.

Introduction

The quality of the extract of a plant material depends on the extraction procedure employed. The extraction procedure employed is reflected in the percentage of a compound of interest present in the extract. Extraction by refluxing is the most common method (1). Recently, many new techniques of extraction have come up. Accelerated solvent extraction (ASE) is one of them, in which extraction of the plant material is carried out under elevated temperature and pressure. The extract quality obtained by this technique is reported to be the same as that obtained by Soxhlet extraction, with an added advantage of utilization of less time and solvent (2). A comparative study was made to check the percentage of hyperforin and hypericin in *H. perforatum* (3,4) as extracted by Soxhlet, ultrasonication, and ASE. Analytical high-performance liquid chromatography (HPLC) was used for the evaluation of the percentage of bioactive compounds in the extracts with respect to the standards (1,5).

Experimental

Materials

Hexane and methanol used for extraction were of HPLC grade from Ranbaxy India (Rankem, Mohali, India). Analytical-grade orthophosphoric acid and ammonium orthophosphate used for HPLC analysis were from SDS Chemicals (Biosar, India). HPLC-grade acetonitrile and methanol were used for HPLC analysis and were obtained from Ranbaxy India.

Extractors

An accelarated solvent extractor was obtained from Dionex (model ASE 300, Dionex, Sunnyvale, CA). Ultrasonic bath was from Toshcon (Ajmer, India).

Analytical HPLC instrumentation

A ThermoFinnigan analytical HPLC system with a P4000 quaternary pump, SCM 1000 degasser, AS 3000 autosampler, UV6000LP photodiode array detector (ThermoFinnigan, San Jose, CA) and Merck (Darmstadt, Germany) Lichrospher RP-18 column (4 × 250 mm and 4 × 100 mm, 5-µm particle size) was used. Data collection was done with Chromquest software (ThermoFinnigan).

Preparation of extracts

In the reflux method, 50 g of shade dried herb (*Hypericum perforatum* L.) was powdered and exhaustively refluxed with hexane (150 mL × 3) for 4 h. The extract was filtered out, concentrated, and dried over a rotavapor in a preweighed flask. The obtained residue was 2.6 g (Extract 1). The defatted marc was again refluxed with methanol (150 mL × 3) for 8 h in the Soxhlet. The extract was filtered out, concentrated, and dried over a rotavapor in a preweighed flask. The obtained residue was 12.4 g (Extract 2).

Cells (100 mL) were used for extraction in ASE. Twenty grams of dried plant material was loaded in the cell. First, the extraction was carried out with hexane at ambient temperature with a static time of 10 min and pressure of 1500 psi. A total of 164 mL of solvent was used. The collected extract was evaporated on rotavapor in a preweighed flask at ambient temperature. The weight of the extract (Extract 3, 0.7 g) was thus calculated. The marc left in the cell was again extracted in ASE with methanol (155 mL) at ambient temperature with a static time of 10 min and pressure of 1500 psi. The extract was dried and the weight was calculated as in the earlier case (Extract 4, 4.6 g).

For extraction by ultrasonication, the dried plant material (20 g) was first sonicated with hexane (100 mL) for 20 min. The extract was filtered out and the marc was dried at room temperature. The marc was again extracted by ultrasonication with methanol (100 mL) for 20 min. Both hexane and methanol extracts (Extract 5 and 6, respectively) were dried at room temperature in a rotavapor and weighed (0.5 and 1.95 g, respectively).

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HPLC analysis of extracts

All six extracts were analyzed on an analytical HPLC (3,5) in order to find the percentages of hyperforin and hypericin in the hexane and methanol extracts, respectively. For hyperforin analysis, a solvent system of acetonitrile–phosphoric buffer (2.5 pH, 85:15) at a flow rate of 1.5 mL/min, at 271 nm was used to elute the extract on C-18 column (4 × 250 mm). Known weights of the three hexane extracts (Extracts 1, 3, and 5) were dissolved in known volumes of methanol and injected by means of an autosampler. The concentrations of the compounds of interest were calculated against the concentration of the hyperforin standard used for preparing the standard curve.

Analysis of hypericin in the three methanolic extracts (Extracts 2, 4, and 6) was carried out at 589 nm using a solvent system of methanol–ethyl acetate–phosphate buffer (2.5 pH, 67:16:17) at a flow rate of 0.4 mL/min in a C-18 column (4 × 100 mm). Known concentrations of the extracts were prepared in methanol and injected by means of an autosampler. The concentrations of the compounds of interest were calculated against the concentration of the standard used for preparing the standard curve.

Table I. Results of Hexane Extraction of *H. perforatum* by Different Extraction Techniques

Extract name	Dried wt. of plant	Extract (wt. and %)	% Detection of hyperforin in extract	% Detection of hyperforin in dried plant
Extract 3 (ASE)	20 g	0.7 g, 3.51%	37.8%	1.32%
Extract 1 (Soxhlet)	50 g	2.6 g, 5.2%	11.6%	0.6%
Extract 5 (ultrasonication)	20 g	0.5 g, 2.5%	0.25%	0.006%

Table II. Results of Methanol Extraction of *H. perforatum* by Different Extraction Techniques

Extract name	Dried wt. of plant	Extract (wt. and %)	% Detection of hypericin in extract	% Detection of hypericin in dried plant
Extract 4 (ASE)	20 g	4.6 g, 23%	0.178%	0.04%
Extract 2 (Soxhlet)	50 g	12.4 g, 24.8%	0.39%	0.097%
Extract 6 (ultrasonication)	20 g	1.95 g, 8.25%	0.18%	0.25%

Table III. Results of Methanol Extraction of *H. perforatum* by ASE at Different Temperatures

Extract name	Dried wt. of plant	Extract (wt. and %)	% Detection of hypericin in extract	% Detection of hypericin in dried plant
Extract 7 (50°C)	10 g	2.02 g, 20.2%	0.21%	0.04%
Extract 8 (100°C)	10 g	2.81 g, 28.1%	0.42%	0.12%

Results and Discussion

As indicated by the percentage of hyperforin in the extract and plant material (Table I), ASE extraction proved to be better than the traditional reflux method, though the extract weight percentage is greater in the reflux method. Hyperforin, being a thermolabile compound, must have degraded in the reflux method. The 20-min extraction by ultrasonication proved to be the least productive method.

The ASE extraction of the plant material for hypericin extraction with methanol has resulted in extract with less percentage of hypericin as compared with traditional reflux method (Table II). The reflux method proved to be far superior because it resulted in less extract with more hyepricin, whereas the ASE method resulted in more extract with less hypericin. Extraction at room temperature in ASE may have resulted in less hypericin percentage in the extract. To check this hypothesis, repetition of the extraction procedure was carried out with methanol at 50°C (Extract 7) and 100°C (Extract 8). Quantitation by analytical HPLC showed that the extraction at 50°C resulted in a marginal increase in the percentage of hypericin. However, the extraction at 100°C resulted in extract quality as good as that by the reflux method (Table III). The extraction by ultrasonication resulted in an extract with hypericin concentrations less than that in extraction with reflux method.

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