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Comparison of Medium Pressure Solid– Liquid Extraction and Rotation Planar Extraction of *Ficus* Leaves with Reference to Optimum Operating Parameters

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ABSTRACT

The effect of three operating variables on the extraction efficiency of medium pressure solid–liquid extraction (MPSLE) was studied using a

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factorial experimental design with three levels. The operating variables tested were medium particle size of the plant material, volume of extractant, and the equilibrium time between consecutive extractions of the same material. The possibility of scaling up the MPSLE extraction process on larger columns was also confirmed. The extraction efficiency did not markedly decrease, and a systematic increase in the extraction yields was observed on scaling up the MPSLE procedure. The results of the MPSLE experiments were compared with those obtained by the rotation planar extraction (RPE) method using a prototype ExtraChrom[®] separation instrument with particle size of the plant material and volume of extractant as the operating variables. Both methods proved to be suitable for the efficient extraction of the leaves of various *Ficus* species. The strong points of RPE were the ease of operation of the ExtraChrom[®] separation instrument and significant time savings achieved, whereas the possibility of scaling up the extraction process made MPSLE a suitable method for even larger scale preparative extractions.

Key Words: Extraction techniques; Plant extraction; *Ficus* sp; RPE; MPSLE; MPLC.

INTRODUCTION

In the isolation of any given compound or compounds from a natural matrix, often very complex in composition, the choice of the initial extraction method is of great importance. The three most commonly used extraction methods for natural products include solvent extraction, steam distillation, and supercritical fluid extraction.^[1] The optimum technique for a particular extraction is chosen by taking into consideration the material to be extracted, the compounds of interest and their chemical and physical properties, the cost-efficiency of the process, and the availability of a suitable level of technology, to name a few. In practice, solvent extraction as a fairly inexpensive and simple method is still the prominent technique in the extraction of most natural products.

Medium pressure solid-liquid extraction (MPSLE), introduced by Nyiredy et al.,^[2] is an extraction method based on the principles of the diffusion-dissolving processes of parametric pumping that have been well characterized.^[2,3] The method involves creating periodic changes in the equilibrium of the process, as well as in the flow directions, in order to separate the components in the treated fluid or gas mixture. A change in the intensive parameter of the system, e.g., temperature, pressure, pH, or electric field, results in a reversible differential alteration of the distribution of the components between the solid and liquid phases. Numerical simulations of parametric



pumping has been in use for over 20 years, and the theory of the method is well known.

Medium pressure solid-liquid extraction can be performed in a medium pressure liquid chromatography (MPLC) system. The same principal factors are valid in MPSLE as in column chromatography, i.e., the geometry of the column, physico-chemical properties of the solvent, flow rate and amount of solvent, pressure, equilibrium time, particle size, compactness, and amount of plant material.^[1] However, the effects of these parameters have not been studied in detail.

Rotation planar extraction (RPE) is an extraction method in which accelerated flow of the extractant is produced by the action of centrifugal force.^[4,5] A novel multi-functional separation instrument, prototype Extra-Chrom[®], even enables the rotation planar extraction of complex matrices, because a planar column can easily be attached to it and then filled with the material to be extracted. The factors affecting the RPE process are basically the same as in MPSLE, with the exception that the solvent is driven by centrifugal force instead of a pump and the geometry of the column differs from that of the MPLC columns used in MPSLE. So far, only few studies concerning the use of RPE in the extraction of plant material have been published.^[6-8]

In this investigation, a factorial experimental design with three levels of three independent operating variables was applied for studying the MPSLE process and for predicting the influence of the operating variables on the extraction yield. The dependences of the extraction yield, the amount of nonpolar compounds in the extract, and the amount of nonpolar compounds obtained from the lower segment of the column on the three independent operating variables, were estimated using multilinear stepwise regression analysis. The effect of plant material particle size and volume of extractant on the extraction efficiency in RPE was studied using a prototype Extra-Chrom[®] separation instrument. The results of RPE are compared with those obtained with MPSLE.

EXPERIMENTAL

Plant Material

Ficus sycomorus L. (Moraceae) and *Ficus bengalensis* L. were grown in the fields of the Faculty of Agriculture, Cairo University, Cairo, Egypt. The material was identified by B. El-Dewan (senior research scientist, Ministry of Agriculture, Cairo, Egypt). The plant material was air-dried and kept in a paper sack in a dry, dark, and cool place and then transferred to the Division of Pharmacognosy, Department of Pharmacy, University of Helsinki.



For this study, the dried leaves were fractionated into different particle sizes (fineness of the powder 0.125–1.68 mm Ph. Eur.) by an automatic shaker (Erweka AR 400, Germany). Fifty grams of the plant material was sieved for 15 min each time. Medium particle sizes of 0.40, 0.67, and 0.87 mm were selected for this investigation.

Medium Pressure Solid–Liquid Extraction of Plant Material

The MPSLE method was applied for the extraction of *F. sycomorus* L. leaves using chloroform as extractant. Medium particle size, equilibrium time, and volume of solvent were chosen as the operating variables. The tested values of the operating variables are listed in Table 1. The study was carried out using a factorial experimental design with three levels. The experiments were performed using a Büchi column (230 × 26 mm, Büchi Laboratoriums-Technik AG, Switzerland) filled with 40.0 ± 1 g of dried and milled leaves. An HPLC pump (A-45, Millipore-Waters) was fitted to the column. The extraction procedure was monitored by a UV detector (LKB Ltd., Sweden) throughout the whole experiment, and a Shimadzu C-R1B integrator (Shimadzu Corp., Japan) was used as recorder. The schematic diagram of the system is shown in Fig. 1. The solvent was pumped through the column at a flow rate of 3.0 mL min⁻¹. The extracts were collected after each equilibrium time for seven successive intervals, evaporated under reduced pressure, and the dry weight determined. The UV tracing of the process is presented in Fig. 2. Each residue was dissolved in 25.0 mL of chloroform, and 1.0 mL of these chloroform extracts were kept at -20°C for TLC analysis. The residues (1.00 g) of

Table 1. Variables used in the extraction experiments. The MPSLE experiments were carried out using a full factorial experimental design with three levels.

	Variable		
	Medium particle size (mm)	Equilibrium time (h)	Solvent volume (mL)
MPSLE	0.40	1	45
	0.67	2	90
	0.87	3	135
RPE	0.40	1	90
	0.67		135
	0.87		



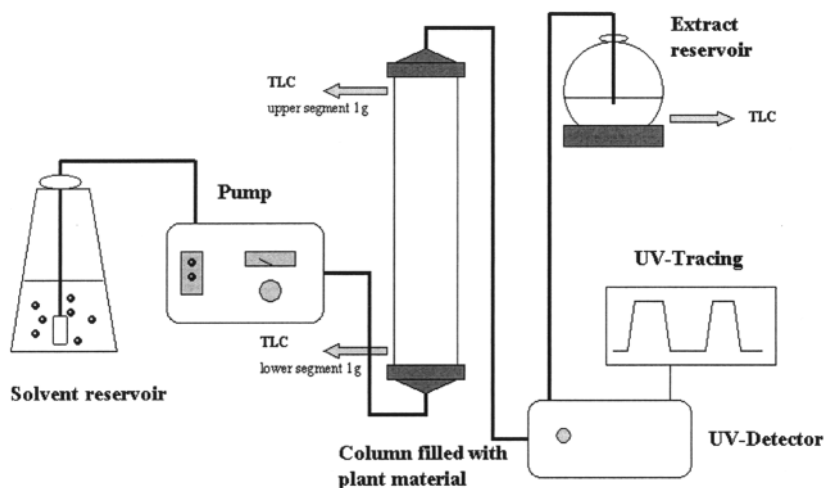


Figure 1. Schematic diagram of the MPSLE system.

plant material obtained from two segments of the column (top and bottom segments of the column in each experiment) were extracted with 10.0 mL of chloroform and filtered. Each filtrate of 1.0 mL was kept at -20°C for TLC analysis. An external standard was prepared by extracting 1.00 g of the milled leaves (medium particle size 0.40 mm) with 10.0 mL of chloroform at ambient temperature for 10 min in an ultrasonicator (USF Finnsonic W 181, Ultra Sonic Finland Ltd., Finland). The extract was filtered and kept at -20°C .

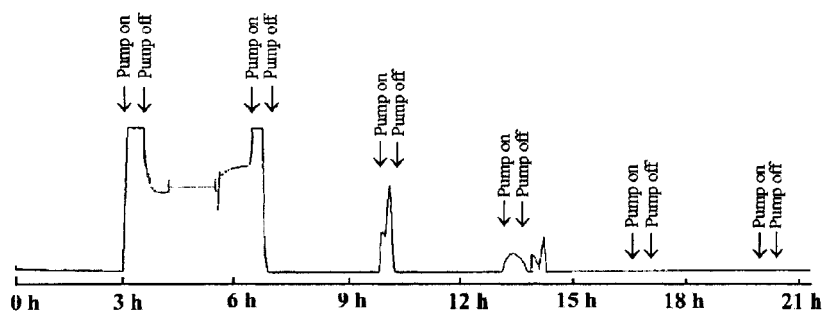


Figure 2. The UV tracing of the MPSLE process ($\lambda = 300\text{ nm}$). Medium particle size of plant material 0.67 mm, equilibrium time 3 h, and the volume of solvent pumped in each cycle 90 mL.



Scaling Up Medium Pressure Solid–Liquid Extraction

The operating variables selected from the small column were utilized for scaling up the extraction process on larger columns. For this purpose, three Büchi columns (460 × 26 mm; 460 × 36 mm; 460 × 49 mm) were filled with 100, 151, and 430 g of the milled leaves, respectively. The amount of filling varied 0.7–1.1% in these columns. The test was conducted two times on each column, using the same procedure described for the initial smaller Büchi column. The linear velocity of the solvent through the columns with larger diameter was kept constant and exactly the same as that used for the smaller column, by adjusting the flow rate according to the following equations:

$$v_1 = \pi d_1^2 \cdot k_i \quad (1)$$

$$v_n = \pi d_n^2 \cdot k_i \quad (2)$$

where v_1 is the flow rate through the smaller column, d_1 is the diameter of the smaller column, k_i is a constant, v_n is the flow rate through a larger column, and d_n is the diameter of the larger column.

In this study, the flow rate was 3 mL min⁻¹ (column I.D. 26 mm), 5.7 mL min⁻¹ (column I.D. 36 mm), and 10.6 mL min⁻¹ (column I.D. 49 mm). A model 6000A pump (Millipore-Waters; 3 mL min⁻¹ and 5.7 mL min⁻¹) and a 600E pump (Millipore-Waters; 10.6 mL min⁻¹) were used for solvent delivery.

Rotation Planar Extraction of Plant Material

The RPE method was studied by ExtraChrom[®], a prototype multi-functional separation instrument with an extraction chamber. The construction of the separation instrument has been described previously.^[6] Dried and milled leaves of *F. bengalensis* L. were used as the plant material and were extracted with chloroform. The medium particle size and volume of solvent were chosen for the operating variables on the basis of the results of the MPSLE method (Table 1). In the experiments the planar column was filled with 40.0 ± 1 g of the plant material, using a rotational speed of 1700 rpm. The plant material was wetted with either 90 or 135 mL of chloroform as the rotational speed was set to 1400 rpm and solvent flow to 6 mL min⁻¹. The time needed for wetting was, therefore, 15 or 22.5 min. After wetting, the planar column was allowed to equilibrate for 1 h. The extracts were collected after the equilibrium time using the rotational speed of 1400 rpm, evaporated to dryness under reduced pressure, and their dry weight determined. Each extract was dissolved in 25.0 mL of chloroform, and 1.0 mL of these chloroform extracts were kept at -20°C for TLC analysis. The residues (1.00 g) of plant material obtained from



two segments of the planar column (inner and outer segments of the column in each experiment) were extracted with 10.0 mL chloroform and filtered. Each filtrate (1.0 mL) was kept at -20°C for TLC analysis.

TLC Conditions

The eluent for TLC separation was optimized by use of the "PRISMA" system according to Nyiredy.^[9,10] TLC was performed on Kieselgel F₂₅₄ TLC plates (Merck, Germany). *n*-Hexane was of technical grade (Oy Exxon Chemicals Ab, Finland) and was filtered prior to use. All other solvents were of analytical grade (Rathburn Ltd., Scotland). The extracts and the standard (5 μL) were applied in the form of bands (3 mm in width) to the 10 \times 20 cm TLC plates using a Linomat IV TLC spotter (Camag, Switzerland). The application distance from the bottom of the plate was 0.8 cm and the distance between the bands was 0.7 cm. The extract serving as a standard was applied twice to each TLC plate. The TLC separations were performed in ascending mode in 20 \times 20 cm unsaturated twin-trough chambers (Camag, Switzerland) at ambient temperature.

The optimized mobile phase consisted of *n*-hexane–diethyl ether–1,4-dioxan–ethanol (78 : 10 : 6 : 6, v/v/v/v). The TLC plates were developed to a distance of 9 cm and the development time was *ca.* 30 min. Visual inspection of the TLC plates was performed under a UV lamp (Camag, Switzerland) at 254 and 366 nm. The densitometric evaluation was carried out with a dual-wavelength flying-spot scanner CS-9001 PC (Shimadzu Corp., Japan) for the analysis of the MPSLE extracts, or with a Desaga CD 60 densitometer (Desaga, Wiesloch, Germany) operating under ProQuant[®] software for the analysis of the RPE extracts. The results achieved by TLC were standardized on the basis of external standards in order to prevent changes in the formation of bands due to factors such as variations in relative humidity and room temperature.

Statistical Evaluation

Factor, cluster, and regression analyses were carried out using Systat[®] 6.0.1 (SPSS Inc., Chicago, IL) software. Cluster analysis was performed by the complete linkage method with Pearson correlation. In factor analysis the common factor model was selected and matrix correlation types with Varimax rotation were used.

RESULTS AND DISCUSSION

The MPSLE extraction procedure is rapid, easy to perform, and yields a particle-free extract.^[2] In the present communication, the effect of operating



variables of the MPSLE technique, i.e., medium particle size of the plant material, equilibrium time, and the volume of solvent, on the extraction efficiency was studied. Furthermore, the RPE technique was compared to the MPSLE technique with medium particle size of the plant material and volume of extractant as the independent operating variables.

Ficus sycomorus L. and *F. bengalensis* L. were selected as examples for modeling the extraction process of nonpolar compounds, primarily of the coumarin type. The physical and chemical criteria of the solvents and their extraction abilities have been studied previously by Härmälä et al.^[11] Chloroform was found to be the most suitable solvent for the extraction of the nonpolar, biologically active coumarins from the roots of *Angelica archangelica* L., and was, therefore, applied for this study.

For analysis of the extracts by TLC, 10 pure solvents according to the Snyder classification^[12] were selected for the mobile phase optimization using the "PRISMA" system.^[9,10] A combination of diethyl ether, 1,4-dioxan, and ethanol at a selectivity point (P_S) of 333 was selected. The solvent strength (S_T) was adjusted with *n*-hexane to 0.8, resulting in a mobile phase consisting of 78.4% *n*-hexane, 9.7% diethyl ether, 5.6% 1,4-dioxan, and 6.3% ethanol.

The off-line UV reflectance spectra of the TLC bands of the tested extracts indicated that the main compounds, which had a strong blue or yellow fluorescence at 366 nm, were of the coumarin type. Most of the investigated compounds had absorbance maxima around 300 nm. In TLC, the compounds remaining at the starting zone were considered polar, while all the other compounds were considered nonpolar (Fig. 3). In this study, the main interest was focused on the nonpolar compounds (coumarins) due to their various biological activities.^[13–20]

The influence of the different operating variables was monitored by determining the extraction yield of each individual experiment and the peak areas of polar and nonpolar compounds of both the extract and the bottom segment of the MPLC column or the outer segment of the RPE column by TLC at 300 nm. The peak area of the nonpolar compounds extracted from these segments of the columns contributes directly to the degree of depletion of the compounds of interest from the plant material.

The Medium Pressure Solid–Liquid Extraction Method

The data from the MPSLE experiments are shown in Table 2. The medium particle size of the plant material clearly affected the extraction yield and the amount of nonpolar compounds detected from the extract by TLC. A pronounced increase in the extraction yield, as well as, in the peak area of the nonpolar compounds present in the extract, were observed with the



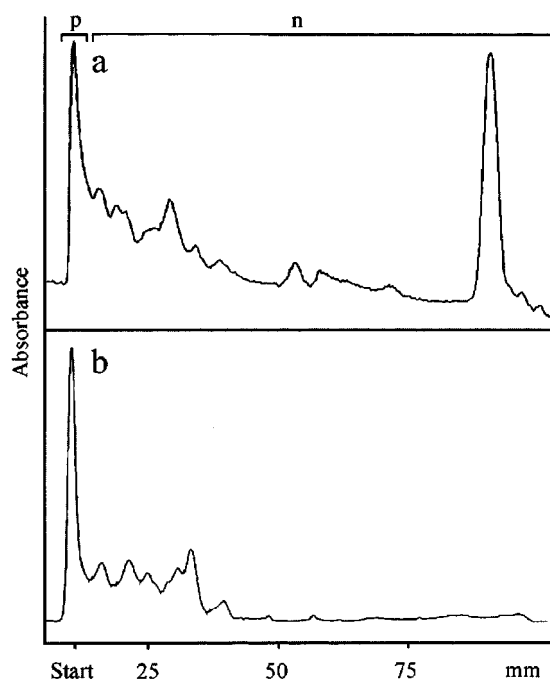


Figure 3. TLC analysis of the studied *Ficus* leaf extracts; UV at 300 nm (A) *F. sycomorus* leaf extract obtained with MPSLE; (B) *F. bengalensis* leaf extract obtained with RPE; p, polar compounds; n, nonpolar compounds. TLC conditions: see Experimental.

smallest medium particle size when compared to the other two medium particle sizes, which did not markedly differ from each other. The influence of the medium particle size of the plant material on the peak areas of the nonpolar compounds extracted from the bottom segments of the columns showed the same tendency. An increase in the equilibrium time increased the extraction yield only slightly and the differences were insignificant. The other parameters did not reveal any significant changes either. Increasing the volume of solvent used for one extraction cycle increased the extraction yields, as well as, the proportion of nonpolar compounds in the extract. The results between 90 and 135 mL were, however, very similar indicating no actual differences.

The data were analyzed using both rotated factor analysis and cluster analysis with the complete linkage method. Rotated factor analysis, presented in Table 3, indicated that the most important factor affecting the extraction efficiency is the volume of the solvent, i.e., a direct dependence was found



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Table 2. Influence of the operating variables in MPSLE ($n = 9$). The amount of polar and nonpolar compounds in the extract and nonpolar compounds in the bottom segment of the column was determined as peak area units after TLC separation of the extracts. Nonpolar compounds left in the column were determined as the percentage ratio of the peak areas of nonpolar compounds in the bottom segment of the column and in the extract.

	Medium particle size (mm)			Equilibrium time (h)			Volume of solvent (mL)		
	0.40	0.67	0.87	1	2	3	45	90	135
Extraction yield (mg)	769	662	645	676	681	719	637	711	729
Polar compounds in the extract (peak area)	87,055	69,140	67,740	75,979	74,645	73,312	77,791	74,750	71,396
Nonpolar compounds in the extract (peak area)	337,579	181,125	211,090	266,960	243,276	219,557	207,082	258,748	263,964
Nonpolar compounds in the bottom segment of the column (peak area)	5,068	2,382	2,936	4,121	2,641	3,624	4,126	3,066	3,194
Nonpolar compounds left in the column (%)	1.5	1.3	1.4	1.5	1.1	1.7	2.0	1.2	1.2



Table 3. Rotated factor pattern for the effect of operating variables on the extraction yield, polar compounds in the extract, and nonpolar compounds obtained from both the bottom segment of the column and the extract.

Factor	1	2	3	4
Polar compounds in the extract	0.87	-0.18	0.03	0.13
Nonpolar compounds in the extract	0.48	0.16	-0.38	0.09
Nonpolar compounds in the bottom segment of the column	0.15	-0.14	-0.96	0.11
Medium particle size	-0.70	-0.08	0.35	0.13
Equilibrium time	-0.02	0.00	0.08	-0.57
Volume of solvent	-0.04	0.88	0.09	0.02
Extraction yield	0.68	0.57	-0.13	-0.44

between the volume of solvent and the extraction yield. The medium particle size of the plant material showed negative correlation to the extraction yield and the amount of both polar and nonpolar compounds in the extract, indicating that smaller particle size results in increased extraction yield and higher amounts of both polar and nonpolar compounds in the extract. The equilibrium time was also directly correlated to the extraction yield. The tree diagram of the cluster analysis in Fig. 4 revealed a strong linkage between

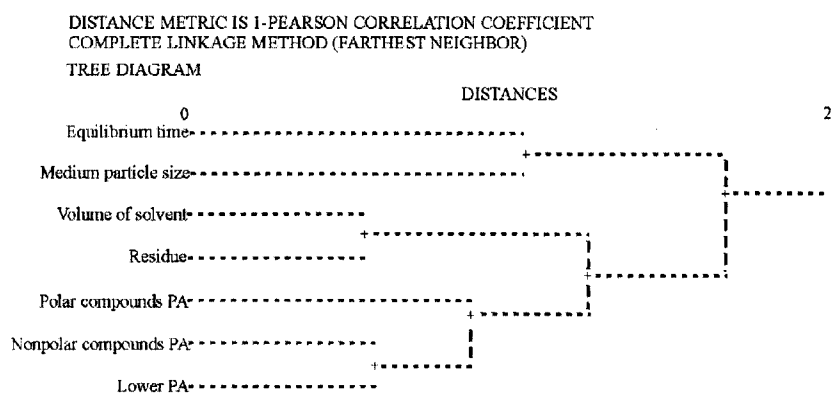


Figure 4. Cluster analysis of the data obtained from the MPSLE experiments. Polar compounds PA, peak area of polar compounds in the extract ($\lambda = 300$ nm); Nonpolar compounds PA, peak area of nonpolar compounds in the extract; Lower PA, peak area of nonpolar compounds obtained from the bottom segments of the column.



the volume of solvent and the extraction yield. The influence of equilibrium time and medium particle size on the extraction process was less pronounced.

The dependence of the extraction yield on the three independent operating variables was studied using multilinear stepwise regression analysis. The obtained regression model that best described the effects of the operating variables on the extraction yield was a second order polynomial, and had the following form:

$$Y = 699.3P^2 - 1144P + 0.98V + 23.3T + 980 \quad (3)$$

where Y is the extraction yield, P is the medium particle size of the plant material, V is the volume of solvent, and T is the equilibrium time. The multiple regression coefficient, R , for the experimental and calculated residue yield was 0.89.

Choice of the most suitable operating variables for the efficient extraction of the nonpolar compounds from *F. sycomorus* L. leaves was evaluated on the basis of the extraction yields and the peak areas of polar and nonpolar compounds in the extracts and the bottom segments of the column by TLC at 300 nm. According to these criteria, a medium particle size of 0.40 mm, a solvent volume of 90 mL, and an equilibrium time of 2 h, were selected as the most efficient conditions for the extraction process.

Scaling Up Medium Pressure Solid–Liquid Extraction

The medium particle size of 0.40 mm, although selected as the most suitable particle size for the MPSLE system, could not be used for the scale-up process due to the increase in back pressure caused by compactness of the plant material. Instead, a medium particle size of 0.67 mm was chosen. A systematic increase in the extraction yields was observed on scaling up the MPSLE procedure for the three larger columns, as can be seen in Table 4. The extraction yields obtained on scaling-up the process confirmed that the ratio between the extraction yield and the amount of plant material extracted did not decrease markedly.

Table 4. Extraction yields of the different-sized columns ($n = 2$).

Column size (mL)	Total yield (mg)	Yield/plant material (mg/g)
130	676.0	16.9
260	1700.7	18.0
470	2422.6	14.6
920	6178.2	14.4



Rotation Planar Extraction Method

The influence of the medium particle size of the plant material on the extraction efficiency using RPE, differed to some extent from that with the MPSLE method. The highest yield was obtained using a medium particle size of 0.67 mm, as shown in Table 5. The amount of nonpolar compounds in the extract was, on the other hand, highest using a medium particle size of 0.40 mm. A direct dependence was observed between the medium particle size and the amount of nonpolar compounds extracted from the outer segment of the planar column, i.e., decreasing the medium particle size of the plant material decreases the amount of nonpolar compounds left in the outer segment of the column, indicating a more efficient extraction. It can be seen from Table 5, that the relative proportion of nonpolar compounds left in the column after the extraction was slightly higher when compared to the MPSLE method, but it should be taken into account that only one extraction cycle was performed with the RPE method. In the extraction of floral stems and leaves of *Gerbera hybrida*, the two methods have previously been shown to be practically equal in terms of extraction yield and the quality of the extracts, when the number of extraction cycles was the same for both methods.^[6] The two extraction methods seemed equal in terms of the volume of solvent used for the extraction. Increasing the volume of solvent led to a slight increase in the extraction yield but, at the same time, the amount of nonpolar components in the extract decreased. TLC chromatograms of the extract, and the extract

Table 5. Influence of the operating variables in RPE ($n = 2$, medium particle size; $n = 3$, volume of solvent). For details see Table 2.

	Medium particle size (mm)			Volume of solvent (mL)	
	0.40	0.67	0.87	90	135
Extraction yield (mg)	986	1184	965	979	1111
Polar compounds in the extract (peak area)	1345.5	1387.9	1331.8	1456.6	1253.6
Nonpolar compounds in the extract (peak area)	1282.4	820.7	943.6	1139.4	891.7
Nonpolar compounds in the outer segment of the column (peak area)	22.7	27.5	45.5	30.3	33.4
Nonpolar compounds left in the column (%)	1.8	3.4	4.8	2.7	3.8



from the outer segment of the planar column after RPE was completed, are shown in Fig. 5.

Based on the same criteria as with MPSLE, the optimum extraction efficiency was achieved with a medium particle size of 0.40 mm and solvent volume of 90 mL, i.e., the same values that were chosen for MPSLE.

CONCLUSIONS

This is the first time, to our knowledge, that the use of a statistical design of experiments has been applied to study the importance of some of the operating variables on the extraction efficiency using MPSLE and RPE methods. In spite of the limited number of RPE experiments performed, the results obtained with ExtraChrom[®] indicated that both methods were suitable for the extraction of nonpolar compounds from *Ficus* leaves with respect to extraction efficiency. The optimum plant material particle size and solvent volume were identical in the two methods, indicating a similar principle of action.

The full factorial experimental design with multilinear stepwise regression was a suitable technique for studying a complicated multivariate process such as extraction, and for predicting the influence of the selected operating

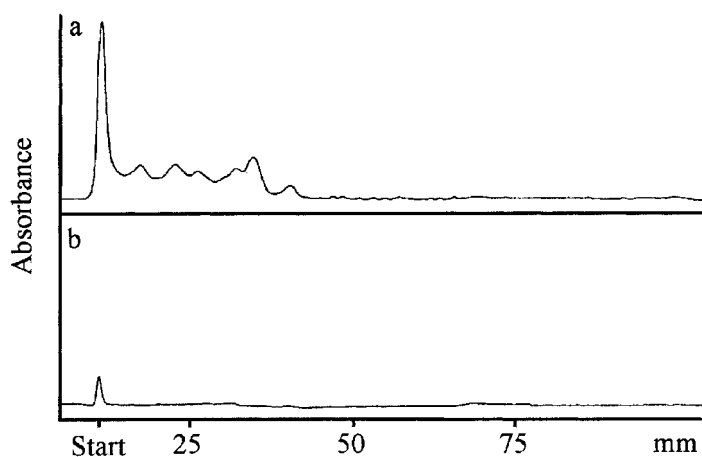


Figure 5. TLC analysis of the RPE extract of *F. bengalensis* ($\lambda = 300$ nm). Medium particle size of plant material 0.67 mm, equilibrium time 1 h, and the volume of extractant 90 mL (a, extract; b, extract from the outer segment of the planar column). TLC conditions: see Experimental.



variables on the residue yield. The methodology used in this study yielded maximum information with a limited number of experiments.

Given the ease of operation and significant time savings achieved, the RPE method with the ExtraChrom[®] separation instrument seems to be well suited for screening purposes: Plant material, 20–50 g at a time, are to be extracted and the number of these samples is fairly large. MPSLE proved to be an exhaustive extraction method, and the possibility of scaling up the extraction process makes it a suitable method for preparative extractions. Since only few reports on the influence of some operating variables in MPSLE and RPE, namely plant material particle size, volume of extraction solvent and equilibrium time, have been published so far, further studies using different plant materials and solvents are still needed in order to gain an understanding of the importance of the many operating variables in the extraction processes of different constituents from complex matrices.

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