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Comparison between sample disruption methods and solid–liquid extraction (SLE) to extract phenolic compounds from *Ficus carica* leaves

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Abstract

Sea sand disruption method (SSDM) and matrix solid phase disruption (MSPD) were compared to solid–liquid extraction (SLE) for extraction of phenolic compounds from the *Ficus carica* leaves. Statistical treatment, ANOVA-single factor, was used to compare the extraction yields obtained by these methods, and for the majority of the extracted compounds, significantly higher yields were obtained by the solid disruption methods. Both solid disruption methods are faster and ecologically friendly, but the sea sand method was more reproducible (RSD < 5% for most compounds), and was also the least expensive method. Recoveries above 85% were obtained for chlorogenic acid, rutin, and psoralen using the sea sand extraction method.

Keywords: Sea sand disruption method; SSDM; MSPD; *Ficus carica*; Phenolic acid; Flavonol; Coumarin

1. Introduction

The fig tree (*Ficus carica* L., Moraceae) is very common in the Mediterranean and in countries with dry and warm-temperate climate like Portugal. Since ancient times the figs have been used for human consumption, but it was only recently that their nutritive and pharmacological value has been investigated. It seems that their consumption helps in the prevention of vein blockage, its high content in fibers has laxative effects, and the fig latex inhibits the growth of carcinoma cells [1]. Despite the fact that other parts of the fig tree, like the fig leaves, have also reported pharmacological properties they have been much less investigated. In 1998, Serraclara et al. [2] reported the hypoglycemic action of a fig leaf decoction in type-I diabetic patients, and in 2000, Canal et al. [3] used a chloroform extract, obtained also from a decoction of *F. carica* leaves, to decrease the cholesterol levels of rats with diabetes. These pharmacological properties are probably in part due to the high content of phenolic compounds in these plant extracts. Some phenolic compounds, with reported pharmacological properties have already been isolated from fig leaves, namely furanocoumarins like psoralen and bergapten [4], flavonoids like rutin [5], phenolic acids like ferrulic acid [6], and also phytosterols like taraxasterol [7].

The extraction of phenolic compounds from plants has been traditionally performed using solvent extraction or steam distillation techniques. Traditional methods of extraction are labour-intensive, time consuming, and require large volumes of solvents. Following the rapid development of analytical techniques, trends in analytical extraction have been a movement toward less (organic) solvent consumption, faster extraction time and improved quantification [8]. In the last years, several new methods have been applied for plant phenolic extraction such as supercritical fluid extraction (SFE) [9–12], pressurized fluid extraction (PFE) [9,13,14] and matrix solid-phase dispersion (MSPD) [15–17], which are less labour intensive and more environmentally friendly. Despite the use of this new extraction techniques solid–liquid extraction (SLE) is still commonly used [18–20].

MSPD is a patented process [21] that permits simultaneous disruption and extraction of semi-solid and solid samples. This technique is based on the blending of a viscous, solid or semi-solid sample with an abrasive solid support material. This

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method has been applied mainly to the analysis of herbicides, pesticides and pollutants from animal tissues, fruits, vegetables and also from other matrices [22–27]. So far, only a few reports have been published using MSPD technique for plant phenolic extraction [15–17]. Only a few families of plant phenolic compounds have been extracted by the MSPD technique namely, phenolic acids [15], isoflavonoids [16], xanthones and flavanones [17]. In 2005, Teixeira and da Costa [17] reported the use of a new method for extraction of xanthones and flavanones from plant material, which evolve an experimental procedure similar to the MSPD, but uses sea sand as the sample disrupting media. This new procedure, the sea sand disruption method (SSDM), compared favorably with MSPD and SLE for xanthones and flavanones extraction from the root bark of *Maclura pomifera* [17]. Higher yields were obtained by the more expedite sample disruption methods, but the lower cost of the sea sand method makes it a very promising extraction procedure.

The aim of this work is to evaluate the use of SSDM to other families of plant phenolics. In order to do it, *F. carica* leaves were extracted by MSPD, SSDM, and SLE, and the extraction yields were evaluated for several extracted compounds which include rutin (flavonol), psoralen (coumarin) and chlorogenic acid (phenolic acid). Several elution media and elution volumes were tested, and the extraction efficiency was evaluated by comparison of the peak areas of the individual compounds obtained by LC analysis using diode array detection. Compound identification was achieved by their UV and mass spectra obtained on-line by LC-DAD and LC-ESI-MS, respectively. The chemical nature of the sea sand used was determined by X-ray diffraction (XRD) and the degree of sample disruption attained by SSDM and MSPD was evaluated by optical microscopy (OM).

2. Experimental

2.1. Materials and reagents

Acetonitrile (HPLC gradient grade) and methanol (analytical reagent) were purchased from SDS (Peypin, France); methanol (HPLC gradient grade) was purchase from Merck (Darmstadt, Germany; n-hexane (analytical reagent) was obtained from Labscan (Dublin, Ireland); formic acid (HPLC gradient grade) was obtained from Merck (Darmstadt, Germany). Water from a MILLIPORE Simplicity^{TM} system was used for sample preparation and LC analysis.

The solid support material used for MSPD was Polygoprep C_{18}, 40 μm, non-end-capped 14% C, (Macherey-Nagel, Germany). Sea sand was collected in Faro Beach, Portugal. The sand particles size was homogenised with a sieve for ≤1 mm diameter.

The *F. carica* leaves were collected in Évora, Portugal in the Spring of 2004. The green leaves were air-dried for one week; a food processor was used to grind the leaves into fine particles, after which they were stored at 4 °C. The same batch of ground bark was used with the different extraction techniques. Standards of chlorogenic acid and rutin were purchased from Agros Organics (New Jersey, USA), and psoralen was obtained from Fluka (Madrid, Spain).

2.2. Preparation of standards

A 5.0 mg amount of each standard (chlorogenic acid, rutin and psoralen) was weighed, dissolved and transferred to 5 mL volumetric flasks with methanol (HPLC gradient grade) to yield three stock solutions (1000 μg/mL). By serial dilution of those solutions with methanol, calibration standards at levels of 100.0, 80.0, 60.0, 40.0 and 20.0 μg/mL of chlorogenic acid, rutin and psoralen were obtained. All the stock solutions and working solutions were stored at 4 °C, and brought to room temperature before use.

2.3. Extraction procedures

2.3.1. Solid–liquid extraction

500 mg samples of dry leaves were soaked in 20.0 mL of methanol:water (7:3, v/v) for 24 h. All extracts were dried under vacuum, redissolved in 5.0 mL of the same mixture, and filtered through a 0.45-μm PTFE filter (Macherey-Nagel, Germany).

2.3.2. Matrix solid phase dispersion (MSPD) and sea sand disruption method (SSDM)

Both C_{18} solid support material and sand were cleaned before use: C_{18} was washed three times with methanol and the sea sand was washed several times with deionised water and three times with methanol. Both materials were air dried before use.

A 500 mg sample of dried leaves was placed in a glass mortar with 2000 mg of the previously cleaned C_{18} or sea sand and 2.0 mL of *n*-hexane. The materials were mixed in the glass mortar using a glass pestle to obtain a homogenous material suitable for column packing. The blend was then quantitatively transferred into a 5 mL syringe with three circles of filter paper on the bottom. The packing material was covered with another circle of filter paper and compressed using the syringe plunger. The filled syringe was then dried under vacuum. The phenolic compounds were eluted with methanol:water (7:3, v/v). All extracts were dried under vacuum, redissolved in 5.0 mL of the same mixture, and filtered through a 0.45 μm PTFE filter (Macherey-Nagel, Germany).

2.3.2.1. Optimal elution volume determination. The determination of optimal elution volume was done using sea sand as solid support and methanol:water (7:3, v/v) as elution media. Four different elution volumes of the methanol:water (7:3, v/v) mixture were tested: 5.0, 10.0, 15.0 and 20.0 mL. All extracts were dried under vacuum, redissolved in 5.0 mL of the same mixture methanol:water, and filtered through a 0.45 μm PTFE filter (Macherey-Nagel, Germany).

2.4. Reproducibility and recovery

The reproducibility of the analytical methods and the repeatability of the extraction procedures were assessed by evaluating the peak area variation of eight compounds present in the extracts which include chlorogenic acid, rutin and psoralen. Five replicates were performed for each extraction assay, and three replicate LC-DAD analyses were performed on each filtrate.

Statistical treatment (ANOVA-single factor, $p < 0.001$, Microsoft Excel® 2000) was performed to the data to determine significant differences whenever they occurred.

The recovery of the sea sand disruption method (SSDM) was assessed by measuring the recovery of chlorogenic acid, rutin and psoralen. 300.0 μL of rutin and psoralen (equivalent to 300.0 μg), and 400.0 μL (equivalent to 400.0 μg) of chlorogenic acid standard stock solutions were added to the mortar with 500 mg of plant and 2000 mg of sand. The extraction was performed with 10.0 mL methanol:water (7:3, v/v). The extract was dried, recovered in 5.0 mL of the solvent mixture used for the extraction, and filtered through a 0.45 μm PTFE filter (Macherey-Nagel, Germany). This assay was repeated five times and three replica analyses were performed on each extract.

2.5. LC-DAD

An Agillent 1100 system (Agilent Technologies, Germany) with a diode-array detector (DAD) and an HP ChemStation (Agilent Technologies, Germany) was used for LC-DAD analyses. The analytical column was a reversed-phase Zorbax Eclipse XDB-C_{18}, 250 mm × 4.6 mm (length × I.D.) and 5 μm particle size (Agilent Technologies, Germany). The analytical guard column was a Zorbax Eclipse XDB-C_{18}, 12.5 mm × 4.6 mm (length × I.D.) and 5 μm particle size (Agilent Technologies, Germany). The mobile phase was: solvent A: acetonitrile; solvent B: water with acetonitrile (2.5%) and formic acid (0.5%). Gradient program was adopted as follows: linear from 0 to 15% of solvent A (0–5 min), 15 to 20% of solvent A (5–25 min), 20 to 40% of solvent A (25–30 min) and from 40 to 45% of solvent A (30–40 min). LC analyses were performed at room temperature; the injection volume was 20 μL, and the flow-rate was 1.0 mL/min; the DAD detector was scanned from 200 to 500 nm, and the chromatographic profile was recorded at 254 nm.

2.6. LC-ESI-MS/MS

LC-ESI-MS/MS analyses were carried out in a LCQ Advantage ThermoFinnigan mass spectrometer equipped with an electrospray ionization source and using an ion trap mass analyser. It was controlled by Xcalibur software (ThermoFinnigan). It was coupled to an HPLC system with a photodiode array detector (DAD) (Surveyor ThermoFinnigan) and an autosampler (Surveyor ThermoFinnigan). The conditions of analyses were: capillary temperature 250 °C; source voltage 4.0 kV, source current 80.0 μA, and capillary voltage 7.0 V in positive mode; source voltage 4.5 kV, source current 80.0 μA, and capillary voltage −45.0 V in negative mode. The elution conditions were similar to those used for the LC-DAD analysis.

3. Results and discussion

3.1. Comparison of extraction procedures

The *F. carica* leaves, rich in plant phenolics families, namely flavonols, coumarins and phenolic acids, were extracted by different extraction procedures, namely MSPD, SSDM, and SLE, in order to evaluate their extraction efficiency.

Several elution media were initially evaluated for the fig leaves phenolic extraction: dichloromethane, ethanol, methanol and mixtures of methanol:water (9:1, 7:3, 4:6, and 1:1, v/v). Considering the yields and number of compounds extracted, methanol: water mixtures were the most efficient eluents for all extraction methods tested. Among the different aqueous methanolic solutions, methanol:water (7:3, v/v) was the preferred eluent because it originated better chromatographic separation, likely because the extracts contained less unwanted matrix components (data not shown). Unlike to what might have been expected due to the chemical properties of octadecyl bonded silica (C_{18}), the use of methanol or methanol:water 9:1 (v/v) as eluents for the MSPD procedure, did not increased the extraction yields of the non-polar compounds.

Using methanol:water (7:3, v/v) as elution media, the *F. carica* leaves were extracted by SLE, MSPD with C_{18} derivatized silica, and SSDM, and the different extracts were analyzed by HPLC-DAD (see Fig. 1). A careful examination of the different extracts chromatograms reveals that compounds number 2 and 5 are not extracted by SLE, and higher amounts of those compounds seam to have been removed when SSDM is used.

LC-DAD and LC-DAD-MS/MS analysis of the extracts was performed and the mass and UV spectra obtained enabled the identification of some of the extracted compounds (see Table 1 for compound identification). Compound number 1 was identified as chlorogenic acid [28–30], and compound number 4 as rutin [28,29,31]. Chlorogenic acid (5-caffeoylquinic acid) is a phenolic acid very common in plants but, as far as we know, it has never been identified in *F. carica* leaves. Compounds number 7 and 8 were identified as psoralen [32] and bergapten [33], respectively, and these had already been identified in *F. carica* leaves [4]. The reported on-line mass spectra for these furanocoumarins has been obtained by the APCI interface [32,33], while here we used an ESI interface, and as expected, more fragments were observed. When a standard of psoralen was analyzed under ESI conditions a fragment at *m/z* 187 $[M + H^+]$ and also the acetonitrile adduct at *m/z* 228 were observed. Further fragmentation of parent ion at *m/z* 187, yield ions at *m/z* 143 $[M + H - CO_2{}^+]$, and *m/z* 115 $[M + H - CO_2 - CO^+]$. The peak in the extracts chromatograms with $r_t = 34$ min (compound number 7) was identified as psoralen as it yield a fragmentation pattern, an UV spectra, and a r_t similar to those obtained for the psoralen standard under similar conditions. The peak with $r_t = 38$ min (compound number 8) yield a parent ion at *m/z* 217. MS^2 of the parent ion yielded fragments at *m/z* 203 $[M + H - OMe^+]$ and *m/z* 173 $[M + H - CO_2{}^+]$. This compound was identified as bergapten, a furanocoumarin similar to psoralen, only with an extra methoxi group on carbon 5. The UV spectrum of this compound is similar to that reported on the literature for bergapten, and a fragment loss of a methoxy group (*m/z* 203) is also observed when the mass spectrum was obtained under APCI conditions [33].

In order to access the different extraction procedures efficiency, the peak areas for the different compounds, known and unknown, were evaluated (see Table 2 and Fig. 2). As it was

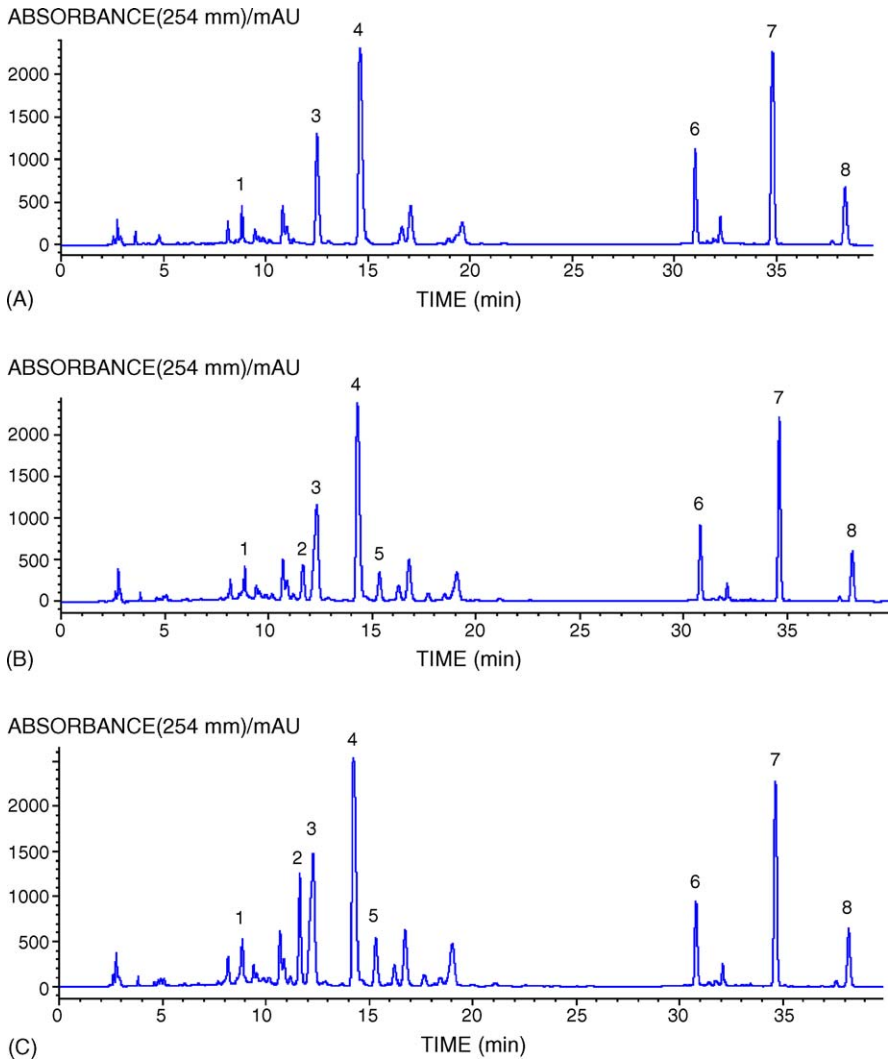

Fig. 1. LC-DAD chromatograms of methanol:water (7:3, v/v) extracts of *Ficus carica* leaves samples, using SLE (A), MSPD (B) and sea sand extraction method (C). Column: Zorbax Eclipse XBD-C_{18}. Elution conditions: Solvent A, acetonitrile; Solvent B water with acetonitrile (2.5%) and formic acid (0.5%). Gradient program: linear from 0 to 15% of solvent A (0–5 min), 15 to 20% of solvent A (5–25 min), 20 to 40% of solvent A (25–30 min) and from 40 to 45% of solvent A (30–40 min). Peak identification: chlorogenic acid (1); rutin (4); psoralen (7); bergapten (8); unknown (2, 3, 5, and 6).

Table 1
Identification of known compounds in the leaves of *Ficus carica* by HPLC-DAD and HPLC-ESI-MS

Compd	Identity	HPLC-DAD λ_{max}	ESI full scan MS (−) *m/z*	ESI-MS^2 (−) *m/z*	ESI full scan MS (+) *m/z*	ESI-MS^2 (+) *m/z*
1	Chlorogenic acid (5-*O*-caffeoylquinic acid)	241 305sh 326	353 $[M-H]^-$ 707 $[2M-H]^-$	191 $[M-C_9H_6O_3-H]^-$	355 $[M+H]^+$	163 $[C_9H_6O_3]^+$
4	Rutin (quercetin-3-*O*-rutinoside)	254 355	609 $[M-H]^-$	301 [M − rutinoside − H]$^-$	611 $[M+H]^+$	465 [M − rhamnose + H]$^+$ 303 [M − rutinoside + H]$^+$
7	Psoralen	252 300 332sh	–	–	187 $[M+H]^+$ 228 $[M+H+CH_3CN]^+$	143 $[M+H-CO_2]^+$ 115 $[M+H-CO_2-CO]^+$
8	Bergapten (5-Methoxypsoralen)	252 256 268 312	–	–	217 $[M+H]^+$	203 [M + H − OMe]$^+$ 173 $[M+H-CO_2]^+$

Table 2
Evaluation of the precision on the SLE, MSPD and sea sand method extraction and LC analysis of plant phenolic compounds from the leaves of *Ficus carica*

Compound (peak number)	SLE: peak area[a] (mAUs)		MSPD C_{18}: peak area[a] (mAUs)		Sea sand extraction method: peak area[a] (mAUs)	
	Mean[b] (SD)[c]	% RSD[d]	Mean[b] (SD)[c]	% RSD[d]	Mean[b] (SD)[c]	% RSD[d]
Chlorogenic acid (1)	2896.10[1] (104.72)	3.62	3353.39[2] (143.68)	4.28	3781.76[3] (89.81)	2.37
Unknown (2)	not extracted	–	10727.22[1] (1360.30)	12.68	11724.30[1] (676.39)	5.77
Unknown (3)	12049.21[1] (493.37)	4.09	16581.06[2] (844.34)	5.09	18803.66[3] (266.74)	1.42
Rutin (4)	26165.62[1] (1117.55)	4.27	22392.37[2] (1994.97)	8.91	26395.22[1] (1523.60)	5.77
Unknown (5)	not extracted	–	5592.16[1] (869.91)	15.56	6043.68[1] (157.70)	2.61
Unknown (6)	3135.77[1] (271.74)	8.67	4380.25[2] (942.75)	21.52	6402.02[3] (178.54)	2.79
Psoralen (7)	21516.19[1] (454.34)	2.11	15117.56[2] (2913.06)	19.27	20050.65[1] (1054.80)	5.26
Bergapten (8)	6348.99[1] (441.13)	6.95	2770.76[2] (361.68)	13.05	5721.95[1] (428.21)	7.48

[a] Normalized to 500 mg of leaves extracted, sample dried and redissolved in 5 mL of methanol:water (7:3, v/v); 20 μL injection.
[b] The values represent the mean of three replicate measurements on the five different extracts. For each compound means with different index numbers are significantly different (ANOVA: single factor Microsoft® Excel 2000, $P < 0.001$).
[c] Standard deviation of a single measurement.
[d] Relative standard deviation.

stated before, unknowns 2 and 5 are only extracted by the disruption methods, and statistical analysis by ANOVA-single factor of the peak areas of these compounds indicate that efficiency of the two methods was not significantly different. This is likely due to the fact that, for these compounds, the difference between average peak areas obtained with the two extraction procedures is smaller then their replicate error. The MSPD extractions were much less reproducible and the average extraction yields for compounds 2 and 5 were affected by the large RSD values for this method. When compared with SLE, both disruption methods were more efficient in the extraction of chlorogenic acid and unknowns 3 and 6, with their extraction yields being significantly higher when the sea sand method was used.

For rutin, psoralen and bergapten the conventional SLE method gave similar results to SSDM, whereas the MSPD C_{18} efficiency was significantly lower. The extraction efficiency for these compounds is probably controlled by the higher contact time with the solvent (in SLE), or by a more effective sample architecture disruption (in SSDM). The lower efficiency of the MSPD method might also be due to strong interactions between the non-polar furanocoumarins with the C_{18} materials.

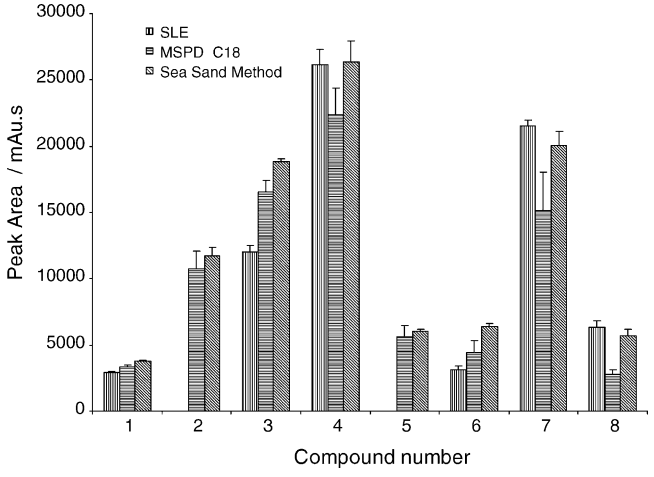

Fig. 2. Comparison between SLE, MSPD (C_{18}) and sea sand extraction of *Ficus carica* leaves samples with methanol:water (7:3, v/v). Conditions: 500 mg of plant; agitation at room temperature with 20 mL of solvent for 24 h in SLE; 2000 mg of C_{18} or sea sand eluted with 20 mL of solvent in MSPD and sea sand extraction method; all extracts dried, redissolved in 5 mL of methanol:water (7:3, v/v) and analyzed by LC-DAD. Compound identification: see Table 1.

The higher extraction efficiency observed for the majority of the most polar compounds (with the exception of rutin) when MSPD and SSDM methods are used is likely due to the sample disruption which, by exposing the cell components to the solvent, tend to yield richer extracts. Another important factor for the SSDM extraction efficiency is probably the lack of chemical interactions between the sand and the analytes. To verify this hypothesis the chemical composition of the sand and the degree of sample disruption were analyzed.

The chemical composition of the sea sand used was determined by petrographic microscopy and X-ray diffraction analysis. The microscopic pictures (data not shown) indicated that the sand was mainly composed by quartz, with minor amounts of mollusc shells and sandstone aggregates. X-ray diffraction analysis confirmed the mineralogical composition of this sand, which is mainly quartz with traces of orthoclase and calcite (data not shown). The degree of sample disruption attained by SSDM and MSPD was evaluated by optical microscopy (OM) (data not shown). The abrasive properties of sand seem to provide a more efficient disruption of the plant material, breaking it in smaller pieces and in this way exposing, in a more efficiently manner, the cell plant components to the eluents. These results seem to corroborate the idea that the high extraction efficiency observed with the SSDM method is probably due to a combination of two factors: very effective sample disruption and lack of chemical interactions between the analytes and the solid support.

3.2. Determination of optimum elution volume for sea sand extraction

The assays performed for the determination of the optimum elution volume were done using the optimized conditions for SSDM extraction using methanol:water (7:3, v/v) as elution solvent. The peak areas of all analyzed compounds were statistically evaluated for various volumes of the elution solvent. The

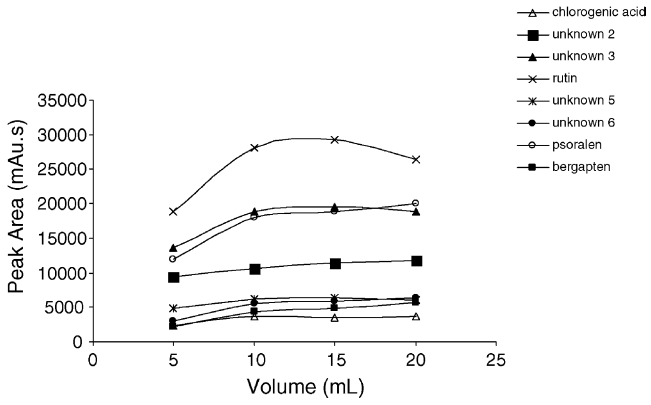

Fig. 3. LC peak area variation of the compounds extracted from *Ficus carica* leaves samples by sea sand method with increasing methanol:water (7:3, v/v) volume. Conditions: 500 mg of plant; 2000 mg of sea sand eluted with 5.0, 10.0, 15.0 and 20.0 mL of methanol:water (7:3, v/v); all extracts dried, redissolved in 5 mL of methanol:water (7:3, v/v) and analyzed by LC-DAD.

data presented in Fig. 3 and Table 3 show that maximum yields for all compounds, except unknown 2, were obtained with 10 mL of solvent. Maximum yields of unknown 2 was obtained with only 5 mL. Extraction of this compound is only accomplished by the disruption methods, and maximum yields are obtained with less solvent. Probably, this due to the fact that unknown 2 is one of the most polar compounds extracted, and it is likely to have a high affinity with the chosen eluent. Chlorogenic acid is another polar extracted compound, and a similar behavior should also be expected. In fact, when elution was performed with 5 and 10 mL of eluent, the difference in peak areas for the chlorogenic acid was so small that could only be recognized when the data was subjected to statistical analysis.

3.3. Validation: reproducibility and recovery

The data presented in Table 2 demonstrate that SLE and SSDM procedures are reproducible as RSD values were less than 5% for almost all compounds. However, when MSPD C_{18} was used the RSD values were higher. The small reproducibility of the MSPD method together with the cost of the C_{18} materials makes it much less attractive than the sea sand method.

To evaluate the sea sand disruption method recovery, spiking experiments were performed for three known compounds, chlorogenic acid, rutin and psoralen. The mean peak area of the three spiked compounds was calculated by subtracting the total peak area after spiking from the mean peak area in the extract of the plant before spiking. Calibration curves for the compounds were constructed using the standard solutions prepared. The characteristic data, the correlation coefficients and the errors of estimation of slope and intercept parameters are listed in Table 4. The recoveries were 97.3, 85.7 and 86.2% for chlorogenic acid, rutin and psoralen, respectively.

The limits of detection (LOD) were estimated as 0.0515, 0.0310 and 0.0342 mg/g for chlorogenic acid, rutin and psoralen, respectively and corresponding to the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank [34]. The limits of quantification (LOQ)

Table 3
Evaluation of the precision on the optimal volume determination in SSDM extraction with methanol:water (7:3, v/v), and LC analysis of plant phenolics from the leaves of *Ficus* carica

Compound (peak number)	Volume of elution media (mL): peak area[a] (mAUs)							
	5.0		10.0		15.0		20.0	
	Mean[b] (SD)[c]	% RSD[d]	Mean[b] (SD)[c]	% RSD[d]	Mean[b] (SD)[c]	% RSD[d]	Mean[b] (SD)[c]	% RSD[d]
Chlorogenic acid (1)	2423.26[1] (82.01)	3.38	3691.46[2] (147.98)	4.01	3581.78[2] (351.14)	9.80	3781.76[2] (89.81)	2.37
Unknown (2)	9414.86[1] (704.70)	7.49	10653.30[1] (1002.62)	9.41	11487.66[1] (937.86)	8.16	11724.30[1] (676.39)	5.77
Unknown (3)	13642.98[1] (213.66)	1.57	18926.54[2] (655.80)	3.47	19442.28[2] (1246.36)	6.41	18803.66[2] (266.74)	1.42
Rutin (4)	18928.77[1] (262.77)	1.39	28016.93[2] (1355.05)	4.84	29318.38[2] (1735.65)	5.92	26395.22[2] (1523.60)	5.77
Unknown (5)	4958.77[1] (229.36)	4.63	6152.11[2] (286.39)	4.66	6439.38[2] (555.75)	8.63	6043.68[2] (157.70)	2.61
Unknown (6)	3090.46[1] (185.07)	5.99	5518.76[2] (231.15)	4.19	5926.72[2] (834.53)	14.08	6402.02[2](178.54)	2.79
Psoralen (7)	11918.90[1] (899.85)	7.55	18043.82[2] (919.82)	5.10	18817.75[2] (2083.83)	11.07	20050.65[2] (1054.80)	5.26
Bergapten (8)	2217.65[1] (242.48)	10.93	4366.32[2] (353.75)	8.10	4866.85[2] (772.74)	15.88	5721.95[2] (428.21)	7.48

[a] Normalized to 500 mg of fig leaves extracted, sample dried and redissolved in 5 mL of methanol:water (7:3, v/v); 20 μL injection.
[b] The values represent the mean of three replicate measurements on the five different extracts. For each compound means with different index numbers are significantly different (ANOVA: single factor Microsoft® Excel 2000, $P < 0.001$).
[c] Standard deviation of a single measurement.
[d] Relative standard deviation.

Table 4
Calibration curves, recovery, LOD and LOQ of spiking experiments of phenolic compounds in the leaves of *Ficus carica*

Compound	*a* (estimation error)[a]	*b* (estimation error)[a]	R^2	Recovery (%)	LOD (mg/g)	LOQ (mg/g)
Chlorogenic acid	−41.744 (−123.770; 40.283)	17.205 (15.851; 18.560)	0.997	97.3	0.0515	0.1888
Rutin	19.945 (−60.753; 100.642)	28.127 (26.795; 29.460)	0.999	85.7	0.0310	0.1137
Psoralen	29.301 (−92.209; 150.812)	38.419 (36.412; 40.426)	0.999	86.2	0.0342	0.1253

[a] Upper and lower 95%confidence limits (regression statistics Microsoft® Excel 2000).

were estimated as the analyte concentration giving a signal equal to the blank signal plus eleven standard deviations of the blank [35]. The corresponding LOQ values were 0.1888 mg/g for chlorogenic acid, 0.1137 mg/g for rutin and 0.1253 mg/g for psoralen.

4. Conclusions

The data presented here show that the solid disruption methods compare favorably to SLE in the extraction of several phenolic compounds belonging to different families, namely, phenolic acids, flavonols and coumarins, from the leaves of *F. carica*. More compounds and higher yields were obtained by these methods, using smaller amounts of solvents, and less sample preparation time.

The optimized extraction procedure involves the use of sea sand as solid support, and methanol:water (7:3, v/v) as elution media. Higher extraction yields and smaller RSD values were obtained with SSDM when compared with MSPD.

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