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Comparison of methods for extraction of flavanones and xanthones from the root bark of the osage orange tree using liquid chromatography[1]

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Abstract

This study compares conventional solid–liquid extraction, supercritical fluid extraction (SFE), and pressurized fluid extraction (PFE) for their efficiency in extracting xanthones and flavanones from the root bark of the osage orange tree (*Maclura pomifera*). Seven compounds were extracted from the plant material by solvent extraction at room temperature for 48 h. The same compounds were removed from the root bark by 45- and 35-min extractions using SFE and PFE, respectively, and under optimized conditions, in same or higher yields than those obtained by the conventional 48-h solvent extraction. Although all seven compounds were present in the SFE extracts when only CO_2 was used as the fluid, the addition of 20 vol.% methanol (MeOH) to the CO_2 proved essential for achieving high yields. Use of SFE with CO_2–MeOH also led to the recovery of an additional flavanone from a wet sample of root bark. This flavanone is absent from the conventional solvent extracts and appears in small amounts in the PFE extracts. An optimized LC separation for the analysis of the different extracts is presented, and it is demonstrated that the separation of xanthones and flavanones is considerably improved by the use of deactivated C_{18} columns in conjunction with a mobile phase containing acetonitrile and a weak organic acid.

Keywords: Flavanones; Xanthones

1. Introduction

Maclura pomifera Raf., Moracea, commonly known as the osage orange tree, grows extensively throughout the midwestern and southwestern regions of the United States. Several phenolic compounds have been isolated and identified from various parts of this plant, namely, isoflavonoids from the fruit [1,2], flavonols and xanthones from the heartwood and stem bark [3–5], and flavanones and xanthones from the root bark [6–10]. Xanthones, unlike the other compounds, are known to occur in only a few families of plants [11], and are of interest not only for their obvious importance in chemotaxonomy, but more importantly for their pharmacological proprieties, including in vitro cytotoxic and antitumor

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activity [12,13], potential as antidepressive drugs, [14] and as potential antimalarial agents [15].

Solvent extraction and steam distillation are the traditional methods for extraction of natural products from plants, but they are labor-intensive, time-consuming, and require large volumes of solvents. With the demand for more environmentally friendly methods and increased productivity, newer extraction techniques have been developed, including supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE). PFE is conducted at elevated pressures allowing liquid extraction at temperatures above the boiling points of the solvents, thereby improving analyte solubilities and the kinetics of their desorption from the matrices [16]. Advantages over the conventional extraction methods include shorter extraction times and a decrease in solvent consumption [16]. PFE has been found to be a suitable alternative to Soxhlet extraction for the removal of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) from environmental samples [17], but we are not aware of previous reports on its use in the extraction of plant material. Supercritical fluid extraction (SFE) is a more widely used technique and, because of its intrinsic features, seems to be particularly amenable to the extraction of natural products from plant sources [18–20]. Degradation through lengthy exposure to elevated temperatures and atmospheric oxygen are avoided, and extracts with fewer unwanted analytes may be obtained by careful manipulation of the SFE conditions (pressure, temperature, and use of modifiers). The majority of reports on natural products extracted with SFE from plant sources [18–20] involve lipids, terpenes and alkaloids. Very few reports appear in the literature concerning the extraction of plant phenolics by SFE. Manabe and co-workers [21–23] applied SFE to extract lignans, chalcones, and prenylflavonoids from crude drugs and plants. Some compounds were extracted with CO_2 alone, while others required the addition of ethanol as a modifier. In 1995 Cocks et al. [24] demonstrated the feasibility of using SFE for extracting xanthones; they recovered Sydowinin B and a related epoxide from the fermentation broth of *Aspergillus fumigatus* using CO_2 modified with 20 vol.% MeOH.

The separation of plant phenolics is conventionally performed by reversed-phase liquid chromatography (LC), and different types of reversed-phase columns and elution systems have been used for separating both flavanones [25] and xanthones [26].

In this report we evaluate three different extraction techniques, namely, solid–liquid extraction, PFE, and SFE for isolating the xanthones and flavanones present in the root bark of the *Maclura pomifera* tree. The extracts obtained by these different methods were compared by LC analysis using diode array absorbance detection. A variety of reversed-phase C_{18} stationary phases and elution conditions were studied to optimize the LC separation of the analytes. The chemical identity of each analyte was inferred from comparison of its absorbance spectrum and mass spectrum to published data for known compounds [6–10].

2. Experimental

2.1. Chemicals

Methanol, acetonitrile and dichloromethane (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Anhydrous diethyl ether (analytical grade) was obtained from EM Science (Gibbstown, NJ, USA). Formic acid 88%, was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Water from a Millipore Milli-Q system (Bedford, MA, USA) was used for sample preparation and LC analysis.

The carbon dioxide (SFE/SFC grade) was obtained from Air Products (Allentown, PA, USA). The drying/dispersing agent, Wetsupport™, a diatomaceous earth, was purchased from Isco (Lincoln, NE, USA) and was extracted with dichloromethane before being used.

2.2. Sample preparation

The roots of the *Maclura pomifera* tree were collected on the grounds of the National Arboretum in Washington, DC, in June of 1997. The bright orange, paper-thin root bark was peeled off, air-dried for 3 days, and then stored in an air-tight container at 4°C. A food processor was used to grind the bark into fine particles. Wet samples of the root bark were

prepared by soaking 150 mg of ground bark in 1 ml of water for 3–6 h.

The samples used for SFE and PFE were prepared by mixing 150 mg of ground root bark (dried or wet) with approximately 2.5 g of drying/dispersing agent (Wetsupport™). To determine if SFE and PFE were complete, the root bark samples were sequentially extracted a second time by the same method, and the extract was collected in a separate vial. All extracts were dried under a stream of nitrogen, redissolved in 1 ml of acetonitrile, and filtered through a 0.45-μm nylon filter. The filtrate was analyzed by LC.

The same batch of ground bark was used with the different extraction techniques, and the data presented herein represent a single extraction for each individual procedure. The reproducibility of the analytical methods and the repeatability of the extraction procedures were evaluated. A new batch of root bark was ground, and six dry samples were prepared. Three samples were extracted by SFE at 80°C with CO_2 modified with 20 vol.% MeOH, while the other three samples were extracted by PFE with dichloromethane at 80°C. Three replicate LC analysis were performed on each SFE and PFE extract.

2.3. Solid–liquid extraction

Samples of 150 mg of dry or wet root bark were soaked in 20 ml of diethyl ether or dichloromethane for 24 h. Each sample was extracted twice and the extracts combined. Two samples of dry root bark were extracted with dichloromethane (3×12 ml) for a total of 35 min. Ultrasonic agitation was applied to one of the samples.

2.4. Pressurized fluid extraction

An Accelerated Solvent Extractor™ from Dionex Corporation (Sunnyvale, CA, USA) was used for the PFE extractions. The samples were placed in an 11-ml stainless steel cell and extracted with dichloromethane. The extractions were performed at 13.8 MPa, with 5 min equilibration, 5 min static time, and a 90-s purge for a total of three cycles. The extractions were done at three temperatures, 40, 80 and 100°C. About 15 ml of solvent were used for each extraction.

2.5. Supercritical fluid extraction

The SFE experiments were performed on a Isco model 3560 SFE (Lincoln, NE, USA). The samples were extracted at a flow-rate of 1.5 ml/min and 40.5 MPa. The extracts were collected in vials containing 15 ml of dichloromethane. Six different extraction methods were used:

Method A (CO_2): extraction chamber temperature, 40°C; restrictor temperature, 40°C; static extraction for 15 min followed by 30 min of dynamic extraction.

Method B: same as method A, but extraction chamber temperature, 80°C; and restrictor temperature, 60°C.

Method C: same as method A, but extraction chamber temperature, 100°C; and restrictor temperature, 60°C.

Methods D, E and F: same as methods A, B, and C, respectively, but using CO_2 modified with 20 vol.% MeOH.

2.6. Liquid chromatography

LC separations were performed with a Varian Model 5000 chromatograph (San Fernando, CA, USA) coupled with a Waters 990 photodiode array absorbance detector (Milford, MA, USA). Five reversed-phase columns were evaluated: Zorbax Rx-C_{18}, Zorbax ODS-C_{18}, and Zorbax Eclipse XDB-C_{18}, obtained from MAC-MOD Analytical (Chadds Ford, PA, USA); SMT OD-5-100 was obtained from Separations Methods Technologies (Newark, DE, USA), and Capcell C-18 was obtained from Phenomenex (Torrance, CA, USA). All columns were 250×4.6 mm (length×I.D.) with 5 μm nominal particle size.

Three elution systems were used to evaluate the LC separation of the analytes.

System A: Solvent A, water–acetonitrile (5:1, v/v); Solvent B, acetonitrile; linear gradient from 40 to 60% B, 0–20 min; and then isocratic at 60% B, 20–30 min.

System B: Solvent A, formic acid–water (5:1000, v/v) and acetonitrile (5:1, v/v); Solvent B, acetonitrile; linear gradient from 40 to 60% B, 0–20 min; and then isocratic at 60% B, 20–30 min.

System C: Solvent A, formic acid–water (5:1000,

v/v) with methanol (5:1, v/v); Solvent B, methanol. Isocratic elution with 25% A and 75% B, 0–30 min.

LC analyses were performed at room temperature (21°C); the injection volume was 10 μl, and the flow-rate was 1.5 ml/min; the absorbance was monitored with a diode array absorbance detector between 240 and 450 nm.

2.7. Identification of the compounds

The chemical identity of each analyte was inferred by comparing the mass spectrometric and UV spectroscopic data with published data for known compounds [6–10]. The diode array detector was scanned between 240 and 450 nm, thus providing the absorbance spectrum of each individual peak. The electron ionization (EI) mass spectrum of each compound was determined on a Jeol JMS-700 mass spectrometer (Peabody, MA, USA). The individual compounds were obtained by collecting and combining the material of each peak from several LC runs and the solvent was removed by lyophilization. The lyophilized compounds were dissolved in dichloromethane and inserted by direct probe. The EI was set at 70 eV, the ion source chamber temperature was set at 200°C and the scan range was 50–600 (m/z).

3. Results and discussion

It was necessary to develop an optimized method for the analysis of the different extracts to permit evaluation of how SFE, PFE and conventional solvent extraction compare as methods for recovery of plant phenolics from the root bark of the *Maclura pomifera* tree.

Although never reported for the separation of extracts from this plant material, reversed-phase LC is a commonly used technique for the determination of flavanones and xanthones [25,26], two families of compounds previously identified in the root bark of the osage orange tree (see Fig. 1). Various reversed-phase supports and mobile-phase systems have been reported [25,26]. Previous work done in our laboratory has shown that the use of acid-containing mobile phases in conjunction with deactivated monomeric C_{18} columns can significantly improve the separation of some classes of bioflavonoids [27]. A variety of C_{18} stationary phases and gradient systems were evaluated to determine whether similar chromatographic behavior was observed for the xanthones and flavanones.

3.1. Comparison of LC columns and optimization of elution systems

The Zorbax RX-C_{18} column, a deactivated reversed-phase C_{18} column, was the first column evaluated, and three different elution systems (systems A, B and C) were developed for separation of the analytes of a root bark extract obtained by a solid–liquid extraction with dichloromethane. The absence of acid in elution system C and use of methanol in elution system C causes coelution and peak broadening (data not shown). Optimum separation of our analytes was achieved with elution system B, containing acetonitrile and 0.5 vol.% formic acid (Fig. 2A). Three other deactivated reversed-phase C_{18} columns (Zorbax Eclipse XDB-C_{18}, SMT OD-5-100, Capcell C_{18}) and a non-deactivated reversed-phase deactivated reversed-phase C_{18} column (Zorbax ODS) were also evaluated using elution system B. Similar peak shapes was observed for all four C_{18} deactivated columns (data only shown for the Zorbax RX-C_{18} column in Fig. 2A). For this particular mixture of analytes each column exhibited different selectivity, and in some columns the elution order of the analytes was reversed while in others coelution occurred. Severe peak tailing was observed with the Zorbax ODS, a non-deactivated column (Fig. 2B). This is probably due to interactions between the flavanones and xanthones with accessible acidic silanols, which can be minimized by using the deactivated columns.

In conclusion, of the systems evaluated, the Zorbax RX-C_{18} column and the acetonitrile-weak organic acid solvent (elution system B) gave optimum separation of the plant phenolics present in the root bark, and they were used to evaluate the extracts obtained by the different extraction procedures.

3.2. Comparison of extraction procedures

The root bark was ground in heterogeneous batches and we observed that, although the relative ratio

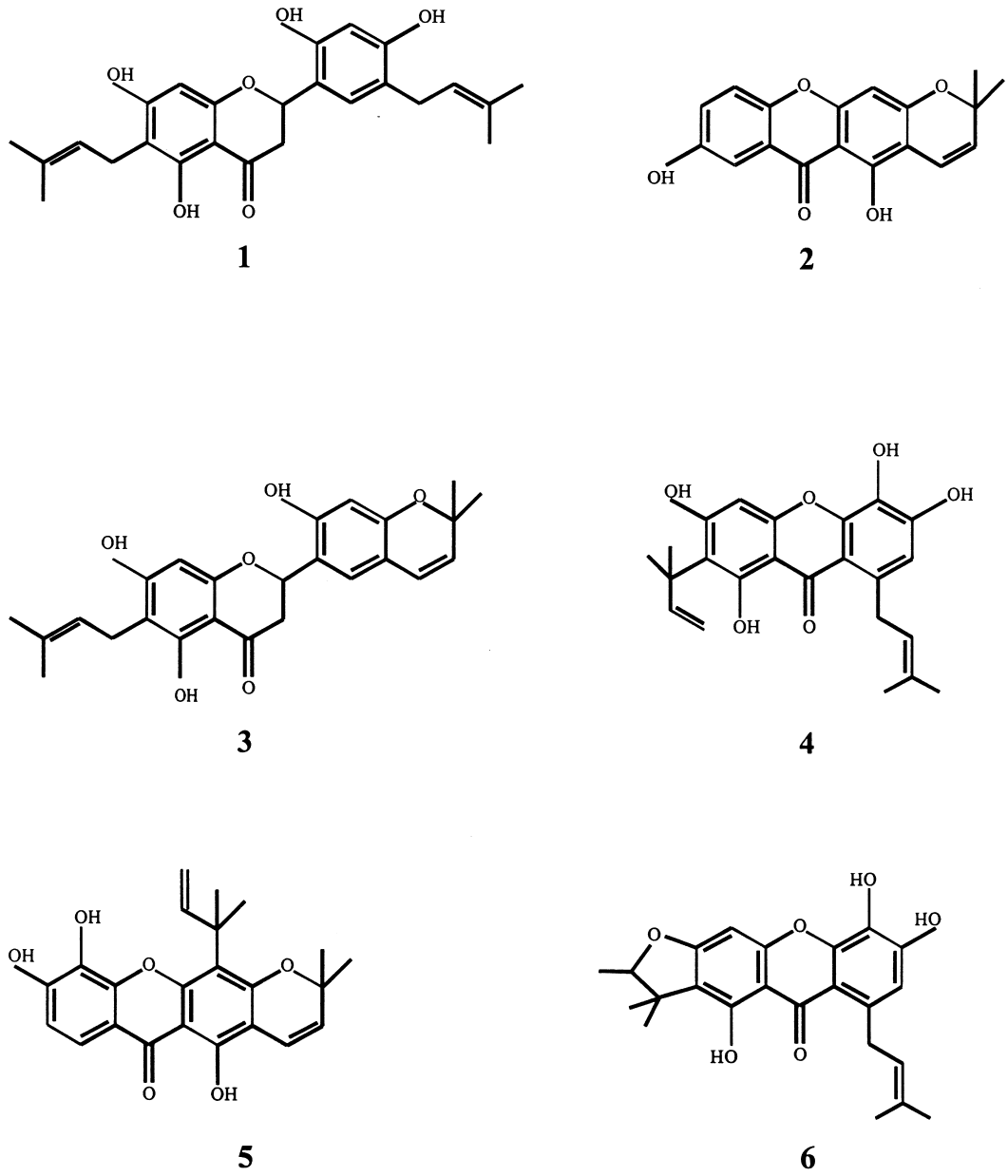

Fig. 1. Structure of flavanones and xanthones from the root bark of osage orange tree, *Maclura pomifera* [6–10]. Euchrestaflavanone B (**1**); osajaxanthone (**2**); euchrestaflavanone C (**3**); alvaxanthone (**4**); macluraxanthone (**5**); 8-prenyltoxyloxanthone (**6**).

of the different compounds extracted was dependent on the extraction procedure, the overall yield was dependent on the batch of bark that was being used. This is probably due to the fact that the root bark was obtained from both large and small roots which may be different in their content of plant phenolics. Therefore, in order to compare the efficiency of the different extraction techniques the same batch of ground root bark was used.

As mentioned in Section 1, solvent extraction is the traditional method for the removal of natural products from plant material. Wolfrom et al. [6] and

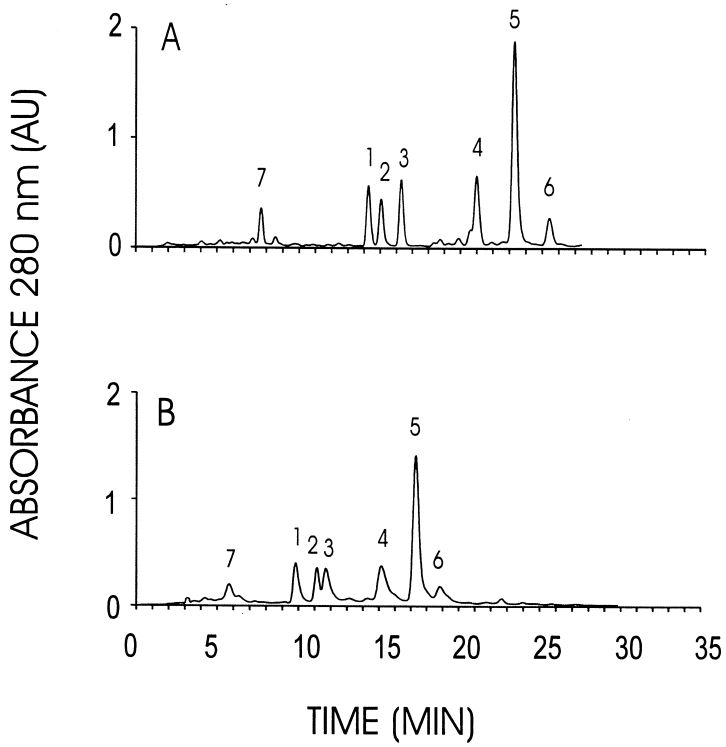

Fig. 2. Comparison of the chromatographic separation of phenolic compounds in a dichloromethane extract of root bark from the osage orange tree on two reversed-phase columns. Elution conditions: solvent A, formic acid–water (5:1000, v/v) and acetonitrile (5:1, v/v); solvent B, acetonitrile; linear gradient from 40 to 60% B, 0–20 min; isocratic at 60% B, 20–30 min. Columns: chromatogram A, Zorbax RX-C_{18}; chromatogram B, Zorbax ODS. Peak identification: euchrestaflavanone B (**1**); osajaxanthone (**2**); euchrestaflavanone C (**3**); alvaxanthone (**4**); macluraxanthone (**5**); 8-prenyltoxyloxanthone (**6**), unknown (**7**).

Delle Monache et al. [10] used diethyl ether and dichloromethane, respectively, for extracting the root bark of the *Maclura pomifera* tree at room temperature. These extractions were repeated in our laboratory, yielding yellow-orange extracts, and both solvents proved to be almost equivalent (see Fig. 3). The nature of the six compounds previously identified in the root bark [6–10] was confirmed by their absorbance spectra and mass spectra (see Fig. 1 for their chemical structures and Fig. 2A for peak identification). A compound that has not been previously identified on this plant material (peak 7 in Fig. 2A) was also extracted; its absorbance spectrum suggests that it is a xanthone, and elucidation of its complete structure is currently under study.

Addition of a small amount of water to a sample prior to SFE extraction has been shown to sometimes improve the recovery of PHAs and PCBs from sediments [28]. We found this procedure useful in our SFE experiments, but there was no significant change on the yields of all seven analytes when both diethyl ether and dichloromethane were used to extract a wet sample of root bark at room temperature (data not shown).

SFE which has been widely applied for the extraction of plant material, has seldom been used to extract plant phenolics [20–24]. This is probably because carbon dioxide, the most commonly used supercritical fluid in SFE, has a very low effective polarity and therefore is not a good solvent for the highly polar plant phenolics. The use of modifiers, such as ethanol or methanol, can improve the solubility of the more polar compounds in supercritical fluid, but sometimes this is still insufficient. Miyachi et al. [21] reported that when more than four hydroxyl groups were present in a pre-

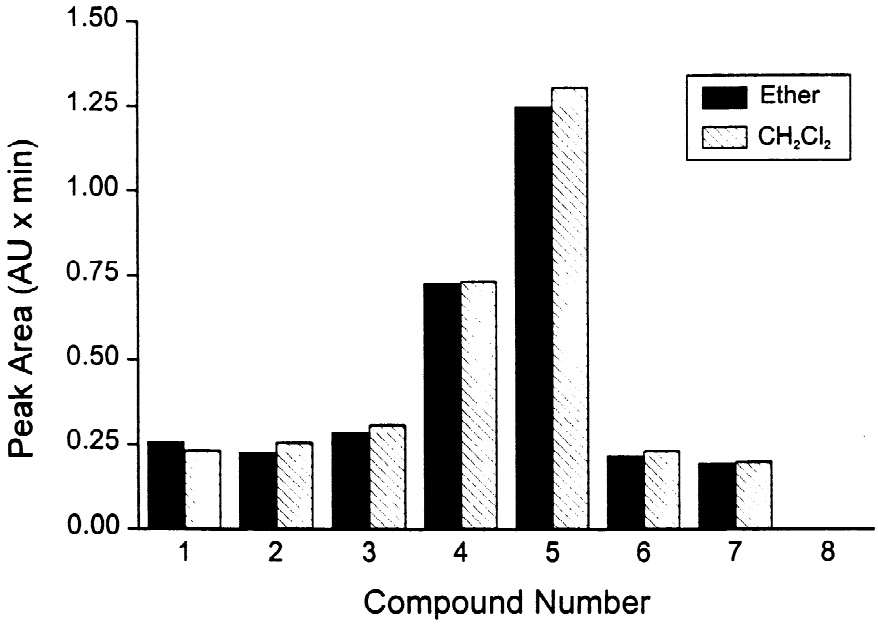

Fig. 3. Solid–liquid extraction of dry samples of root bark from the osage orange tree with ether or dichloromethane. Conditions: room temperature; 2×20 ml solvent; 2×24 h. Compound identification: see Fig. 1, compounds (**7**) and (**8**) are unknown.

nylflavonoid structure, not even the addition of ethanol to the supercritical CO_2 permitted their extraction from plant material. The compounds already identified from the root bark of *Maclura pomifera* (see Fig. 1) are all relatively non-polar (they have fewer than four hydroxyl groups) and therefore they should be extractable by SFE.

The first SFE experiments were performed with CO_2 alone at three temperatures, 40, 80 and 100°C. The extracts obtained were light yellow. When a dry sample of root bark was used, all seven compounds that had been extracted by conventional solvent extraction were also extracted by this technique, although less efficiently (compare Fig. 3 with Fig. 4A and B). The extractions performed at 80 and 100°C were more efficient than those conducted at 40°C (Fig. 4A). The addition of water in the sample before extraction did not seem to have a major effect on the extraction efficiency, except for compound 5 (compare Fig. 4A with 4B).

Each sample was sequentially re-extracted by the same method and the extracts were collected in separate vials. At 40°C, and for both dry and wet root bark samples, the amount of material collected in the second extraction vial (data not shown) was almost identical to that collected in the first extraction vial (Fig. 4); as the extraction temperature increased, the amount of material collected in the first vial increased and that collected in the second vial decreased (data not shown). This is probably due to the fact that with CO_2 at 40°C the kinetics of desorption of the compounds from the plant material are very slow. As the temperature increases the desorption is faster and almost all of the material that is extracted under this conditions is collected in the first vial.

In a second set of experiments, CO_2 modified with 20 vol.% methanol was used for extracting both dry and wet samples of the root bark, at 40, 80 and 100°C (Fig. 5A and B). The dry samples yield yellow-orange extracts, whereas the wet samples yield turbid, dark-brown extracts. The improvement in solubilizing power of this fluid can be seen by comparing Fig. 4A with Fig. 5A, and Fig. 4B with Fig. 5B. When a dry sample of root bark is extracted with the 20 vol.% MeOH, not only are the compounds extracted the same as those recovered by conventional solvent extraction, but their relative ratios are almost the same (compare Fig. 3 with Fig. 5A).

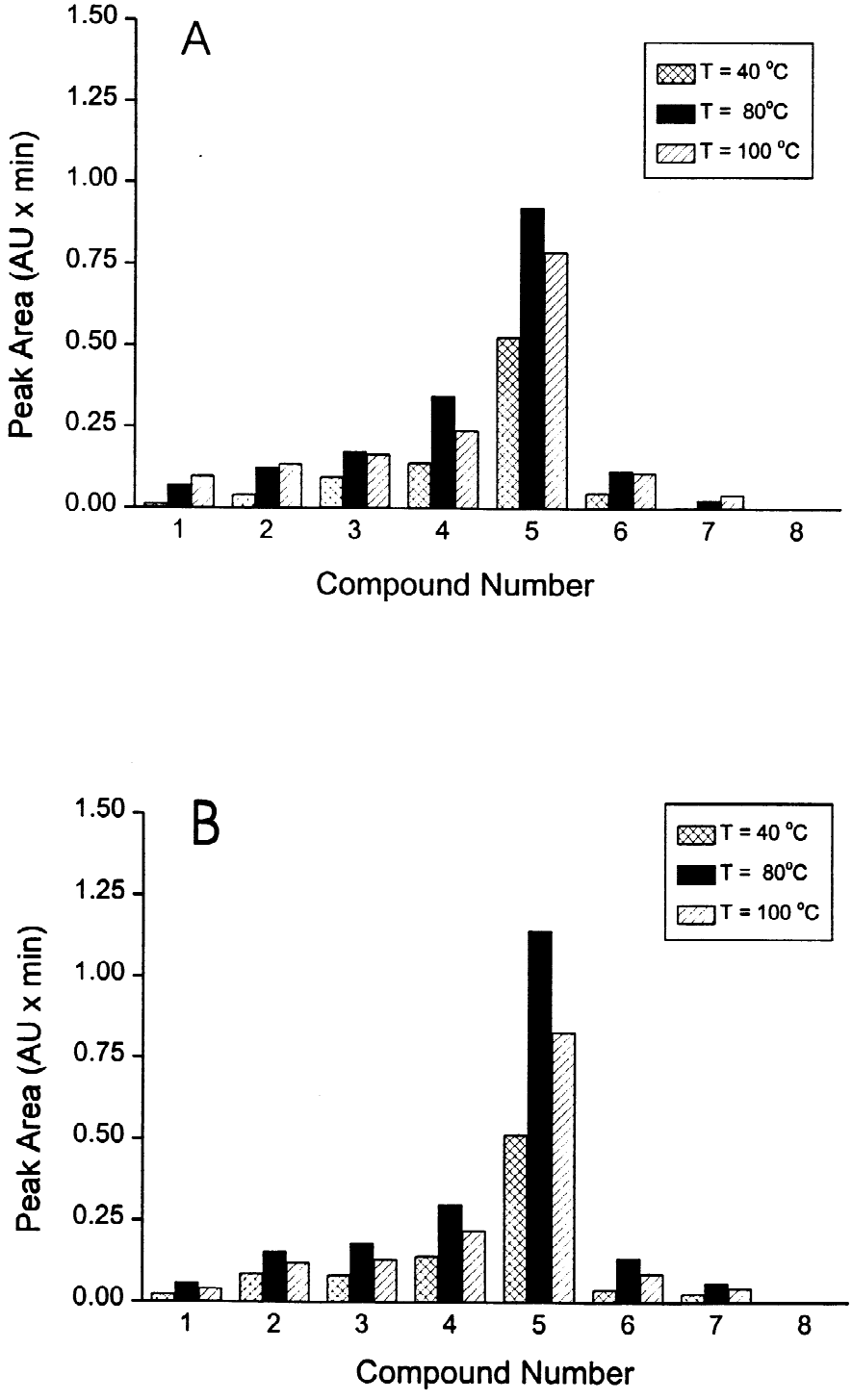

Fig. 4. SFE extraction of dry and wet samples of root bark from the osage orange tree with carbon dioxide at different temperatures. Conditions: 40.5 MPa; variable temperature; 0–15 min, static extraction, 15–45 min dynamic extraction. Bar graph A, dry sample. Bar graph B, wet sample. Compound identification: see Fig. 1, compounds (**7**) and (**8**) are unknown.

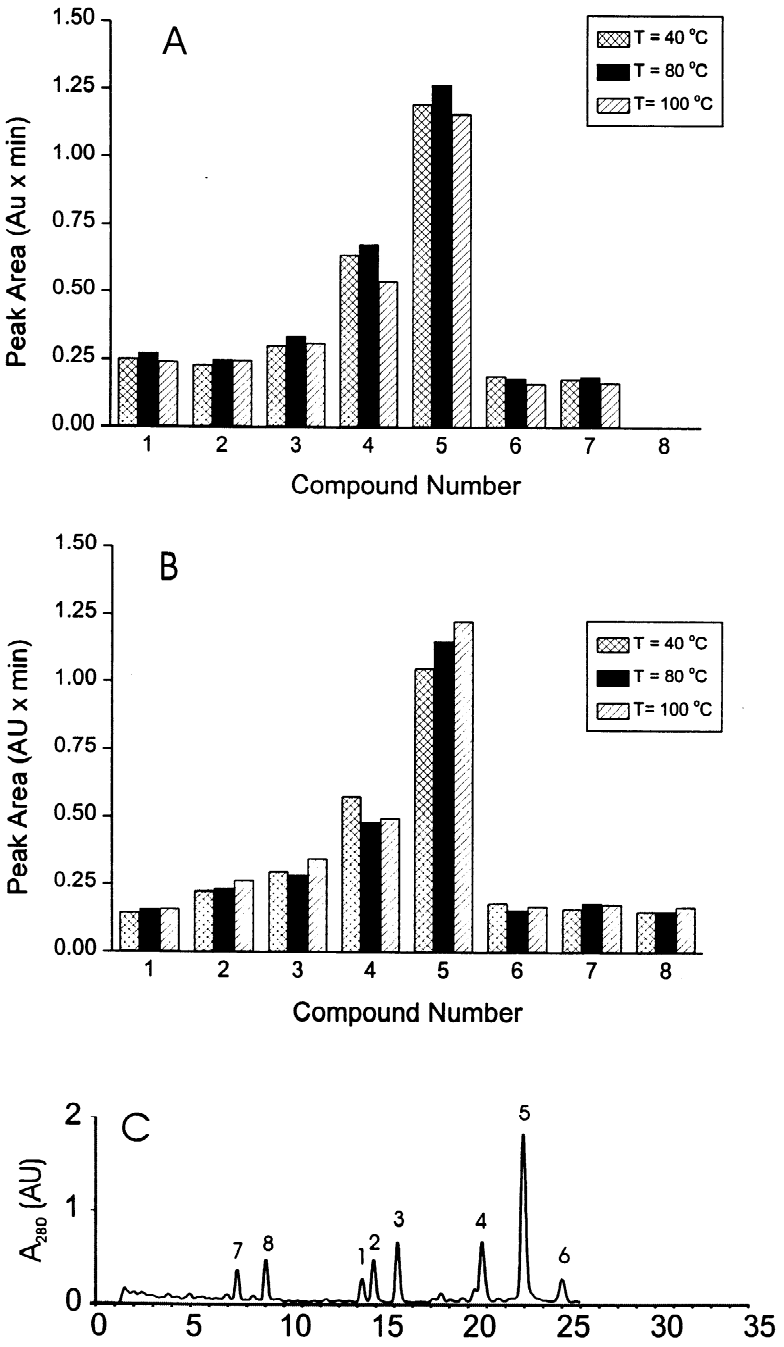

Fig. 5. SFE extraction of dry and wet samples of root bark from the osage orange tree with carbon dioxide–20% methanol at different temperatures. Conditions: 40.5 MPa; variable temperature; 0–15 min, static extraction, 15–45 min dynamic extraction. Bar graph A, dry sample. Bar graph B, wet sample. Compound identification: see Fig. 1, compounds (**7**) and (**8**) are unknown. Chromatogram C, LC analysis of extract from a wet root bark sample. Column, Zorbax RX-C_{18}; elution conditions, see Fig. 3. Peak identification: see Fig. 2 and compound (**8**) unknown.

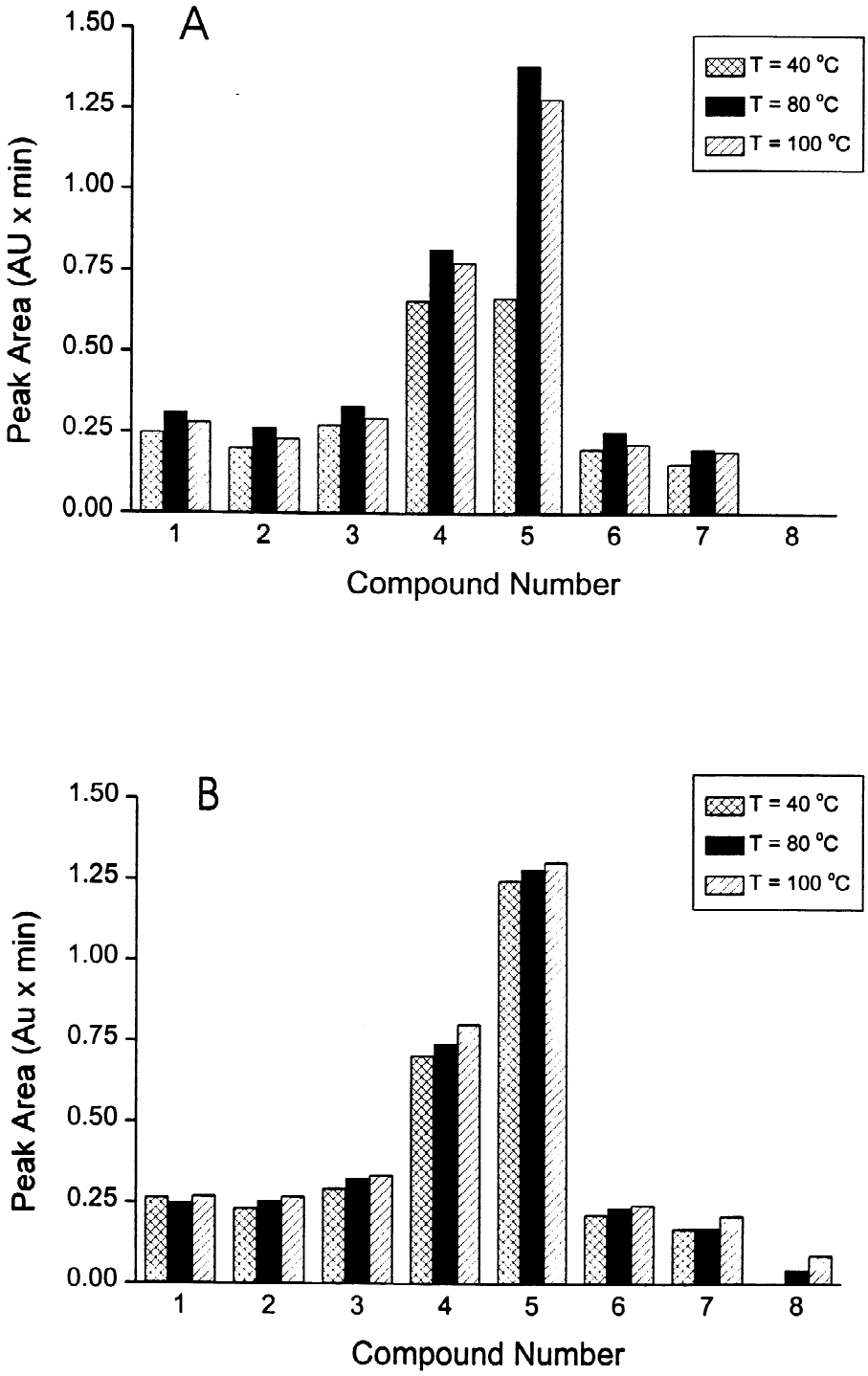

Fig. 6. PFE extraction of dry and wet samples of root bark from the osage orange tree with dichloromethane at different temperatures. Conditions: 13.8 MPa; variable temperature; three cycles: 5 min equilibration, 5 min static, and 90 s purge. Bar graph A, dry sample. Bar graph B, wet sample. Compound identification: see Fig. 1, compounds (**7**) and (**8**) are unknown.

When a wet sample of root bark was extracted with the 20 vol.% MeOH the efficiency of recovery for some of the compounds diminished (compare Fig. 5A with 5B). This is particularly observed for euchrestaflavanone B (compound 1), with the amount extracted being only half of the value obtained for a dry sample. There is, however, an advantage for using a wet root bark sample with this SFE procedure, and this is the recovery of a new component, peak 8 in Fig. 5C. This new component has an absorbance spectrum that corresponds to a flavanone and its complete structure elucidation is under study.

When the root bark samples are sequentially extracted with CO_2 modified with 20% MeOH, for each compound less than 10% of the amount collected in the first vial was collected in the second vial (data not shown). The modifier addition is essential for fast and complete removal of the analytes from the root bark by SFE.

Although we are not aware of previous reports of the use of PFE for the extraction of plant material, it has the potential to give results similar to those obtained with conventional solid–liquid extraction. The same organic solvents can be used in both techniques and, therefore, the problems of low recovery that may arise with the SFE fluid do not occur with PFE. When a dry sample of root bark was extracted by PFE with dichloromethane at 40, 80 and 100°C, the extracts obtained were yellow-orange. The extracts recovered at 80 and 100°C were very similar to those obtained by SFE with the modified fluid and solvent extraction (compare Fig. 6A with Fig. 5A and Fig. 3). When a wet sample was extracted with this technique, the extracts obtained were turbid and dark brown. The compound corresponding to peak 8 is present only in the extracts obtained at 80 and 100°C, but in smaller amounts than obtained by SFE with CO_2 modified with 20 vol.% MeOH (compare Fig. 5B with Fig. 6B).

The PFE and SFE required 35 and 45 min, respectively, while the solvent extraction required 48 h. Two samples of dry root bark were extracted with dichloromethane at room temperature for a similar period of time, 35 min. Ultrasonic agitation is known to improve extraction yields and was used in one of the samples. Compared to the 48-h static extraction, the yield was only approximately 50% for the 35-min sonicated sample, and 5% for the 35-min static extraction procedure.

To evaluate the precision on both the extraction procedure and the LC analytical technique, a series of replicate extractions and LC analyses were performed. The data presented in Table 1 demonstrate that the extraction procedures are reproducible at typically 1–5% within the same batch of ground root bark.

The results presented in this study prove that both

Table 1
Evaluation of the precision on the extraction and LC analysis of plant phenolics from the root bark of the osage orange tree

Compound no.	Extraction method			
	PFE[a]: peak area[c] (AU×min)		SFE[b]: peak area[c] (AU×min)	
	Mean[d] (S.D.)[e]	% R.S.D.[f]	Mean[d] (S.D.)[e]	% R.S.D.[f]
1	0.217 (0.008)	3.7	0.215 (0.005)	2.5
2	0.192 (0.006)	3.5	0.203 (0.007)	3.7
3	0.235 (0.011)	4.8	0.241 (0.005)	2.2
4	0.586 (0.025)	4.3	0.553 (0.013)	2.4
5	1.054 (0.049)	4.7	1.054 (0.025)	2.4
6	0.182 (0.010)	5.6	0.175 (0.002)	8.9
7	0.146 (0.004)	3.1	0.150 (0.003)	2.5
8	Not extracted		Not extracted	

[a]Dry samples extracted with dichloromethane at 80°C.
[b]Dry samples extracted with CO_2 modified with 20 vol.% MeOH at 80°C.
[c]Normalized to 150 mg of root bark extracted, sample dried and redissolved in 1 ml of acetonitrile; 10 μl injection.
[d]The values represent the mean of three replicate measurements on the three different extracts.
[e]Standard deviation of a single measurement.
[f]Relative standard deviation.

SFE with modifier, and PFE with dichloromethane, remove flavanones and xanthones from plant material at similar or slightly higher yields than obtained with solid–liquid extraction, in a much shorter period of time and with decreased amount of solvent.

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References

[1] M.L. Wolfrom, W.D. Harris, G.F. Johnson, J.E. Mahan, S.M. Moffet, B. Wildi, J. Am. Chem. Soc. 68 (1946) 406.
[2] G. Delle Monache, R. Scurria, A. Vitali, B. Botta, B. Monacelli, G. Pasqua, C. Palocci, E. Cernia, Phytochemistry 37 (1994) 839.
[3] M.L. Wolfrom, H.B. Bhat, Phytochemistry 4 (1965) 765.
[4] V.W. Deshpande, A.V. Rama Rao, M. Varadan, K. Venkataraman, Indian J. Chem. 11 (1973) 518.
[5] R.A. Laidlaw, G.A. Smith, Chem. Ind. (1959) 1604.
[6] M.L. Wolfrom, E.E. Dickey, P. McWain, J.H. Looker, O.M. Windrath, F. Komitsky Jr., J. Org. Chem. 29 (1964) 689.
[7] M.L. Wolfrom, F. Komitsky Jr., J.H. Looker, E.E. Dickey, P. McWain, A. Thompson, P.M. Mundell, O.M. Windrath, J. Org. Chem. 29 (1964) 692.
[8] M.L. Wolfrom, F. Komitsky Jr., J.H. Looker, J. Org. Chem. 30 (1965) 144.
[9] M.L. Wolfrom, F. Komitsky Jr., P.M. Mundell, J. Org. Chem. 30 (1965) 1088.
[10] F. Delle Monache, F. Ferrari, M. Pomponi, Phytochemistry 23 (1984) 1489.
[11] V. Peres, T.J. Nagem, Phytochemistry 44 (1997) 191.
[12] K. Hostettmann, M. Hostettmann, in: P.M. Dey, J.B. Harborne (Eds.), Methods in Plant Chemistry, vol. 1, Plant Phenolics, ch. 14, Academic Press, San Diego, CA, 1989, p. 496.
[13] C.-N. Lin, S.-Y. Liou, T.-H. Lee, Y.-C. Chuang, S.-J. Won, J. Pharm. Pharmacol. 48 (1996) 539.
[14] K. Hostettmann, M. Hostettmann, in: P. M. Dey, J.B. Harborne (Eds.), Methods in Plant Chemistry, vol. 1, Plant Phenolics, ch. 14, Academic Press, San Diego, CA, 1989, p. 495.
[15] M.V. Ignatushenko, R.W. Winter, H.P. Bächinger, D.J. Hinrichs, M.K. Riscoe, FEBS Lett. 409 (1997) 67.
[16] T.S. Reighard, S.V. Olesik, Crit. Rev. Anal. Chem. 26 (1996) 61.
[17] M. Schantz, J.J. Nichols, S.A. Wise, Anal. Chem. 69 (1997) 4210.
[18] P. Castioni, P. Christen, J.L. Veuthey, Analusis 23 (1995) 95.
[19] R.M. Smith, LC·GC 13 (1995) 930.
[20] C.D. Bevan, P.S. Marshall, Nat. Prod. Rep. 11 (1994) 451.
[21] H. Miyachi, A. Manabe, T. Tokumori, Y. Sumida, T. Yoshida, S. Nishibe, T. Agata, T. Nomura, T. Okuda, Yakugaku Zasshi 107 (1987) 435.
[22] H. Miyachi, A. Manabe, T. Tokumori, Y. Sumida, T. Yoshida, M. Kosawa, T. Okuda, Yakugaku Zasshi 107 (1987) 357.
[23] A. Manabe, T. Tokumori, Y. Sumida, T. Yoshida, T. Hatano, K. Yazaki, T. Okuda, Yakugaku Zasshi 107 (1987) 506.
[24] S. Cocks, S.K. Wrigley, M.I. Chirarelli-Robinson, R.M. Smith, J. Chromatogr. A 697 (1995) 115.
[25] R.J. Grayer, in: P.M. Dey, J.B. Harborne (Eds.), Methods in Plant Chemistry, vol. 1, Plant Phenolics, ch. 8, Academic Press, San Diego, CA, 1989, p. 301.
[26] K. Hostettmann, M. Hostettmann, in: P.M. Dey, J.B. Harborne (Eds.), Methods in Plant Chemistry, vol. 1, Plant Phenolics, ch. 14, Academic Press, San Diego, CA, 1989, p. 502.
[27] J.J. Dalluge, B.C. Nelson, J.B. Thomas, L.C. Sander, J. Chromatogr. A 793 (1998) 265.
[28] H.-B. Lee, T.E. Peart, R.L. Hong-You, D.R. Gere, J. Chromatogr. A 653 (1993) 83.