Mohammad Hudaib[a], Roberto Gotti[b], Romeo Pomponio[b], Vanni Cavrini[b]

[a] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan
[b] Dipartimento di Scienze Farmaceutiche, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

Recovery evaluation of lipophilic markers from *Echinacea purpurea* roots applying microwave-assisted solvent extraction versus conventional methods

In the present study the recovery from roots of lipophilic markers (mainly alkamides) of *Echinacea purpurea* (L.) Moench for analysis by GC/MS was evaluated by applying microwave-assisted solvent extraction (MASE), as a new extraction approach, versus two other conventional methods (Soxhlet and ultrasonic extraction). A preliminary screening of the three methods, using the best-reported parameters (solvent and extraction time) for Soxhlet (as a reference method) and ultrasonic extraction, showed MASE and ultrasonic extraction (using 70% methanol as the solvent system in both) to be superior methods to Soxhlet extraction in two solvent systems. Both methods, MASE and ultrasound, were further evaluated applying different ratios of methanol-water (60 to 100% methanol) as the solvent system. In these investigations, MASE showed significantly higher recoveries than the ultrasonic technique over the 70–100% methanol range while comparable values were obtained at 60% methanol. The best recovery of the individual alkamides and the whole lipophilic fraction was obtained at 70% methanol. The MASE method could serve as good alternative procedure for the preparation of more chemically potent samples and/or crude extracts from *Echinacea* species.

Key Words: Sample preparation; Microwave-assisted solvent extraction (MASE); Ultrasonic extraction; *Echinacea purpurea*; Alkamides; Sesquiterpenes; GC/MS analysis

Received: March 15, 2002; accepted: July 22, 2002

1 Introduction

The main lipophilic components of *Echinacea purpurea* and *E. angustifolia* species are alkamides, typically alkyl-isobutylamides (# **1–3**, **5**, **6**, **8–15**, **18**, **19**, **Figure 1.A,B**) or alkylmethylbutylamides (# **4**, **7**, **16**, **17**, Figure 1.A, B) [1–3]. Alkamides from *E. purpurea* roots possess mainly a 2,4-diene moiety (Figure 1.A), while most amides of *E. angustifolia* are characterized by a monoene moiety (#**12–14**, **16**, and **17**, Figure 1.B) with the isomeric mixture of **8/9** (dodeca-2*E*, 4*E*, 8*Z*, 10*E*-tetraenoic acid isobutylamide) as the main alkamide constituent of the roots and aerial parts in both species [1]. These components are mainly localized in roots and to lesser extent in the aerial parts and their concentrations, in particular the principal components *trans/cis* isomers (**8/9**), were reported to be over the range 0.04–7.20 (mg/g, dry weight) and 0.01–1.50 in the roots [1, 4–9] and the aerial parts [1, 8, 9] of *E. purpurea*, respectively.

Correspondence: Mohammad Hudaib, Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan. Phone: +96 277 495792.
Fax: +96 265 339649. E-mail: hudaib1@yahoo.com.

Extracts from *E. purpurea* are widely used in medicine for their immunostimulant activities [10, 11] and reports have shown that the lipophilic constituents, in particular the alkamides, play an important in vivo and in vitro immunoactive role [1, 12–14]. More recently the alkamides and polyacetylenic components of *Echinacea* were also found to possess light-mediated antifungal activity [9]. On account of these essential pharmacological properties, the search for appropriate sample preparation methods and effective extraction techniques enabling high recoveries of these constituents is, therefore, of particular importance. Most of the analytical studies performed until recently [1–4, 8, 15–17] utilized Soxhlet extraction with organic solvents (hexane, chloroform, or methanol) to recover the lipophilic or the total (lipophilic and hydrophilic) fractions. Some other researchers [9] utilized mother tinctures, prepared by maceration of roots in aqueous-alcoholic solution, as the starting material for subsequent liquid-liquid extraction prior to analysis. Leinert et al. [18] have evaluated the influence of three extraction methods (maceration, Soxhlet, and supercritical fluid extraction (SFE)) on the GC patterns of *Echinacea* species and recommended maceration (in dichloromethane-pentane, 1:1 *v/v* mixture) as the favorable solvent. Recently Ber-

Original Paper

 1615-9306/2003/0101–0097$17.50+.50/0

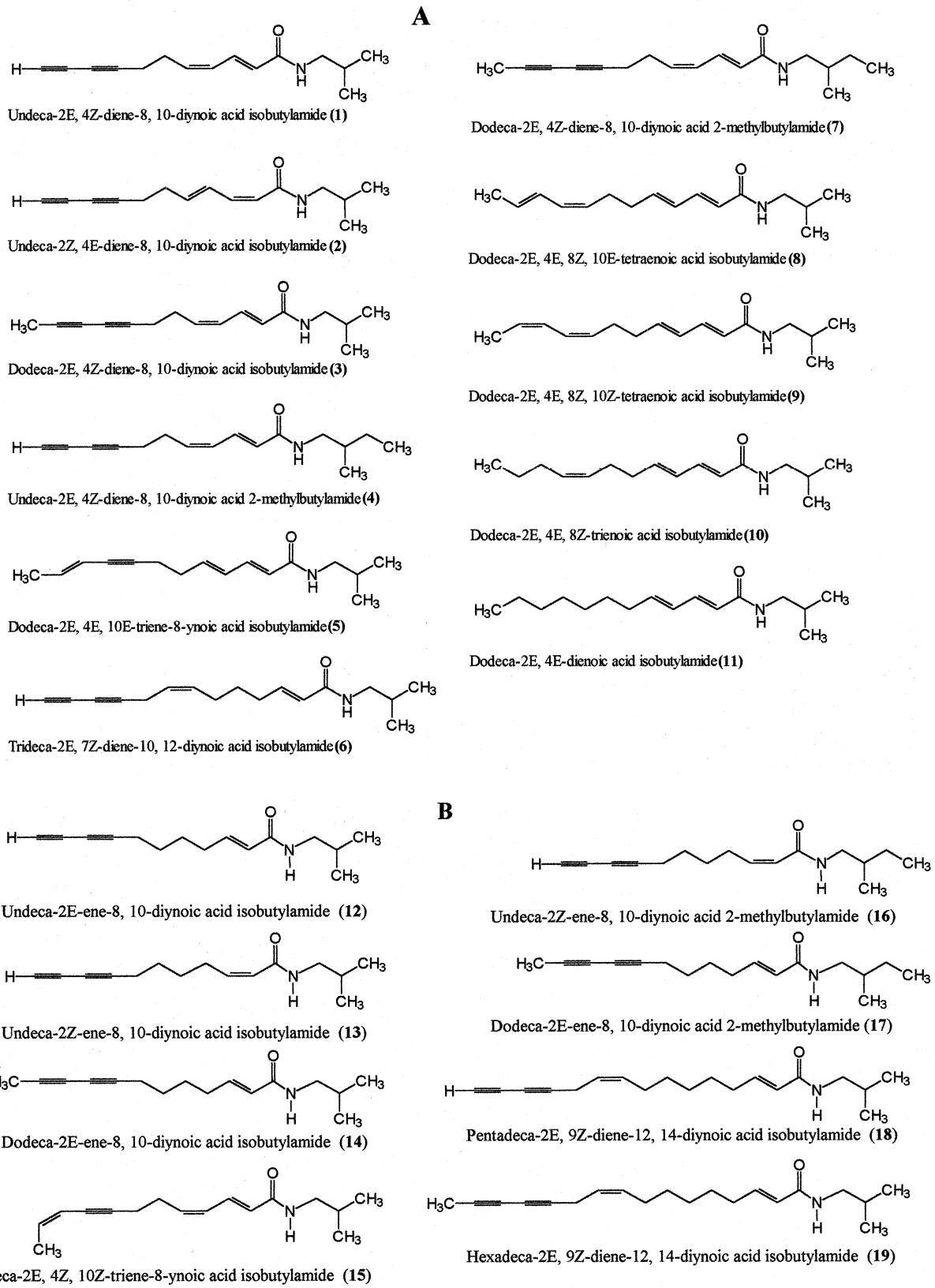

Figure 1. Chemical structures of major alkamides from **(A)** *E. purpurea* and **(B)** *E. angustifolia*. Compound numbering according to Bauer et al. [1].

geron et al. [8] reported ultrasound extraction (in 70% methanol or ethanol) as a simple method providing better recoveries compared to Soxhlet for both the polar and apolar fractions. For the recovery of phenolic components a modified sonication procedure, using ethanol-water (70:30) mixture, has been used by Perry et al. [19] for standardization of *Echinacea*. The same researchers [5, 6] utilized homogenization with CH_3CN, using an Ultraturrax treatment, coupled with C18 solid phase clean up procedure to recover the alkamide fraction.

Microwave-assisted solvent extraction (MASE) has been successfully applied to extraction of naturally derived components [20–23]. Since its first application to extraction of organic compounds from different matrices [24], in recent years many investigations have been concerned with the applicability of MASE as a powerful alternative technique to other conventional methods [25, 26]. The technique, in closed and opened vessel systems [27, 28], showed many advantages over other techniques for the extraction of compounds of medium to high polarity [22] from solid matrices. Microwave assisted extraction consists of heating the solvent (extractant) in contact with sample (e.g. ground roots) with microwave energy [20, 29]. The process involves disruption of hydrogen bonds, as a result of microwave-induced dipole rotation of molecules, and migration of ions, which enhance the penetration of solvent into matrix, allowing the solvation of components to be extracted [30]. The temperature and the nature of the solvent play particular roles in partitioning of the compounds of interest from the matrix to the solvent system [20].

In the present study, as a part of our research on sample preparation methods to integrate GC/MS analysis of the lipophilic constituents of *E. purpurea* roots [31], we evaluated the use of microwave-assisted solvent extraction (MASE) in a closed vessel system as an alternative procedure. To our knowledge, the use of MASE has not been previously reported to extract marker constituents from *Echinacea* roots. As a preliminary investigation, using different solvent systems, a selected MASE method was compared to both Soxhlet and ultrasonic extractions under the conditions reported in the literature for these latter methods. In order to asses the effect of solvent on the recovery of the lipophilic fraction by MASE and ultrasonic extraction and for the purpose of comparative evaluation of both methods, different proportions of methanol in water (60–70% *v/v*) were used as extracting solvent systems. The lipophilic (mainly alkamide) content in these extracts was then analyzed by GC/MS, using the internal standard method, and the recovery data were statistically evaluated.

2 Experimental

2.1 Plant materials

About 1–2 kg entire healthy plant materials (roots and tops) of *E. purpurea* plants grown in the open field at the Herb Garden of Casola Valsenio (Ravenna, Italy) were collected in October, 2000 at almost full growth of roots. Root materials were separated, washed, cut into small parts, air-dried, and ground into small particles (0.5 mm) just before extraction.

2.2 Apparatus

Microwave-assisted solvent extraction was carried out using a Microwave Accelerated Reaction System, model MARS X® (CEM, Matthews, NC, USA). The system is a closed-vessel, provided with a GreenChem Plus vessel set that permit simultaneous processing of up to 14 samples under pressure (max. 350 psi) and temperature (max. 210°C) controlled microwave extraction.

2.3 Extraction protocols

2.3.1 Soxhlet extraction

About 1 g accurately weighed dried root materials were extracted in a Soxhlet apparatus for 48, 3, and 2 h with *n*-hexane, methanol, and chloroform, respectively. The final extract obtained in each solvent was filtered and the filtrate was concentrated under vacuum to 25 mL, for the methanol and chloroform filtrates, and below 10 mL volume for the *n*-hexane one. To the *n*-hexane filtrate, a 100 mL volume of the internal standard solution (propyl *p*-hydroxybenzoate, 0.1% *w/v* in *n*-hexane-ethyl acetate (1:1 *v/v*)) was added and the volume was adjusted to 10 mL with the solvent. The final solution was dried over anhydrous sodium sulphate before being subjected to GC/MS analysis.

2.3.2 Ultrasonic extraction

About 1 g accurately weighed amounts of dried ground root materials were extracted in an ultrasonic bath (Sonorex Super RK103H Bandelin, Berlin, Germany), for 7 min at room temperature, three times with 8 mL solvent (60–100% *v/v* methanol in water). The extracts ($n = 3$) were collected, filtered through a 0.20 mm membrane filter (GyroDisc, Orange Scientific, Waterloo, Belgium), and the volume was completed up to 25 mL to a final concentration equivalent to 0.04 g roots per mL.

2.3.3 MASE approach

About 1 g accurately weighed amounts of dried ground root materials were transferred into a vessel containing

20 mL solvent. Solvents used were either chloroform, absolute methanol, or methanol-water at different *v/v* ratios (60–100% methanol). In each run only three extractions (i.e. 3 vessels containing different solvents) were performed always using the control vessel. The microwave power was applied in a pulsed manner using 400 W (75%) as the maximum applied energy. The samples were processed for a 10 min total extraction time, being heated by the microwave energy to 100°C final temperature (150–200 psi extraction pressure) in 5 min, and maintained at that temperature for another 5 min. The system was allowed to cool to room temperature (RT) for an additional 5 min prior to opening the vessels. The extract obtained in each vessel was then filtered through a 0.20 mm membrane filter and further processed as in Section 2.3.1, for the *n*-hexane solution, and as in Section 2.3.2 for the others. Generally the following parameters and conditions were maintained constant in all the MASE experiments performed: (a) volume of solvent (20 mL); (b) sample weight (1 g), particle size (0.5 mm) and moisture content (fully air-dried) of roots; (c) number of vessels in each run (3); (d) applied energy (400 W, 75%); and (e) temperature program (raised to 100°C in 5 min, isothermal for 5 min).

2.3.4 Liquid-liquid extraction (clean up procedure)

To recover the lipophilic fractions a 10 mL volume of each of the solutions, obtained in methanol or chloroform by ultrasonic, MASE, and Soxhlet extraction, was subjected to a threefold extraction with 20 mL *n*-hexane-ethyl acetate (1:1 *v/v*). The organic phases were collected, concentrated under vacuum, and a 50 mL volume of the internal standard working solution was added before making up the volume to 5 mL with *n*-hexane-ethyl acetate (1:1 *v/v*). The final solution, corresponding to 0.08 g roots per mL and containing 10 mg/mL of the internal standard, was dried over anhydrous sodium sulphate prior to GC/MS analysis.

2.4 GC/MS analysis

The gas chromatograph was a TRACE GC 2000 SERIES (ThermoQuest CE Instruments, Austin TX, USA) equipped with a split-splitless injector (split ratio of 20:1). The column was an Rtx®-5MS (30 m × 40.25 mm, fused silica capillary column, 0.25 μm film thickness) consisting of Crossbond® (5% diphenyl 95% dimethyl polysiloxane). Helium (He) was the carrier gas at a flow rate of 1.0 mL/min. The GC was interfaced with a GCQ plus (ThermoQuest-Finnigan, Austin TX, USA) mass detector operating in the EI^+ mode (70 eV) with a 1.5 min filament delay. The mass spectra were generally recorded over 40–650 amu full-scan mode (scan time: 1.44 s) revealing the total ion current (TIC) chromatograms.

GC/MS operation conditions and quali-quantitative analyses were as reported in reference [31]. Briefly, a linear temperature program was adapted to separate the different components of the extracts as follows: the column temperature was raised, from an initial value of 100°C, at 10°/min to 200°C and held there for 2 min; a second ramp was then applied at 3 °/min up to a final temperature of 250°C which was maintained for 5 min. For quantitative analyses, percentage contents of the various components, identified in the total ion current (TIC) GC/MS traces, were calculated by integrating their corresponding peak areas (relative to the internal standard area) and assuming a uniform response for all the components.

3 Results and discussion

3.1 Preliminary evaluation of extraction protocols

In a previous study [31] we developed a GC/MS method for the analysis of the lipophilic constituents of *E. purpurea* obtained from roots by an ultrasonic extraction method modified from that reported by Bergeron et al. [8]. Ultrasonic extraction was chosen as it was reported to provide the best recovery of the polar and apolar fractions from the plant [8, 19]. The many advantages reported so far for microwave-assisted solvent extraction (MASE), as a modern extraction technique for natural products and environmental sample preparations [20–22], has prompted us to apply this method to the extraction of lipophilic marker constituents from *E. purpurea* roots.

As a preliminary evaluation, three extraction methods, including MASE, with several solvent systems were compared. The other two methods utilized were ultrasonic and Soxhlet extraction employing the general procedures summarized in **Table 1** and as described in Section 2. Soxhlet extraction was used with two solvent systems: *n*-hexane (or chloroform) and methanol (absolute), which are the solvents most commonly used to extract the lipophilic and total (lipophilic and hydrophilic) fractions, respectively [2, 3, 8, 15–17]. Ultrasonic extraction was performed with 70% methanol (*v/v* in water), a system that was reported to afford the best recovery of these fractions as mentioned above. Finally, using MASE, 70% methanol was selected as the starting point for the preliminary study. The obtained data are shown in **Table 2** and **Figure 2**, where Soxhlet extraction with *n*-hexane is considered as a reference method with an assumed 100% relative recovery for each component class (alkamides, sesquiterpenes, and total lipophilic fractions). As can be observed, the MASE and ultrasonic approaches, both with 70% methanol, afforded the highest recovery of the alkamide- and the whole lipophilic-fractions, providing significantly higher levels of these fractions compared to conventional Soxhlet extraction.

Table 1. Conditions and schematic procedures of the extraction of *E. purpurea* roots by different protocols.

Extraction technique	Solvent system, total volume	Procedure scheme
Soxhlet	*n*-Hexane, 25 mL	1 g dried roots treated in Soxhlet for 48 h
	Methanol, 25 mL,	1 g dried roots treated in Soxhlet for 3 h
	Chloroform, 25 mL	1 g dried roots treated in Soxhlet for 2 h
Ultrasonic	Methanol-water (70 : 30 – 100 : 0.0, *v/v*) 25 ml	1 g dried roots sonicated 3 times, each for 5 min, using 7 – 8 mL solvent
MASE (closed-vessel system)	Methanol-water (70 : 30 – 100 : 0.0, *v/v*),	1 g dried roots, 400 W (75%), 200 psi max., temperature program (extraction time: 10 min; ramped to 100°C in 5 min, maintained at 100°C for 5, cooled to RT in 5 min)
	Chloroform and *n*-hexane, 20 mL	*as above*

Table 2. Relative recovery (R.R.) of the lipophilic fractions from *E. purpurea* roots by different extraction protocols.

Extraction method	Sesquiterpenes R.R.	Alkamides R.R.	Total lipophilic R.R. (RSD)[a)]
Soxhlet (*n*-hexane, 2 days)*	100	100	100 (0.93)
Soxhlet (absolute methanol, 3 h)	55.04	69.96	67.74 (1.33)
Soxhlet (chloroform, 2 h)	52.25	57.02	56.43 (2.12)
Ultrasonic (methanol 70% *v/v* in water, 3 × 5 min)	49.70	119.63	103.28 (1.42)
MASE (methanol 70% *v/v* in water, 10 min)	42.88	121.57	104.15 (0.95)
MASE (*n*-hexane, 10 min)	31.49	56.97	52.20 (1.13)
MASE (Chloroform, 10 min)	34.59	72.85	63.99 (1.75)

a) Relative standard deviation (RSD) values obtained by inter-day (4 days) analysis of sample replicates (4 replicates per day).

* This method, mostly reported to extract alkamides, is considered here as a standard (100% relative recovery) reference method for comparative purposes.

R.R.: Mean relative recovery value of 6 determinations.

Moreover, for better and more reliable comparison of the extraction protocols and as a part of the preliminary evaluation study, the stability of alkamides in different solvents has been taken in consideration. Previous studies [8, 19, 32] showed that alkamides, compared to the polar phenolic fractions, are stable compounds in solution (e.g. methanol, ethanol, and *n*-hexane) and also in already prepared alcoholic mixtures [32] and under different storage conditions (time and temperature). In the present study, the stability of the lipophilic components from *Echinacea* in solution in different solvents has been assessed by inter-day analysis of random samples during the preliminary study as shown in Table 2. High stability of these fractions can be concluded and confirmed by the low RSD values obtained for each extract (in a given solvent) repeatedly analyzed in different days. On the other hand, the repeatability values (RSDs), which will be shown later in **Table 3**, obtained in the comparative evaluation of the relative recovery of alkamides by both ultrasonic and

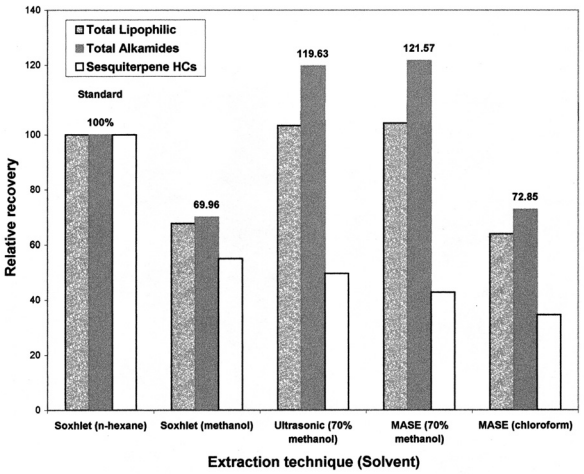

Figure 2. Relative recovery of the lipophilic fractions from *E. purpurea* roots applying different extraction protocols. Recoveries are compared to that of Soxhlet extraction (*n*-hexane) assumed here to provide 100% relative recovery.

Table 3. Relative recoveries of the lipophilic fractions from *E. purpurea* roots extracted by ultrasonic and MASE. Relative recovery (R.R.) values are derived as the ratio of total compounds (fraction) area to internal standard area.

Methanol% (in water *v/v*)	Alkamides R.R.[a)]		*P*	Sesquiterpenes R.R.		*P*	Total lipophilic R.R.		*P*
	MASE	Ultrasonic		MASE	Ultrasonic		MASE	Ultrasonic	
60%	14.39 (0.26;1.83)	14.97 (0.55;3.64)	0.0417 *	1.19 (0.07;5.79)	1.35 (0.09;6.89)	0.0064 **	15.58 (0.29;1.89)	16.33 (0.61;3.74)	0.0216 *
70%	16.54 (0.44;2.67)	15.02 (0.19;1.24)	<0.0001 ***	1.40 (0.12;8.69)	1.64 (0.13;7.87)	0.0077 **	17.94 (0.40;2.24)	16.66 (0.17;1.00)	<0.0001 ***
80%	12.13 (0.52;4.25)	8.99 (0.41;4.60)	<0.0001 ***	1.74 (0.11;6.60)	1.50 (0.24;16.0)	0.0501 ns	13.87 (0.48;3.44)	10.35 (0.48;4.67)	<0.0001 ***
100%	11.47 (0.21;1.85)	7.55 (0.35;4.64)	<0.0001 ***	1.83 (0.05;2.91)	1.46 (0.08;5.38)	<0.0001 ***	13.30 (0.24;1.81)	9.01 (0.37;4.16)	<0.0001 ***

[a)] Mean relative recovery value of 6 determinations, between brackets (SD; RSD) are the standard deviation and the relative standard deviation, respectively.

P: Probability value from unpaired *t*-test.

ns Non-significant, * significant, ** very significant, *** extremely significant relative recovery difference.

MASE can also give an indication of their stability under extraction conditions.

3.2 MASE versus ultrasonic extraction and influence of extraction-solvent polarity

To ascertain the potential advantages of MASE over other techniques and for comparison between MASE and ultrasonic extraction, the influence of solvent polarity on the recovery provided by both techniques was evaluated. The MASE method utilized here was optimized from selected applications, provided by the manufacturer, for extraction of alkamide-related compounds from root materials. Since the only variable between the two compared methods was the solvent (different% methanol in water, 60–100% *v/v*) the other experimental parameters were maintained constant for all the experiments in the MASE procedure (Section 2.3.3).

3.2.1 Recovery of volatile components (sesquiterpenic fraction)

A cooling stage (5 min) was utilized in each MASE run in order to minimize the possibility of losing the essential oil-typical volatile components (germacrene D and beta-caryophyllene). The phenomenon is very common in closed MASE systems [20] because of the high temperature applied that facilitates the escape of readily volatile components into the headspace compartment. Cooling before opening the vessels will, therefore, allow condensation of these compounds and thus reliable evaluation of extraction performance will be achieved. Representative GC/MS chromatograms that briefly describe the effect of solvent (type and polarity) on the relative recoveries of the sesquiterpenes as well as the alkamides fractions are shown in **Figure 3**. Increased recovery of sesquiterpenes

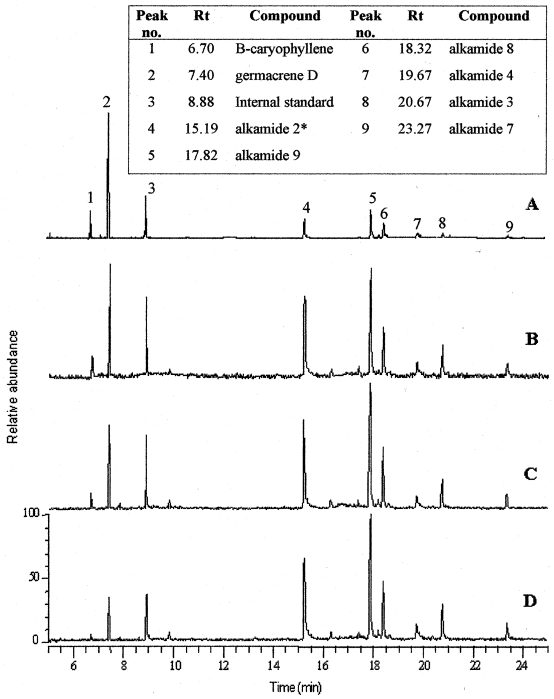

Figure 3. Typical GC/MS chromatograms of the lipophilic fractions from *E. purpurea* obtained from roots by MASE extraction, showing the relative recoveries of the different components using: (A) *n*-hexane, (B) absolute methanol (100%), (C) 80% (*v/v*) methanol/water, and (D) 70% (*v/v*) methanol/water, as the extracting solvent. For peak identification refer to Table insert. For chromatographic and MS conditions refer to Experimental. Alkamides (**2**, **3**, **4**, **7**, **8**, **9**) are according to Figure 1.A.

with increased methanol% was observed as these components are non-polar and their extraction efficiency (solubility parameter) is directly proportional to lipophilicity of the extraction solvent. This effect is also shown in **Figure 4.A**

and is in agreement with the best recovery of these compounds using Soxhlet extraction with hexane (Figure 2 and Figure 3.A). The possibility of losing these volatile compounds in the less-controlled open extraction systems such as in ultrasonic extraction can, accordingly, be an explanation for their lower and irregular recoveries versus increased methanol% (Figure 4.A).

3.2.2. Recovery of alkamides and the total lipophilic fractions

The recovery values of the alkamides and the whole lipophilic fractions by MASE and ultrasonic methods, using different methanol-water mixtures as extracting solvents, are collected in Table 3 and the recovery profiles are shown in **Figure 4.B, C**. Significance values (at 95% confidence intervals, unpaired *t*-test) of the recovery differences between both methods, at each methanol%, are also reported in Table 3. Compared to the ultrasonic method, MASE was found to be superior, providing significantly higher levels over the whole concentration range of methanol, but less significant at 60%. The obtained data suggest that the level of water in the extraction solvent plays a critical role in determining the recovery in both techniques. Although the lipophilic components of *E. purpurea* are considered here, the alkamides combine both polar (amide acid moiety) and apolar (olefinic hydrocarbon chain moiety) properties, which largely determine their recovery in different solvents. Two factors can be considered, therefore, to influence the recovery of these compounds. The first is that increasing the water content leads to an increase in polarity and the dielectric constant of the solvent, thus enhancing the microwave extractive mechanism (absorption of microwave energy and conversion to heat) according to Camel [20]. This factor is valid only for MASE and not for ultrasonic extraction. The second is that increasing the water (polar solvent) content, while maintaining a considerable level of methanol, as a less polar solvent, will lead to a system with an overall polarity tending to enhance the solubility of alkamides. This last factor (solubility parameter) is applicable to both techniques and explains the increasing alkamide recovery by both (Figure 4.B) up to 70% methanol as the best solvent. Accordingly, the MASE method, combining both mentioned factors, provided significantly higher alkamide recoveries relative to the ultrasonic technique over the whole methanol% range, although comparable values were obtained at the 60% methanol level. The solvent polarity therefore influenced the efficiency of extraction by both techniques; however, this effect was enhanced in MASE in the presence of the microwave energy.

The data presented for MASE and ultrasonic extraction (Table 3 and Figure 3.B,C) showed that 70% methanol was an appropriate solvent for best recovery of both the

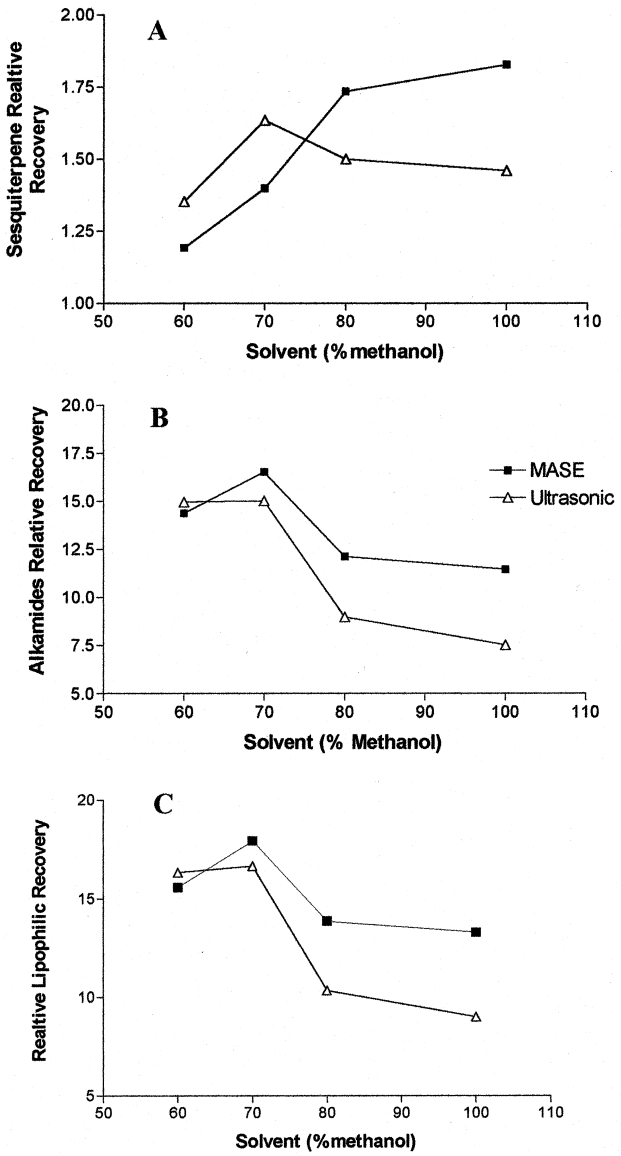

Figure 4. Comparison between MASE and ultrasonic extraction methods showing the relative recoveries of the (A) sesquiterpenes, (B) alkamides, and (C) total lipophilic fractions extracted from *E. purpurea* roots by both methods using different methanol-water ratios.

alkamides and the whole lipophilic fractions, in substantial agreement with the results obtained by Bergeron et al. [8]. Higher water levels (>30%) were associated with comparable ($P = 0.838$) (in ultrasonic) or significantly ($P < 0.0001$) lower (in MASE) recoveries of either fractions and individual components. The lipophilicity of alkamides possibly played the key role in explaining the latter behaviour. Most likely a 70% methanol content offers the best environment able to solvate alkamides, whose polarity (solubility parameter) is close to that of the solvent system.

4 Concluding remarks

Complex participation of many factors was found to control the relative recovery of the lipophilic fractions (mainly alkamides) from *E. purpurea* extracts by both MASE and ultrasonic methods using methanol-water as extracting solvent. In particular, the polarity of both the solvent system and the alkamide components and their relationship to the overall solubility of these compounds (targets), the ability of the solvent to reach these targets, interact with and liberate them from the complex matrix structures, all determine to a great extent the best recovery solvent (methanol/water ratio) by both the procedures. Moreover, the microwave effect in MASE was an additional extraction-enhancing factor, increasing the extraction efficiency of the solvent, and thus providing better recoveries, relative to ultrasonic extraction, over the different methanol/water ratios used in the study (Figure 4 and Table 3). For the alkamide recovery, both methods, in particular the MASE, showed good reproducibility in intra-day analysis of three separately prepared samples (each analyzed in duplicate) with each solvent system, as expressed by the RSD values reported in Table 3 for the different lipophilic fractions. For the sesquiterpenic fraction, the recovery reproducibility was found to be less than that obtained for alkamides, probably due to the higher volatility of these components (Table 3). On the whole, under the various experimental conditions, the MASE method appears to offer the best recovery, with a good reproducibility, of the alkamide components from *E. purpurea* extract.

Acknowledgement

Thanks are due to Dr. Sauro Biffi from the Herb Garden of Casola Valsenio (Ravenna) for supplying the plant materials. This work was supported by a grant from MURST (Cofin, 2000), Rome, Italy.

References

[1] R. Bauer, P. Remiger, *Planta Med.* **1989**, *55*, 367–371.

[2] R. Bauer, P. Remiger, H. Wagner, *Phytochemistry* **1988**, *27*, 2339–2342.

[3] R. Bauer, P. Remiger, H. Wagner, *Phytochemistry* **1989**, *28*, 505–508.

[4] X.-g. He, l.-z. Lin, M.W. Bernart, L.-Z. Lian, *J. Chromatogr. A* **1998**, *815*, 205–211.

[5] N.B. Perry, J.W. Van Klink, E.J. Burgess, G.A. Parmenter, *Planta Med.* **1997**, *63*, 58–62.

[6] N.B. Perry, J.W. Van Klink, E.J. Burgess, G.A. Parmenter, *Planta Med.* **2000**, *66*, 54–56.

[7] R.B.H. Wills, D.L. Staurt, *Food Chemistry* **1999**, *67*, 385–388.

[8] C. Bergeron, J.F. Livesey, D.V.C. Awang, J.T. Arnason, J. Rana, B.R. Baum, W. Letchamo, *Phytochem. Anal.* **2000**, *11*, 207–215.

[9] S.E. Bins, B. Purgina, C. Bergeron, M.L. Smith, L. Ball, B.R. Baum, J.T. Arnson, *Planta Med.* **2000**, *66*, 241–244.

[10] S.S. Percival, *Biochem. Pharmacol.* **2000**, *60*, 155–158.

[11] J. Pepping, *Am. J. Health-Syst. Pharm.* **1996**, *56*, 121–122.

[12] R. Bauer, H. Wagner, in: *Economic and Medicinal Plant Research*, H. Wagner (Ed.), Academic Press, New York 1991, p. 253–321.

[13] R. Bauer, *Z. Arztl. Fortbild. Jena* **1996**, *90*, 111–115.

[14] R. Bauer, *ACS Symposium Series* **1998**, *691*, 140–157.

[15] P. Pietta, P. Mauri, R. Bauer, *Planta Med.* **1998**, *64*, 649–652.

[16] R. Bauer, I.A. Khan, H. Wagner, *Planta Med.* **1988**, *54*, 426–430.

[17] K. Glowniak, G. Zgorka, M. Kozyra, *J. Chromatogr. A* **1996**, *730*, 25–29.

[18] D. Lienert, E. Anklam, U. Panne, *Phytochem. Anal.* **1998**, *9*, 88–98.

[19] N.B. Perry, E.J. Burgess, V.L. Glennie, *J. Agric. Food Chem.* **2001**, *49*, 1702–1706.

[20] V. Camel, *Trends Anal. Chem.* **2000**, *19*, 229–248.

[21] L. Jassie, R. Revesz, T. Kierstead, E. Hasty, S. Matz, in: *Microwave-Enhanced Chemistry. Fundamentals, Sample Preparation, and Applications*, H.M.S. Kingston, S.J. Haswell (Eds.). Am. Chem. Soc., Washington D.C. 1997, pp. 569–609.

[22] B. Kaufmann, Ph. Christen, J.-L. Veuthey, *Phytochem. Anal.* **2001**, *12*, 327–331.

[23] M. Spiro, S.S. Chen, *J. Microwave Power Electromag. Energy* **1994**, *29*, 231–241.

[24] K. Ganzler, A. Salgo, K. Valko, *J. Chromatogr.* **1986**, *371*, 299–306.

[25] K. Ganzler, A. Salgo, *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 274–276.

[26] A. Siquin, T. Gorner, E. Dellacherie, *Analusis* **1993**, *21*, 1–10.

[27] M. Letellier, M. Butzinski, *Analusis*, **1999**, *27*, 259.

[28] C. Demesmay, M.Olle, *Spectra Analyse* **1993**, *175*, 27.

[29] J.R.J. Pare, J.M.R. Belanger, S.S. Stafford, *Trends Anal. Chem.* **1994**, *13*, 176.

[30] K. Ganzler, I. Szinai, A. Salgo, *J. Chromatogr.* **1990**, *520*, 257–262.

[31] M. Hudaib, J. Fiori, M.G. Bellardi, C. Rubies Autonell, V. Cavrini, *J. Pharm. Biomed. Anal.* (in press).

[32] J. Livesey, D.V. Awang, J.T. Arnason, W. Letchamo, M. Barrett, G. Pennyroyal, *Phytomedicine* **1999**, *6*, 347–349.

[JSS 1221]