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Dispersive Liquid-Liquid Microextraction of Floral-water Constituents of Rosmarinus officinalis

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Abstract

Dispersive liquid-liquid microextraction (DLLME) followed by gas chromatography-mass spectroscopy (GC-MS) was used to analyses of components of floral-water of Rosmarinus officinalis . Some effective parameters such as volume of extraction, disperser solvents and salt effect were studied by a full factorial design for obtain the optimum condition. The main compounds of the floral-water of Rosmarinus officinalis were a-pinene (16.7%), camphr (9.1%) and 1,8-cineole (8.5%).

Keywords: Dispersive liquid-liquid microextraction, Rosmarinus officinalis Floral- water, a-pinene, 1,8-cineole

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INTRODUCTION

Rosemary (Rosmarinus officinalis Linn. Fam. Labiatae) is an evergreen branched bushy shrub, attaining a height of about one metre with upright stems, whitish-blue flowers and dark green leaves which are small with edges turned over backward. Underneath these rolled edges are little glands containing aromatic oils. It grows wildly along the north and south coasts of the Mediterranean sea, and also in the sub-Himalayan areas [1-3]. Rosemary (Rosmarinus officinalis Linn.) is a common household plant grown in many parts of the world. It is used for flavouring food, a beverage drink, as well as in cosmetics; in folk medicine it is used as an antispasmodic in renal colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Extract of rosemary relaxes smooth muscles of trachea and intestine, and has choleretic, hepatoprotective and antitumerogenic activity. The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid. These compounds have antioxidant effect. Rosemary is a widely used aromatic and medicinal plantnowadays. Rosmarini folium has antibacterial, antioxidantand antiphlogistic effect. The essential oil enhances theblood-circulation of the limbs, has antirheumatic effect andrelieves the neuralgic pains. Besides the therapeuticalapplication, the essential oil is widely applied in the cosmeticindustry producing various Cologne waters, bathing essences, hair lotions and shampoons. The leaf of rosemary is anindispensable spice of the French, Italian and Spanish cuisine [4].

Liquid-liquid extraction is the most oldest extraction methods. But this method is time-consuming and needs high volum of solvents. Recently Assadi et al developed dispersive liquid-liquid microextraction (DLLME) [5].this method consists of two main step: (1) injection of an appropriate mixture of extraction and disperser solvent to a aqueous sample containing analytes and (2) centrifugation of cloudy solution. After centrifugation , the analysis of sample in sediment can do using instrumental method [6].

EXPERIMENTAL

Reagents and material. The Rosmarinus officinalis floral-water was purchased from Alavi Co. Kashan, Iran in may 2010. Chemicals such as ethanol, methanol, acetone, acetonitrile, chloform, dichloromethane , carbon tetracholoride and normal alkanes (C8-C21) with the purity above 99% were purchased from Merck (Darmstadt, Germany).

Instrumentation. GC-MS analyses were carried out using a HP 6890 gas chromatograph coupled with HP 5973 (30 m; 0.25 mm ID and 0.32 μm filmtickness, 5%phenyl methyl polysiloxane). The temperature program initiated at 60˚C for 3 min then raised at 5˚C / min to 230˚C, held for 3 min. The carrier gas was He (99.999%) at 1 ml/min flow rate. injector temperature was 250˚C with a split ratio of 1:30. Mass spectra were achieved at 70 eV from 30 to 500 amu. The injection into GC-MS was carried out using a 10 μl micro syringe Hamilton 7001.

Identification of components. The constituents of the Rosmarinus officinalis floral – water were characterized by comparing their mass spectra with those stored on a Wiley 275 MS computer library. The Kovats retention indices of constituents were obtained by interpolating between bracketing n-alkanes [7].

in tube. The mixture was centrifuged for 5 min at 8000 rpm. The fine particle of solvent were sedimented in the bottom of tube. 1 μl of sedimented phase was injected to GC-MS using a 10 μl micro-syringe.

RESULTS AND DISCUSSION

Extraction solvent selection. The extraction solvent should have a density higher than water, good extraction capacity for analytes, appropriate GC behavior and immiscibility with water. Carbon tetrachloride (density: 1.59 gml-1), dichloromethane (density: 1.33 gml-1) and chloroform (density: 1.48 grm-1) were selected for this purpose (fig 1).

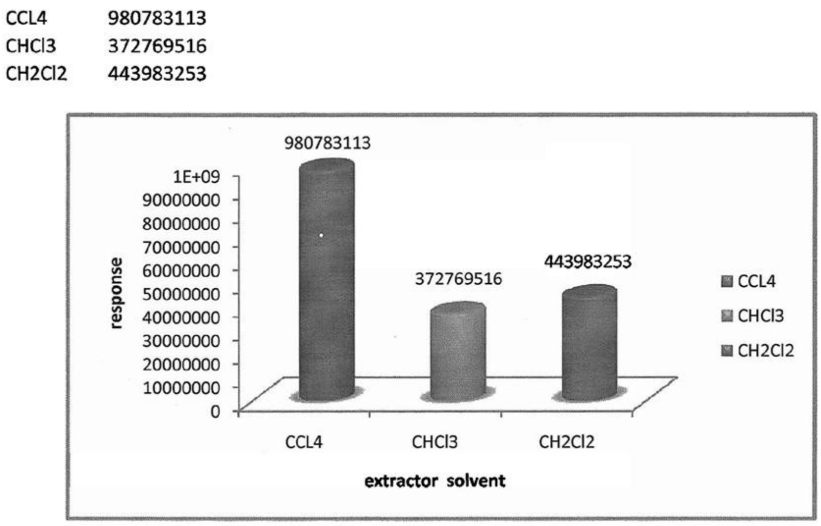

Figure 1: Effect of various extraction solvent

Disperser solvent selection. The extraction solvent will disperse as fine particle when it rapidly injected into aqueous sample solution with extraction solvent. For this purpose, the main issue is miscibility of disperser solvent with extraction solvent and aqueous phase. Acetone, ethanol, methanol [5]. And acetonitrile were subjected to study for selection of disperser solvent. 500 μl of each disperser solvent and 20 μl of carbon tetra chloride (extraction solvent) were injected to 5 ml of Rosmarinus officinalis aqueous floral water. The effect of disperser solvent on extraction efficiency is shown in fig 2. The best extraction yield was obtained by using ethanol as disperser solvent due to maximum sum of GC area peaks.

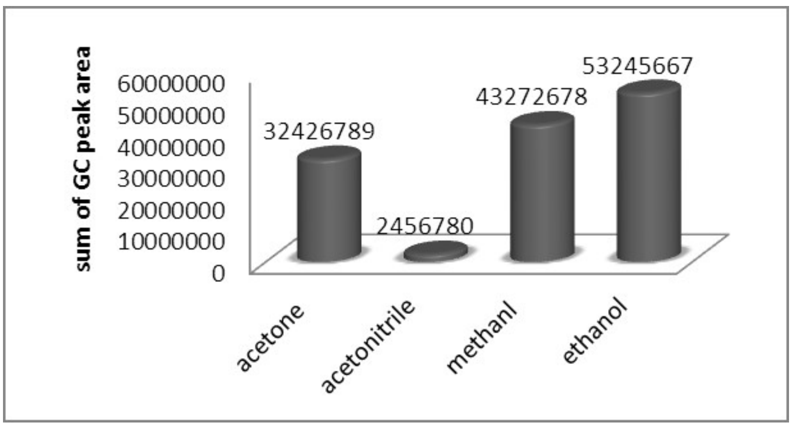

Figure 2: Effect of various disperser solvent

Optimization of Dispersive liquid-liquid microextraction

Full factorial design. In order to evaluate procedure of study, the sum of peak area of the extracted constituents was considered as experimental response. A full factorial design was included 2f=16 experiments (f is the number of factors) which defined to explore main factors and their interactions. The experiments were done in a random manner for minimizing the effect of uncontrolled variables. Since the experiments cannot done in one day, they were divided into two blocks, each with 8 experiments. To find the most important effects and their relationship, analysis of variance (ANOVA) was done using Design-Expert 8.1 software (table 1). Since only two levels are used in factorial designs, the related models are somewhat restricted. Sophisticated second-order models (response surface designs) were required for location of an optimum set of experimental conditions. A CCD (central composite design) was combined a two – level factorial design with additional star point and at least one point at the center of experimental region to obtain properties such as rotatability or orthogonality, in order to fit quadratic polynomials. Under the optimum conditions which achieved by response surface method; sample size; 5 ml; disperser solvent volume (ethanol): 422 μl; extraction solvent (carbon tetrachloride): 20 μl.

Characterization of Rosmarinus officinalis floral water. The chemical constituents of Rosmarinus officinalis floral water were identified by their mass spectra and their relative retention indices with those are in authentic references. The identified compounds were listed in Table 1.

Table 1: composition and percents of the constituents in Rosmarinus officinalis floral water

Compound	RRI	%
a-pinene	*939*	16.7
Camphene	*954*	6.6
Verbenene	*968*	0.9
3-octanone	*984*	4.6
Myrcene	*991*	3.8
p-cymenen	*1221*	1.4
Limonene	*1029*	4.8
1,8-cineol	*1031*	8.5
g-tepinene	*1060*	0.8
a-terpinolene	*1089*	0.9
Linalool	*1097*	2.5
Chrysanthenone	*1128*	1.4
Camphor	*1146*	9.1
Trans- pinocamphone	*1160*	1.4
Borneole	*1169*	7.6

Conclusion

In this study dispersive liquid- liquid microextraction method was used to extract water soluble compound of Rosmarinus officinalis floral water which used in folk medicine. Experiments showed that ethanol and carbon tetracholoride were the most suitable as disperser and extraction solvent, respectively.

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