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EXTRACTION AND CHROMATOGRAPHIC DETERMINATION OF ESSENTIALS OILS FROM *OCIMUM BASILICUM L.* LEAVES

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ABSTRACT. Three different techniques (maceration, sonication and microwave assisted extraction) were used for extraction of essential oils from *Ocimum basilicum* L. The extracts were analyzed by TLC/HPTLC technique and the fingerprint information were obtained. The GC-FID was used to characterized the extraction efficiency and for identify the terpenic bioactive compounds. The most efficient extraction technique was maceration followed by microwave and ultrasound. The best extraction solvent system was ethyl ether - ethanol (1:1, v/v). The main compounds identified in *Ocimum basilicum* L. extracts were: α and β-pinene (mixture), limonene, citronellol, and geraniol.

Keywords: *essential oils, Ocimum basilicum L., extraction, chromatography*

INTRODUCTION

Basil (*Ocimum basilicum L*.) is an aromatic herb that is used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics [1]. The interest in medicinal plants and their biologically active derivatives has increased in recent years, in relation to the possible development of novel potential drugs [2]. Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction. Major aroma compounds from volatile extracts of basil present anti-oxidative activity [1].

Any changes of metabolism equilibrium cause the alteration of volatile oil extracts compositions, which might be called 'aroma profile characteristics' by analogy with the fingerprint [3].

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Conventional essential oil extraction techniques include maceration (passive extraction) [4], steam distillation (SD) [5-7], purge and trap (P&T) [8], static and dynamic headspace [9] and head-space solid-phase microextraction (HS-SPME) [10, 11]. Steam distillation is the routine method recommended by pharmacopoeias for controlling the quality of plant materials as essential oil sources [12]. These classical methods require long extraction time, large amounts of solvents and multiple steps. Moreover, many unstable volatiles compounds would be thermally decomposed and degraded during thermal extraction. Due to the relative simplicity SD and SDE are still extensively used for essential oils extraction. In recent years, some advanced extraction techniques, such as headspace solvent drop microextraction (HSME) [13], pressurized liquid extraction (PLE) [14], supercritical fluid extraction (SFE) [15], solvent free microwave extraction [16], microwave assisted hydrodistillation extraction and ultrasound-assisted extraction [17] were used. Among these extraction techniques, high-temperature water extraction of herb like basil is of particular interest because the water extraction is performed around to 100°C and therefore may mimic the cooking process in the kitchen. The extraction in the microwave field has the same practical importance, as well.

The essential oils extracts are analyzed by various chromatographic techniques such as: high - performance liquid chromatography (HPLC) [18, 19] and thin layer chromatography (TLC) [19, 20]. Due to the high volatility of the analytes, the specific technique is GC. The more precisely information in qualitative analysis are obtained by gas-chromatography coupled with mass spectrometry (GC-MS) [21]. For quantitative determination gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred [1, 2, 20, 22].

The main goal of our investigations was to evaluate the extraction efficiency of essential oils from basil using various techniques and solvent systems. The chromatographic determinations were performed by TLC and GC-FID. The resulting chromatograms were analyzed. Some essential oils were identified using standard solutions

RESULTS AND DISCUSSION

In the case of plant extracts TLC/HPTLC is used to provide fingerprint information [23, 24]. Only when a very good resolution and no doubt about the identity of compounds are achieved a quantitative analyze is possible.

For identification of some essential oils, HPTLC was used. The E6 extraction solvent was chose because it has the capacity to extract the compounds with different polarities. After separation the plates were pulverized with anisaldehyde, heated for spots coloring and inspected in UV at 366 nm (figure 1a) and in visible range (figure 1b).

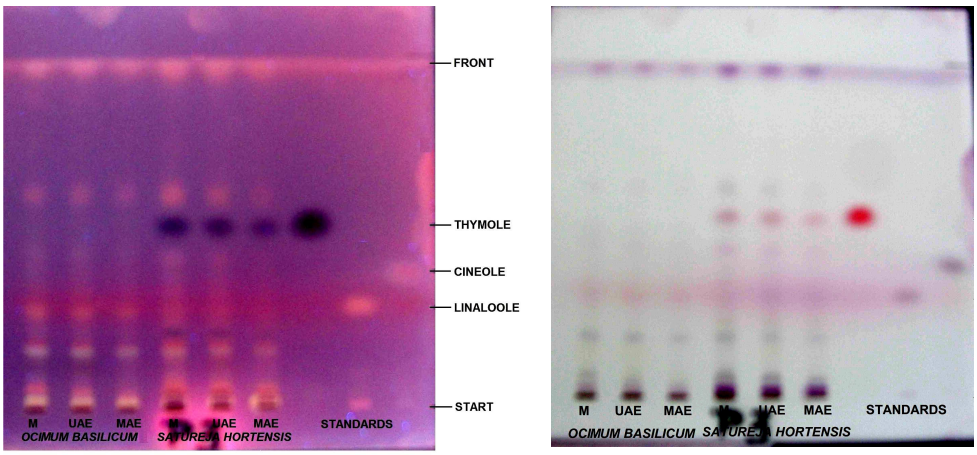

Figure 1. Identification chromatograms of linalool and cineole in *Ocimum basilicum* L. extracts E6 after reaction with anisaldehyde, UV at 366 nm and daylight inspection.

The R_f values were determined and compared with those existent in literature (table 1). Based on this it is possible that the *Ocimum basilicum* L. extracts to contain cineole and linalool. The presence of linalool is confirmed by [23, 25]. The TLC fingerprint of extracts obtained with different techniques – M, MAE and UAE showed to be similar, that means that no degradations processes happens or no new compounds were extracted.

Table 1. Identification of some terpenoidic compounds from *Ocimum basilicum* L. extracts

Compound	R_f value		
	Standard	Sample	Reference [25]
Cineole	0.39	0.38	0.40
Linalool	0.30	0.3	0.30

Another goal was to determine which solvent and which technique is more suitable for extraction of essential oils from *Ocimum basilicum* L. leaves. In figure 2 is presented the chromatogram of all extracts.

It can be observed that E1 shows the lowest efficiency. Better results were obtained with E3, E4 and E6, the spots being more intense. Even so we cannot choose the best extraction condition (solvent and technique), because that together with essential oils, some other compounds were extracted. In this case is indicated to be employed GC as analytical technique.

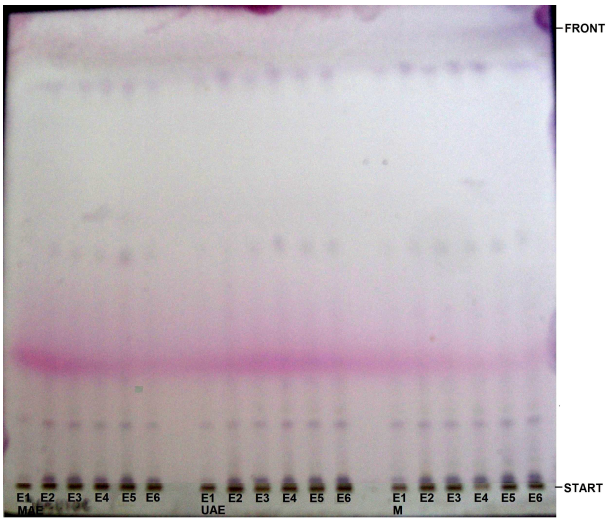

Figure 2. The chromatogram of *Ocimum basilicum* L. extracts obtained with solvent system E1-E6 using M, MAE and UAE techniques

The chromatograms of extracts E1-E6 obtained by MAE and some standards were registered by GC-FID (figure 3).

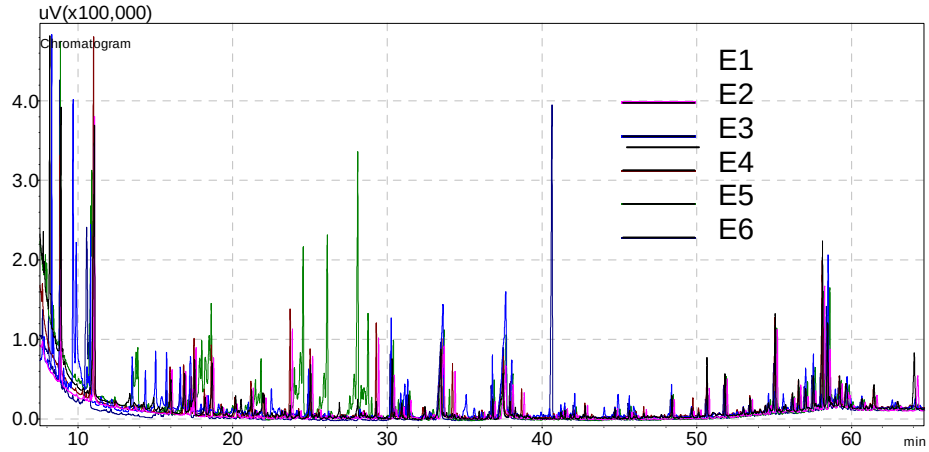

Figure 3. The chromatograms of *Ocimum basilicum* L. extracts obtained by MAE in various solvents mixtures.

It is very obvious that the extracts E5 has a different fingerprint, beginning with a R.T. = 15 min the shape of chromatogram is changed. The maximum area of the peak was obtained using the E5 mixture as extraction solvent. The chromatograms for E5 extracts obtained with the studied techniques were compared (figure 4).

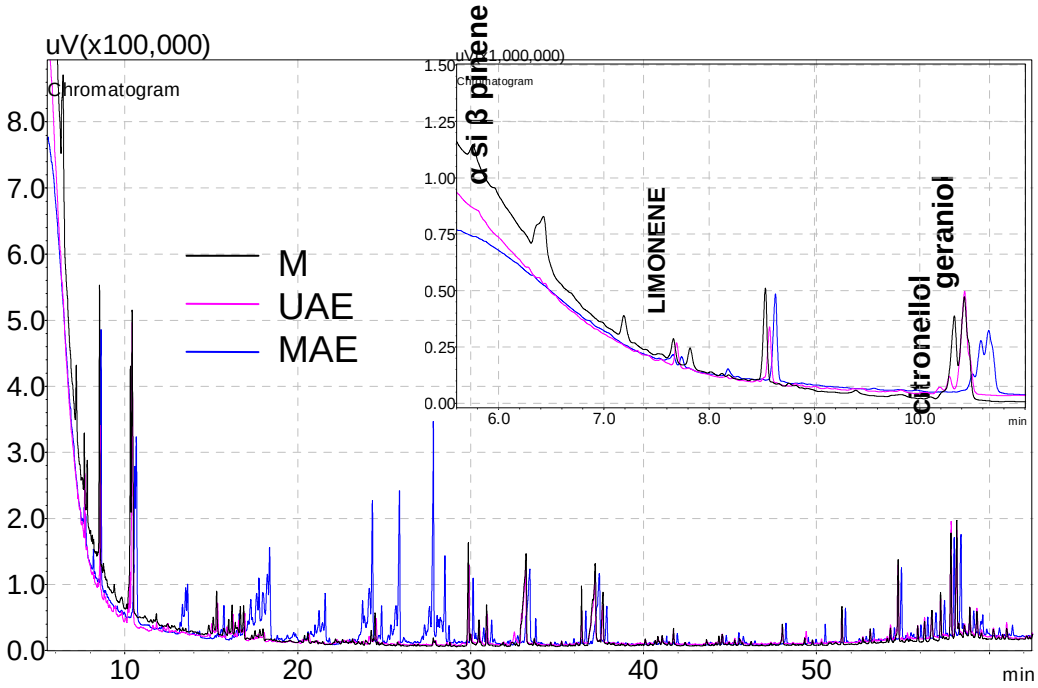

Figure 4. The chromatograms of *Ocimum basilicum* L. extracts E5 obtained by MAE and M, extracts E6 obtained by UAE

This reveals that M and UAE extracts are similar. Maceration is more efficient but more time consuming, which is a huge drawback. First part of chromatogram of the extracts obtained by MAE and M (the reference technique) are similar, but in the second one, after 14 min, are quite different. More probably this is done by some decomposition reaction induces by microwave than because some new extracted compounds.

Good results were obtained by MAE and M techniques. A great advantage of extraction in microwave field was the short time for extraction comparing with maceration or sonication.

By over layering the standards and extracts chromatograms some essential oils were identified: α and β-pinene (mixture), limonene, citronellol and geraniol.

There may be observed from other papers as well, that the essential oils fingerprint depend on extraction technique, solvent system and operation conditions. The modern techniques are advantageous, but they can change the composition of extract, enhancing some compounds and depleting other [26, 27].

CONCLUSIONS

The extracts were analyzed by TLC/HPTLC technique and the fingerprint information was obtained. The GC-FID was used for to establish the best conditions for extractions and for identifying the essential oils. The best extraction of essential oils was obtained by maceration using the mixture ethyl ether + ethanol (1:1, v/v) as extraction solvent. In *Ocimum basilicum* L. extracts were identified: α and β-pinene (mixture), limonene, citronellol, and geraniol.

EXPERIMENTAL SECTION

Materials

The plant material was commercially purchased. The essential oils standards were obtained from Fluka (Germany). The chromatographic plates were from Merck (Germany). All the solvents were from Chimopar (Bucharest, Romania). All chemicals were of analytical grade. Stock solutions were prepared in ethanol at 100 μg ml^{-1}.

Extraction Procedure

The vegetal material of *Ocimum basilicum* L. for culinary purpose was purchase from Kotany, Austria as dried leaves. After grinding with a hand mill (grinder), the powder was exactly weighed in portions of 0.5 g and subjected to solvent extraction with different systems and techniques. Following solvent were chose to perform the extraction: E1 – hexane; E2 – ethyl ether; E3 – ethanol; E4 – hexane + ethyl ether (1:1, v/v); E5 – ethyl ether + ethanol (1:1, v/v) and E6 – hexane + ethyl ether + ethanol (1:1:1, v/v). Each extraction procedure was optimized with respect the principal factors.

Maceration (M) was performed 14 days at room temperature with 15 ml extraction solvents E1-E6. After filtration and washing the final volume was adjusted at 25 ml.

Ultrasound solvent assisted extraction (UAE) was performed in two steps using a Transsonic T 310 bath at 35 kHz and an installed power of 95 W. In the first step sample was soaked 10 min with 10 ml extraction solvent (E1-E6). After 15 min of sonication the extract was separated (by decantation) and the sample was once again subjected for other 15 min sonication with 10 ml solvent (E1-E6). The sample was finally filtered and the residuum washed. The extracts were reunited and than the final volume were adjusted at 25 ml. For avoiding solvent leaks the extraction temperature was established at 4℃ (ice bath) and the extraction vessels were tightly closed.

Microwave solvent assisted extraction (MAE) was performed using a home made apparatus [28]. The device has the possibilities to control the operation time, temperature and duty cycle. Sample (0.5g) together with extraction solvent (20 ml) was placed into the extraction cell. In concordance with sonication the extraction procedure consist in two steps, 10 min soaking followed by microwave extraction. Taking in account the specificity of plant material the following parameter were selected: maximum temperature 30℃, action time 1 min and duty coefficient of 40% at an installed power of 900 W. Depending of the absorption capacity of the solvent, the entire extraction time takes more than 1 min because the cell needs to cool down bellow 30℃. Because of the low operation temperature, the solvent systems used do not boiled, so the extraction can be conducted at atmospheric pressure. After filtration and washing the final volume was adjusted at 25 ml.

TLC analysis

TLC analyses were performed on two kinds of plates: TLC Sil G F_{254} and HPTLC Sil G F_{254} pre-coated plates. Prior using, the TLC plates were conditioned with methanol and dried at 110℃ for 3 h. The samples were applied with a Linomat 5 device as 5 mm bands, 20 µl for plant extracts and 7 µl for standards. In the case of HPTLC plates the applied volume was decreased at 10 µL for extracts and at 5 µl for standards. Every time a mixture of toluene-ethyl acetate (93:7, v/v) was used as mobile phase. The developed plates were sprayed with anisaldehyde and than heated 3 min at 110℃ when red-bluish bands appear. The plates were inspected in daylight and also at 366 nm in UV range [25].

GC-FID analysis

GC analysis was performed with a Shimadzu GC-2010 gas chromatograph with flame- ionization detection (FID). Compounds were separated on a methyl silicone column OV-17 (2m x 3.16 mm, 80-100 Mesh). Helium was used as carrier gas at 15 ml/min flow rate. The oven temperature was programmed 2 min at 80℃ increased to 200℃ wit h 4℃/min, maintained 1 min and then with 20℃/min to 260℃ and held for 35 min. The injection port and detector temperature were 260℃ and 240℃ respectiv ely. The injection volume was 2 µl for extract samples and standards (100 mg/ml).

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