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QUALITATIVE ANALYSIS OF EUGENOL AND POLYPHENOLIC COMPOUNDS IN BASIL

BY

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Abstract. This study aims to analyze phenolic compounds and to identify the amount of eugenol from basil alcoholic extracts, obtained by three different methods, namely: maceration, Soxhlet apparatus and ultrasound extraction. Experimental data show that the alcoholic basil extract macerated at 50°C (EA M50) has a much higher polyphenol content than the other extracts, and is the only extract that also contains eugenol as evidenced by thin layer chromatography (TLC). This study aims to highlight the fact that a spicy plant, such as basil, can be used to obtain various alcoholic extracts that can be applied in the preparation of cosmetic and pharmaceutical products, due to the content of important active substances such as: eugenol and phenolic compounds.

Keywords: alcoholic extracts; basil; eugenol; natural extracts; phenolic compounds.

1. Introduction

Basil (*Ocimum basilicum*) is a spicy plant, extremely well known worldwide and is frequently used in gastronomy (Goncearіuc, 2008; Zeggwagh

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et al., 2007). The antioxidant, antiseptic and antibacterial properties are given by the chemical composition which is very diverse, including: volatile oil, polyphenolic compounds, vitamins, tannins, saponosides, triterpenes, mucilages, etc. (Ijeh *et al*., 2004). Basil oil contains a number of compounds such as: linalool, eucalyptus, eugenol, camphor, etc. (Gebrehiwot *et al*., 2015; Özcan and Chalchat, 2002) that are responsible for the taste and the unmistakable aroma. The importance of phenolic compounds has been highlighted by many researchers; their studies have highlighted the antioxidant activity of basil compounds. (Xiuzhen *et al*., 2007). Moreover, the antibacterial properties of plant-derived polyphenols are a topic of interest because they could become an important source of effective substances against antibiotic-resistant pathogens (Hubert *et al*., 2003; Murphy, 1999; Rao *et al*., 2010; Sakagami *et al*., 1999).

Eugenol is an important phenolic compound found in some plants that is used in low concentrations in the pharmaceutical, food and cosmetics industries. Studies on eugenol have shown that it can have a wide range of uses due to its properties, such as: antimicrobial, anti-inflammatory, analgesic and antioxidant (Grespan *et al*., 2012; Leem *et al*., 2011; Oyedemi *et al*., 2009; Pei *et al*., 2009; Qiu *et al*., 2010).

Considering these aspects, in this study the amount of eugenol was identified and phenolic compounds from alcoholic basil extracts obtained by three different methods, namely: maceration, Soxhlet apparatus and ultrasound were analyzed. Thus, we want to highlight the fact that from a spicy plant, such as basil, can be obtained various alcoholic extracts that can be used in the preparation of cosmetics and pharmaceutical products due to the properties of the active substances contained.

2. Experimental

2.1. Preparation of the Plant Material

Dried and chopped basil, without other additives, that were purchased commercially was used for this study. In order to obtain alcoholic basil extracts, ethyl alcohol of agricultural origin, 96º, producer Prodvinalco, purchased from supermarket, was used. For the alcoholic macerates in 50º, 60º and 70º ethanol, the concentrated alcohol of 96º was diluted according to the specialized literature (Horoba and Horoba, 2010).

2.2. Obtaining Alcoholic Extracts

2.2.1. In order to obtain the *basil extracts* by *maceration in ethyl alcohol*, basil, previously ground in a mortar with a pistil for the release of volatile oils, was introduced in three 500 mL flasks then, ethyl alcohol of 50º, 60º and 70º was added to a 1:10 mass ratio plant: solvent. The three vials were left to soak for 4

weeks, at room temperature, each being stirred periodically. After this, the mixture in each vial was filtered to finally obtain three alcoholic extracts of 50, 60 and 70°.

2.2.2. In order to obtain the *basil extract using the Soxhlet apparatus*, a plant material: solvent in 1:20 ratio was used. The Soxhlet extraction was performed in a laboratory installation, and the extraction occurred for 5 hours, with 2 refluxing / hour.

2.2.3. In order to obtain the *ultrasonic basil extract*, a plant material: solvent in a 1:10 ratio was used. The extraction was performed for 30 minutes in a Digital Ultrasonic Cleaner, with a power of 50W and a frequency of 42000 Hz.

The concentration of the basil extracts obtained by maceration, Soxhlet apparatus and ultrasound was performed using a rotary evaporator.

2.3. Analysis of Alcoholic Extracts

The analysis of polyphenols in alcoholic basil extracts was performed using the Folin-Ciocalteu method (Becze *et al*., 2017), based on the fact that polyphenols in plant extracts react with specific redox reagents (Folin-Ciocalteu reagent) and form a blue complex which can be quantified by visible light spectrophotometry. Thus, 0.5 mL of the sample was measured and added into a 10 mL volumetric flask with 0.5 mL of Folin-Ciocalteu solution, 5 mL of distilled water and 1.5 mL of sodium carbonate solution (20%). The flask was filled to the mark with distilled water. The volumetric vial samples were allowed to stand for 90 minutes and then the absorbance was measured at 765 nm wavelength using a Perkin Elmer Lambda 25 UV/VIS Spectrophotometer. The measurements were compared with a gallic acid calibration curve (25, 50, 100, 250, 500 ppm), and the results were expressed in mg of gallic acid (GAE)/mL.

Qualitative analysis of eugenol from alcoholic basil extracts was performed by thin layer chromatography (TLC) according to the literature method (Cernatescu and Cobzaru, 2014).

3. Results and Discussions

As mentioned above, the alcoholic basil extracts were obtained by three methods, namely maceration, Soxhlet apparatus and ultrasound. Table 1 presents the values of extraction yields by each method studied.

As observed from Table 1, the ultrasonic extraction was performed with the highest efficiency, due to the fact that the extraction by this method was performed with a small amount of energy and in a very short time (30 minutes).

The extraction with alcohol by maceration occurred with lower yields also due to the fact that in time, part of the solvent evaporated since the process was carried out 4 weeks, at room temperature.

Table 1
Extraction Yield for the Methods Applied

No.	Type of Extract	Method	Yield, %	Abbrevation
1.	Basil alcoholic extract 50°C	maceration	42.91	EA M50
	Basil alcoholic extract 60°C		50.03	EA M60
	Basil alcoholic extract 70°C		51.49	EA M70
2.	Basil extract	Soxhlet apparatus	63.5	EA S
3.	Basil extract	ultrasound.	75	EA U

Table 2 presents the values of the yields obtained at the concentration of alcoholic basil samples using the rotary evaporator.

Table 2
Yield for Concentrating the Basil Alcoholic

No.	Extract type	Method	Yield, %
1.	EA M50	samples concentration of using the rotary evaporator	48.82
	EA M60		36.96
	EA M70		26.89
2.	EA S		6.77
3.	EA U		2.66

Table 2 show that yields for the concentration step are very low, due to the losses that took place during the process.

Table 3 presents the quantities of polyphenols existing in the concentrated extracts.

Table 3
Polyphenol Content of Analyzed Concentrated Extracts

No.	The sample	Polyphenols, mg/mL
1.	EA M50	2.16
2.	EA S	0.48
3.	EA U	0.63

Although the extraction yield was low, the alcoholic macerated basil extract (EA M50) has a much higher polyphenol content than the other extracts. This can be explained by the extraction process, which occurs at room temperature, for four weeks. Even if the extraction was carried out in a very short amount of time (30 minutes), the ultrasonic alcoholic basil extract (EA U) still has a content of polyphenols lower than that obtained by maceration (EA M50), but higher than that obtained to the Soxhlet. The result may be explained because the ultrasound extraction method enhances the amount of polyphenols extracted

even in the shorter extraction time (30 minutes) compared to that of the Soxhlet device (5 hours).

According to the literature, the best-known basil extract is the volatile oil containing linalool, eucalyptol, eugenol, estragol, camphor, anethole (Gebrehiwot *et al*., 2015) which explain the pharmacological action of basil oil. Thus, eugenol was also identified from the alcoholic basil extracts obtained by the three extraction processes using thin layer chromatography (TLC).

Figure 1 shows the two plates in which the spots corresponding to the active compounds in the basil alcohol extracts were highlighted, identified by the value of the retention coefficient (R_f), calculated using the equation from the literature (Cernatescu and Cobzaru, 2014).

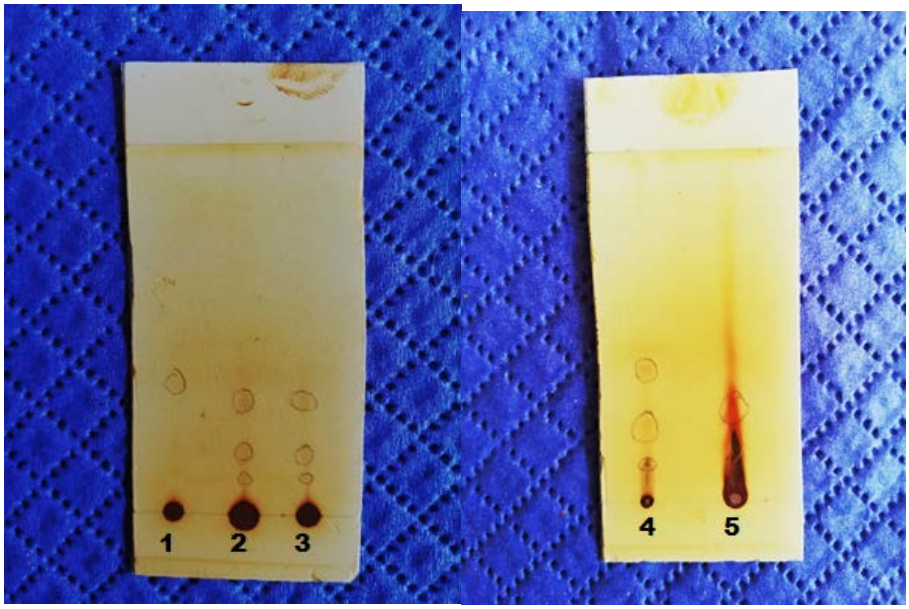

Fig. 1 – TLC for the alcoholic basil extracts.

Table 4 shows the values (R_f) calculated for the alcoholic basil extracts.

Table 4

R_f values, for the Analyzed Basil Extracts

Sample	Rf_1	R_{f2}	R_{f3}
EA M50	0.34	-	-
EA M60	0.30	0.16	0.10
EA M70	0.30	0.16	0.10
EA S	0.36	0.20	0.10
EA U	0.24	-	-

According to the literature (Cernatescu and Cobzaru, 2014), the R_f value for eugenol in basil extracts is 0.34, a value that is also found in the alcoholic macerated basil extract 50º (Table 4).

4. Conclusions

Alcoholic basil extracts were analyzed for polyphenols and eugenol. It was found that the alcoholic macerated basil extract 50º (EA M50) has a much higher polyphenol content than the other extracts. Moreover, in this extract there is eugenol, a fact highlighted by thin layer chromatography (TLC). Also, even if the extraction was performed at high temperatures, the ultrasonic basil alcohol extract still has a higher polyphenol content than that obtained with the Soxhlet device. This study aims to highlight the fact that from a spicy plant such as basil can be obtained various alcoholic extracts that can be used in preparation of cosmetics and pharmaceuticals due to the content of important active substances: eugenol and phenolic compounds.

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IDENTIFICAREA CALITATIVĂ A EUGENOLULUI ŞI ANALIZA COMPUŞILOR POLIFENOLICI DIN BUSUIOC

(Rezumat)

Acest studiu îşi propune să analizeze compuşii fenolici şi să identifice calitativ eugenolul din extractele alcoolice de busuioc obţinute prin trei metode diferite şi anume: macerare, aparat Soxhlet şi ultrasunete. Datele experimentale arată faptul că, extractul alcoolic macerat de busuioc la 50° (EAM50) are un conţinut de polifenoli mult mai mare decât celelalte extracte şi este singurul extract care conţine şi eugenol fapt pus în evidenţă de cromatografia în strat subţire (TLC). Prin acest studiu se doreşte să se evidenţieze faptul că dintr-o plantă condimentară precum busuiocul se pot obţine diferite extracte alcoolice care pot fi utilizate la rândul lor în prepararea unor produse din industria cosmetică şi farmaceutică datorită conţinutului unor substanţe active importante: eugenol şi compuşi fenolici.