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Optimization of Thymol Extraction from Aerial Parts of *Thymus vulgaris* by the Response Surface Method

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ABSTRACT: Thyme is widely used in the food and pharmaceutical industry as an antimicrobial and antioxidant aromatic component, mainly due to its essential oil composition especially the presence of Thymol. In this research, the essential oil of *Thymus vulgaris* collected from Hamedan was obtained by hydro-distillation method. The response surface method was employed to determine the optimal values of the evaluated factors. The proposed optimal conditions were 1.5 to 2.5 hours for 60 to 80 grams of sample per 100 ml. The optimum extraction time of essential oil from the aerial parts and leaves of thyme were 2.5 hours for 77.63 grams and 2.5 hours for 64.66 grams per 100 ml respectively. The results of experiments in optimum conditions showed that the highest amount of Thymol was found in the essential oil obtained from aerial parts of thyme.

Keywords: *Essential oil, Extraction, Thymus vulgaris, Thymol.*

Introduction

Essential oils are a category of volatile oils found in the leaves, flowers, skin, stem, seeds, fruits, roots and underground stems of plants and also in oily gum in some cases. They show different acquaintances in their flavor and scent depending on type and number of carbons in major and minor chains connected as well as aliphatic and aromatic components. Preparation of these substances is accomplished in two types of laboratory synthesis and natural separation of plants where the extraction methods are divided in two groups of traditional and novel. Traditional method consists of distillation and novel extraction methods consists of microwave and ultrasound (Meshkatalsadat *et al*., 2013).

An essential oil that is practical in medical and food industry is the Thyme essential oil. *Thyme vulgaris* a plant of the family Lamiaceae has fourteen aromatic species and perennial where four of them are exclusive of Iran (Davazdahemami and Majnoonhosini, 2008).

The fourteen species reported in Iran is mainly disposed in the north and west of the country. There are 10 species in northern provinces (Gorgan, Gilan, Mazandaran), 11 species in western provinces (Azarbayejan, Kermanshah, Hamedan, Kordestan, Lorestan, Chaharmahal O Bakhtiari and Esfehan), 7 species in center (Tehran, Semnan, Qazvin, Arak and Yazd), one

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specie in Fars and two species in Kerman. *Thyme vulgaris* culture in Tehran, Semnan, Kermanshah, Markazi, East Azarbayejan, Khorasan and Esfehan provinces has been accomplished (Naghdi Badi *et al*., 2003).

Essential oil, an efficacious compound of *Thymus vulgaris*, is a dark reddish yellow or brown liquid with strong pleasant smell and hot flavor which is extracted from leaves and flowered trimmings (Momeni and Shahrokhi, 1991).

Thymus vulgaris includes 0.8% to 2.6% (usually 1%) essential oil where most of it is composed of monoterpen hydrocarbons especially p-cymene and y-terpinene and alcohols like linalool, α-terpinene and thujan-4-ol that sometimes any of these compounds are composed of 80% of essential compounds. In an experiment by distillation with water, essential oil was extracted from aerial parts (branch, leaf, flower) of the plant and after recognition of its components by gas chromatography-germ spectroscopy, it was designated that the Thymol concentration is significantly different.

Thymus vulgaris as a smelling component with an anti-microbial property which is mainly due to Thymol and Karvakril has been used in large quantities in mouthwashes, toothpastes, soaps, detergents and different medical products and it has been advised for treatment of whooping cough, tuberculosis and bronchitis in dosage of 0.3 up 0.6 ml (Leung & Foster, 1996; Dhifi, 2016; Kashiri, 2016). Now in Iran, different medical productions of *T. vulgaris* have been made and are used by patients widely such as thymarta drop, thymex tablet and syrup and thymean syrup that are used as anti-cough and spit syrup (Leung & Foster, 1996). *T. vulgaris* is also used in foods for flavoring and as anti-microbial and anti-oxidant agent (Sharafzadeh & Alizadeh, 2012). Therefore due to various applications of *T. vulgaris*, this study and research work has focused on optimization of essential oil extraction from aerial parts of *T. vulgaris* and its purification.

Materials and Methods

- *Preparation*

In this study, *T. vulgaris* was obtained from botanical garden in Hamadan province. The plants were primarily dried in shadow at room temperature (for 3 days). *T. vulgaris* aerial parts (10 g) was weighted, the root and leaves were separated and were separately weighted. The leaves and stem accounted for 55 and 45 percent respectively. Leaves and aerial parts samples were separately grinded and transmitted through 30 mesh size sieve.

- *Essential oil extraction by water distillation method*

Quantities of 60, 70 and 80 g of aerial parts or leaves were poured in to 2000 ml volumetric flask containing 750 ml water. Clevenger apparatus was applied and essential oil was obtained, and the product was kept in dark vial in the refrigerator.

- *Recognition and determination of Thymol concentration in T. vulgaris*

In order to determine Thymol concentration in essential oils, a calibration curve was drawn and the concentration of Thymol was then determined by standard. Therefore standard solution of Thymol with different concentrations were prepared and applied to Shimadzu gas chromatography equipped with DB-5 capillary column. The thermal program of column was started from 60˚C and after 5 minutes staying in that temperature, it was gradually increased by speed of 7 ˚C in minute until it reached to 220 ˚C. The temperature of injector and detector was regulated on 250 ˚C.

- *Statistical Analysis*

In this study the response surface method in central compound plan model was used for anticipation of changeable effect.

Analysis of data and drawing the graph was performed by Design Expert 7.0.1 program. Designed treatments have been presented in Table 1 by response level for preparing essential oil method. Selection of suitable model and evaluation of model correctness and effect of different ratio of solid to solution and time to output and Thymol concentration (mg/100g) of essential oil were calculated by the program and the optimum condition was defined.

Table 1. The treatment designed for preparing the essential oil based on the response surface method

Row	Solid weight (g/100ml)	Time (h)
1	60	2
2	60	1.5
3	60	2.5
4	70	2
5	70	1.5
6	70	2
7	70	2
8	70	2
9	70	2.5
10	70	2
11	80	1.5
12	80	2.5
13	80	2

Results and Discussion

The minimum obtained quantity of essential extraction from Thymus leaves for 80 g sample in 100 ml of solution in laboratory condition during 2 hours was 0.62%, while the maximum quantity obtained for 60 g sample per 100 ml of solution during 2.5 hours of extraction was 1.16% (Table 2). Regarding the whole sample of plant (stem-leaves), the minimum quantity of obtained of essential extraction through 1.5 to 2 hours of extraction of 60 g sample in 100 ml of solution was 0.33% and the maximum for 80 g sample in 100 ml of solution for extraction time of 2-2.5 hours was 0.62 (Table 3). The results of GC analysis (Figure 1) showed that Thymol was the predominant component in stem-leaf (53.63%) and leaves (59.47%) essential oils. The calibration graph was drawn by using the different standard concentration of Thymol (10, 100 and 250 ppm) and the coefficient of correlation was calculated for the thymol R^2 = 0.9999 while the equation of direction was y= 0.000000004x-0.00001 (Figure 2).

Table 2. Essential oil extraction output and Thymol levels of *Thymus vulgaris*

Thymol level (mg in 100 g of plant)	Output (%)	Essential oil level (g)	Extraction time (h)	Weight (g/ 100 ml solution)
0.1930	1	0.6	1.5	60
0.2655	1	0.6	2	60
0.3115	1.16	0.7	2.5	60
0.2646	0.85	0.6	1.5	70
0.3187	0.71	0.5	2	70
0.3188	1	0.7	2	70
0.2933	0.71	0.5	2	70
0.3034	0.71	0.5	2	70
0.3436	0.85	0.6	2	70
0.3882	0.85	0.6	2.5	70
0.2236	0.75	0.6	1.5	80
0.3200	0.62	0.5	2	80
0.4548	0.87	0.7	2.5	80

Table 3. Essential extraction output and Thymol levels of stem-leaves essential of *Thymus vulgaris*

Thymol level (mg in 100 g of plant)	Output (%)	Essential level (g)	Extraction time (h)	Weight (g/100 ml of solution)
0.1930	1	0.6	1.5	60
0.2655	1	0.6	2	60
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0.2236	0.75	06	1.5	80
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0.4548	0.87	0.7	2.5	80

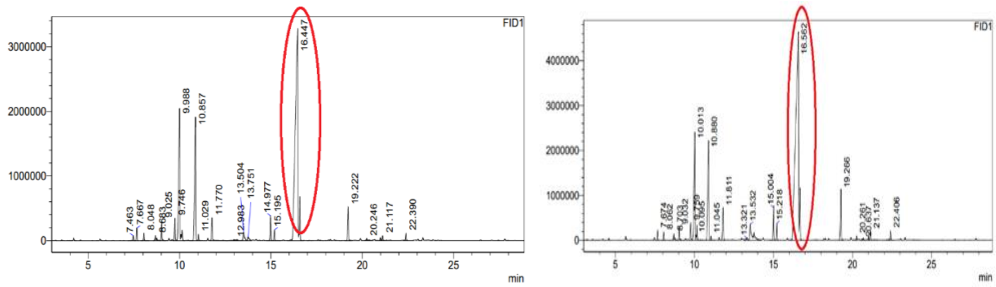

Fig.1. Chromatogram analysis of leaf (right) and stem-leaves (left) essential oil samples.

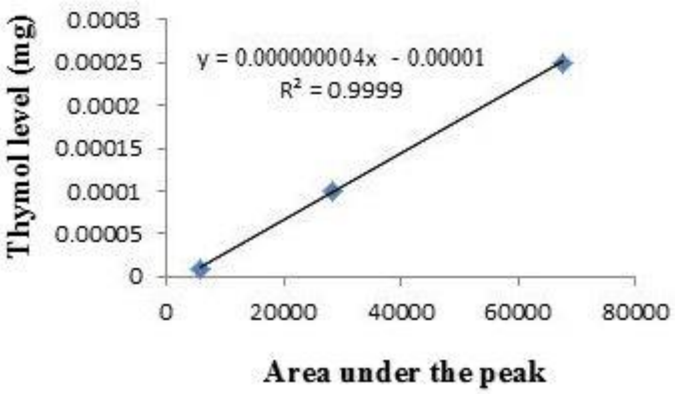

Fig. 2. Calibration graph (mg Thymol in area under the peak)

Figure 3 illustrates that the essential oil output level decreased by increasing of the leaf proportion (60 to 80gr) in 100 ml solution of sample. In fact the leaf sample weight in 100 ml solution has reciprocal on final level of essential output. Time effect on mg Thymol in 100 g leaf samples (Figure 4) shows that by increasing the time (1.5 to 2.5 hours) and Thymol leaf sample in 100 ml of solution (60 to 80 g), Thymol level in essential oil is increased. The obtained result is in agreement with many other studies in this field. Among them, Kashiri *et al*. (2016) that investigated the deliverance of anti-microbial shiraz Thymus essential components of active zein biology composite film in food models. The result of their research defined that by increasing the extraction time, the released Thymol level is also increased. Figure 5 shows the reciprocal effect of variables on Thymol mg level on 100 g of leaf sample. As it is presented by increasing the time (1.5 to 2.5 hours) and initial weight of leaf samples (60 to 80 g), Thymol weight in essential samples is increased.

Figure 5 present the reciprocal effect of variants on Thymol level in leaves essential oil samples.

Operational conditions to define the parameters were analyzed by using the numerical optimization technique with design export program version 8. The response variant included leaves essential oil numerical output level. The suggested optimization with program as presented in Figure 6 is time of 2.5 hours and 60 g sample in time range of 1.5 to 2.5 hours and weight level range of 60 to 80 g of sample in 100 ml solution. In these conditions, the optimization level for numerical output level was estimated 1.05%.

Figure 7 shows the effect of leaves-stem weight in 100 ml of solution in constant time of 2 hours in extraction of essential oil obtained. By increasing the sample weight (60 to 80 g) in 100 ml of solution, the product obtained is increased and also by increasing the solid weight (60 to 80 g) in 100 ml of solution, the level of Thymol weight in leaves-stem samples is increased and then slightly decreased.

The suggested optimization condition for time range of 1.5 to 2.5 hours and weight level range of 60 to 80 g stem and leaves in 100 ml of solution is shown in Figure 8. These conditions include time of 2.5 hours and 77.63 g weight of sample in 100 ml of solution. In these conditions, optimization level for numerical response range, 0.6059 % has been estimated.

Experimental data, for determining the Thymol weight level in stem-leaf showed that the minimum level of this variant, in this laboratorial conditions with 60 g solid sample in 100 ml solution and extraction time of 1.5 hours, 0.2984 mg was obtained while the maximum concentration (0.5625 mg) by 70 g in 100 ml of solution and extraction time of 2.5 hours, was obtained. Figure 9 illustrates the direct effect of time on thymol weight. As it shows time of 2.5 hours for extraction gives the best result of thymol level.

The suggested optimization conditions by program in time range of 1.5 to 2.5 hours and level range of 60 to 80 g of aerial parts sample in 100 ml of solution is according Figure 10. These conditions include time of 2.5 hours and 77.63 g weight of sample in 100 ml of solution. In these conditions, the optimized level for numerical response level was estimated 0.5625.

Thymol (2-isopropyl-5-methylphenol) belongs to the phenolic monoterpenes and mostly exists in thyme species. It is one of the original compounds of thyme essential oil. Both thyme essential oil and Thymol have long been used in traditional medicine as antibacterial, anti- inflammatory antiviral, expectorant and antiseptic agents (Kowalczyk *et al*., 2020; Jannati *et al*., 2021; Aghamohammadi *et al*., 2016; Nickavar *et al*., 2005; Escobar *et al*., 2020). Thyme (*Thymus vulgaris*) is an annual herbaceous shrub that belongs to the Lamiaceae family with 200 genus and 2000-5000 species of aroma. It grows throughout the Mediterranean region (Jannati *et al*., 2021).

Most of the biological properties of the thyme are mediated by thymol, the main monoterpene phenol components of the thyme. Different extraction techniques like steam distillation and hydrodistillation are conducted on thyme to obtain essential oil of it (Mnayer *et al.*, 2017). Generally essential oils consisted of many chemical compounds of different structures and functional properties, employed as an ingredient in many foods, drug formulations, and products.

Due to the economic importance of *Thymus vulgaris'* essential oil, it is of interest to optimize its extraction from the aerial parts. In this research, aerial parts essential oil of cultivated *Thymus vulgaris* L. was isolated by hydrodistillation method. The response surface methodology (RSM) that is a widely used mathematical and statistical method for modeling and analyzing a process has been applied in this research work (Aydar, 2018). The results showed that the suggested optimized conditions were in time range of 1.5 to 2.5 hours and weight level range of 60 to 80 g of leaves sample in 100 ml of solution. The

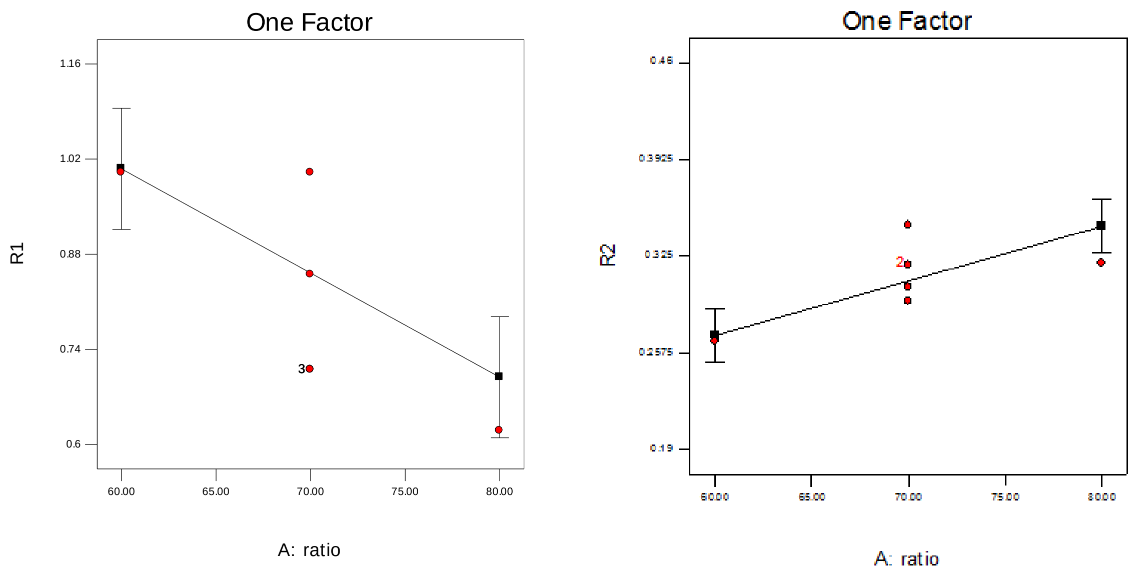

Fig. 3. Reciprocal effect of leaf sample weight in 100 ml solution on essential output level (right) and Thymol level (left).

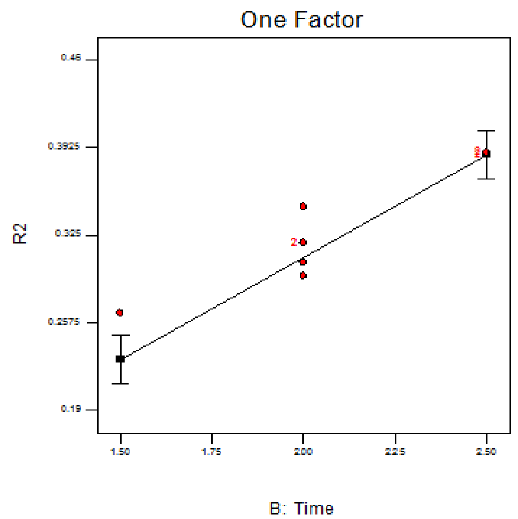

Fig. 4. Reciprocal effect of time on Thymol leaf essential level.

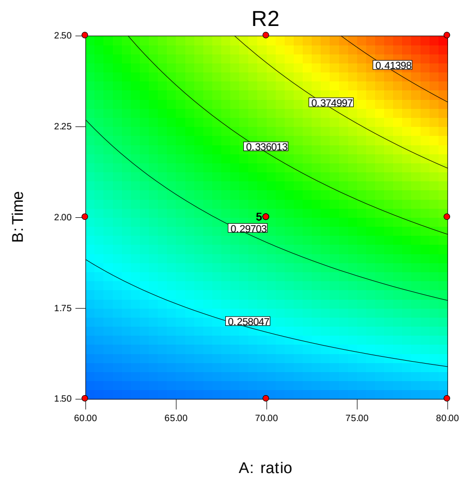

Fig. 5. Reciprocal effect of variants on Thymol level in leaves essential samples.

optimized time and weight of sample levels for achieving the best result for Thymol level and extraction output for different samples has been separately explained in following.

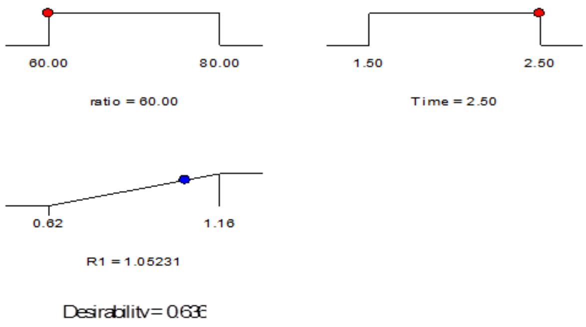

Fig. 6. Optimization for achieving to independent and dependent parameter.

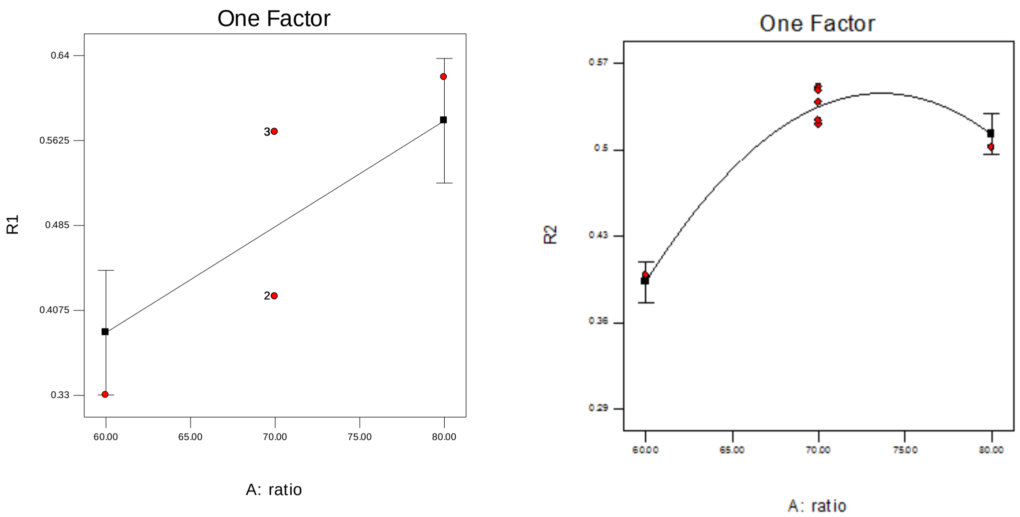

Fig. 7. Reciprocal effect of lead-stem weight in 100 ml of solution in extraction essential output (right) and Thymol level (left).

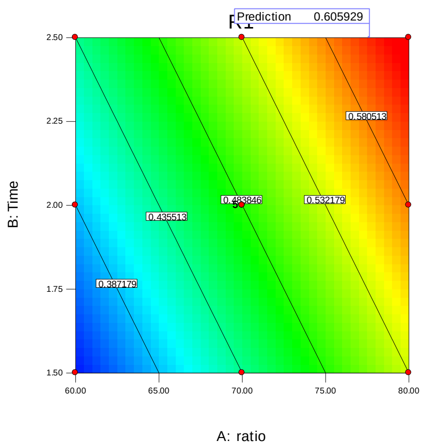

Fig. 8. Two dimensional optimization graph for achieving to independent and dependent parameters of lead-stem essential output

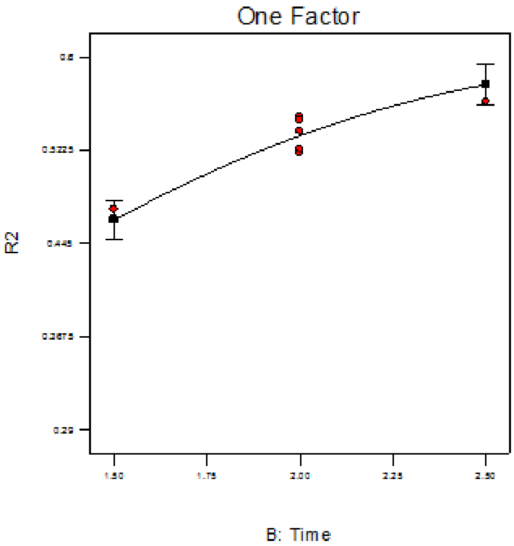

Fig. 9. Effect of time on Thymol weight in aerial parts samples.

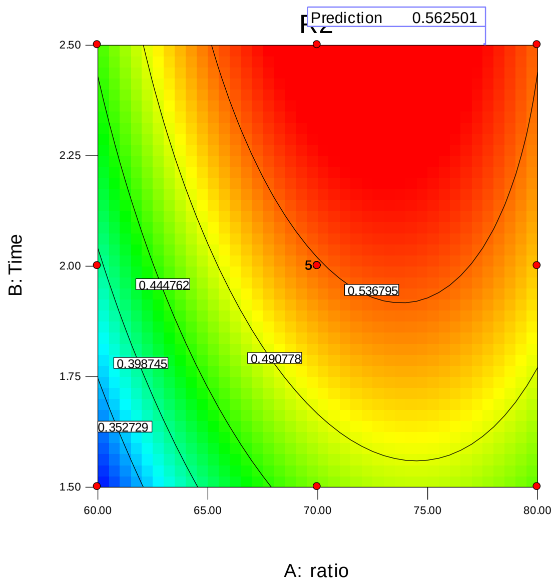

Fig. 10. Two dimensional optimization graph for achieving to in depended and depended for Thymol weight in aerial parts essential oil.

-For Thymol leaf-stem:

<u>The best essential oil output and coincidently the best Thymol level:</u>

Time of extraction: 2.5 hours

Sample weight: 77.63 g in 100 ml of solution

- For Thymol leaves:

<u>The best essential oil output:</u>

Time of extraction: 2.5 hours

Sample weight: 60 g in 100 ml of solution

<u>The best Thymol level:</u>

Time of extraction: 2.5 hours

Sample weight: 64.46 g of per 100 ml solution.

Finally After definition of optimization conditions, available Thymol level in obtained essential oil of 77.63 gr of aerial parts in optimized conditions was 0.3142 g (0.4047 gr in 100 g of leaf-stem) and the available Thymol level in obtained essential oil of 60 gr of leaf in optimization conditions was 0.3641 g (0.6068 g in 100 g of leaves).

Conclusion

Response surface methodology was applied to optimize the extraction time and sample concentration to obtain essential oils from leaves and areal parts of the sample. The final result demonstrated that the maximum amount of Thymol can be obtained from aerial parts of thyme. As it was described the 77.63 g of aerial parts sample in 100 ml of solution by the time of 2.5 hours for extraction gained the best result.

References

Aghamohammadi, A., Azadbakht, M. & Hosseinimehr, S. J. (2016). Quantification of Thymol content in different extracts of Zataria multiflora by HPLC method. Pharmaceutical and Biomedical Research, 2 (1), 8-13.

Aydar, A. Y. (2018). Utilization of Response Surface Methodology in Optimization of Extraction of Plant Materials. 157-169

Davazdahemami, S. & Majnoonhosini, N. (2008). Cultivation and Production of Certain Herbs and Species. Tehran University publications, pp 300.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A Critical Review. Medicines, 3 (25), 1-16.

Escobar, A., Pe´rez, M., Romanelli, G. & Blustein, G. (2020). Thymol bioactivity: A review focusing on practical applications. Arabian Journal of Chemistry, 13, 9243–9269.

Jannati, N., Honarvar, M. & Gharachorloo, M. (2021). Extraction of Thymol Compound from *Thymus vulgaris* L. Oil. Journal of Medicinal Plants and By-products, 1, 81-84.

Kashiri, M., Maghsoudlou, Y. & Khomeiri, M. (2016). Release of antimicrobial components of Zataria multiflora Boiss. essential oil from bio active zein film into food simulants. Iranian Journal of Medicinal and Aromatic Plants, 32 (2), 322-332.

Kowalczyk, A., Przychodna, M., Sopata, S., Bodalska, A. & Fecka, I. (2020). Thymol and Thyme Essential Oil—New Insights into Selected Therapeutic Applications. Molecules, 25(18), 4125.

Leung, A. Y. & Foster, S. (1996). Encyclopedia of common natural ingredients: used in food, drugs, and cosmetics. A Wiley Inter Science Publication - John Wiley & Sons, Inc.

Momeni, T K. & Shahrokhi, N. (1991). Herbal essentials and their therapeutic effects. Tehran university publications, pp 12-18 [In Persian].

Meshkatalsadat, M., Rabiei. K. & Shabaninejad, Y. (2013). Chemical characterization of volatile oils of different parts of Satureja bachtarica Bunge. Journal of Advances in Chemistry, 5(2), 678-684.

Mnayer, D., Fabiano-Tixier, A. S., Petitcolas, E., Ruiz, K., Hamieh, T. & Chemat, F. (2017). Extraction of green absolute from thyme using ultrasound and sunflower oil. Resource-Efficient Technologies, 3, 12–21.

Naghdi Badi, H., Yazdani, D., Nazari, F. & Mohammad Ali, S. (2003). Seasonal variation in oil yield and composition from *Thymus vulgaris* L. under different dense cultivation. Journal of Medicinal Plants, 1(5), 51-56.

Nickavar, B., Mojab, B. F. & Dolat-Abadi, R. (2005). Analysis of the essential oils of two Thymus species from Iran. Food Chemistry, 90, 609- 911.

Sharafzadeh, Sh. & Alizadeh, O. (2012).

Some Medicinal Plants Cultivated in Iran, Journal of Applied Pharmaceutical Science, 2 (1), 134-137.