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Research Article

Technological factors of lipid extraction from flowers and seeds of *Ocimum basilicum* L. and *Ocimum tenuiflorum* L.

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Abstract

This study investigated the technological factors affecting the extraction of lipids and lipophilic compounds from the flowers and seeds of *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. Solid–liquid extraction with ethanol was performed using different ethanol concentrations, plant material particle sizes, extraction temperatures, and extraction duration. Extractions were carried out at room temperature and under thermostatically controlled conditions in a round-bottom flask equipped with a reflux condenser and a water bath. The maximum yield of total extractive substances was obtained with 70% ethanol, whereas the highest yield of lipids and lipophilic compounds among the tested ethanol concentrations was obtained with 96% ethanol, reaching 8.3% for *O. basilicum* and 8.1% for *O. tenuiflorum*. Reducing the particle size to 0.5 mm increased the yields to 9.1% and 8.9%, respectively. Increasing the extraction temperature to 60 °C further increased the yield to 11.2% for *O. basilicum* and 11.4% for *O. tenuiflorum*. Based on the obtained results, 96% ethanol, a particle size of 0.5 mm, and an extraction temperature of 60 °C were selected as the most suitable conditions among those investigated. A two-stage extraction scheme with durations of 3 to 2 h was established.

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1. Introduction

Plants of the *Ocimum* L. genus belonging to the *Lamiaceae* family are widely used in the pharmaceutical, food, and cosmetic industries due to their high content of biologically active compounds of diverse chemical nature [1]. The pharmacological and economic value of *Ocimum* species is primarily attributed to the presence of aromatic compounds, including methyleugenol, eugenol, and linalool, which have been reported to exhibit nematocidal activity and are used as pro-oxidants, antioxidants, dental anesthetics, disinfectants, and protective agents against the toxic effects of nicotine on mouse peritoneal macrophages [2, 3]. Particular attention has been given to *Ocimum basilicum* L. and *Ocimum tenuiflorum* L., which are important sources of phenolic compounds, terpenoids, essential oils, lipids, and other biologically active substances. In recent years, increasing interest has been directed toward the investigation of lipids and other lipophilic constituents of *O. basilicum* and *O. tenuiflorum*, as these compounds exhibit a wide range of biological activities and have considerable potential for the

development of pharmaceuticals and functional food products [4–7].

Previous studies have mainly focused on the extraction of phenolic compounds, antioxidants, and essential oils from *Ocimum* species, whereas the recovery of lipids and lipophilic fractions has received comparatively less attention [7-10]. Various solvent-based extraction approaches have been used to obtain biologically active constituents from basil, and the reported results indicate that the extraction efficiency depends on the nature and concentration of the solvent, as well as the technological conditions of the extraction process [8, 9]. However, systematic studies on the extraction of lipids and lipophilic compounds from *O. basilicum* and *O. tenuiflorum*, particularly with respect to the effects of solvent concentration, particle size, extraction temperature, and extraction time are limited.

The efficiency of extracting lipophilic compounds depends on several factors, including the nature of the extraction solvent, extraction temperature, particle size of the plant material, and extraction time. Among the various solvents employed, ethanol occupies a special position owing to its relatively low toxicity, environmental safety, and ability to extract a broad spectrum of compounds with different polarities [11, 12].

Despite the considerable number of studies devoted to the phytochemical composition of *Ocimum* species, the optimization of extraction conditions for isolating lipids and other lipophilic compounds from plant material containing the flowers and seeds of *O. basilicum* and *O. tenuiflorum* remains insufficiently investigated. Therefore, the present study was undertaken to investigate the effects of ethanol concentration, plant material particle size, extraction temperature, and extraction time on the yield of lipids and lipophilic compounds from the combined flowers and seeds of *O. basilicum* and *O. tenuiflorum*, and to identify suitable extraction conditions under the investigated experimental parameters. The objective of this study was to determine the extraction conditions that provide a high yield of lipids and lipophilic compounds from the flowers and seeds of *Ocimum basilicum* L. and *Ocimum tenuiflorum* L.

2. Materials and methods

2.1. Plant materials

The study was conducted using the flowers and seeds of *O. basilicum* and *O. tenuiflorum* cultivated in the Tashkent Region of the Republic of Uzbekistan (41.429405° N, 69.234515° E). The plant materials were collected during the flowering stage and at full seed maturity. Following collection, the samples were air-dried to a constant weight and subjected to pharmacognostic (macroscopic and organoleptic) analysis prior to extraction. The authenticated plant materials were then ground and used for the extraction experiments.

The analysis showed that the raw material consisted predominantly of seeds, which accounted for approximately 80% of the total mass, while flowers represented approximately 20%. Based on these results, the plant material was used as a combined mixture of flowers and seeds with a constant mass ratio of seeds to flowers of approximately 80:20. This ratio was maintained throughout all extraction experiments to ensure comparability and reproducibility of the obtained results.

To determine the yield of lipids and lipophilic compounds, the solvent was first evaporated from the extracts until a viscous residue was obtained. The resulting residue was then extracted three times with extraction-grade hexane at a solvent-to-residue volume ratio of 2:1. The three hexane fractions were combined and evaporated to constant weight. The resulting residue was taken as the yield of lipids and lipophilic compounds.

To determine the dry residue content in the solutions and extracts, a 5 mL analytical aliquot was transferred to a pre-weighed porcelain dish, evaporated to dryness in a water bath, and then dried in a drying oven at 102.5 ± 2.5 °C for 3 h. After drying, the dish was cooled in a desiccator for 30 min and weighed again. The dry residue content (%) was calculated as the ratio of the difference between the mass of the dish containing the dried residue and the mass of the empty dish to the mass of the analyzed sample.

The effects of ethanol concentration, plant material particle size, extraction temperature, extraction kinetics of lipids and lipophilic compounds from the combined flowers and seeds of *O. basilicum* L. and *O.*

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tenuiflorum L. were investigated according to previously described methods [13, 14].

2.2. *Selection of extraction solvent concentration*

Plant material samples (500.0 g) were subjected to five successive extractions with ethanol at different concentrations (60–96% v/v) using a plant material-to-extractant ratio of 1:4 (w/v) at room temperature. Each extraction step was performed for 6 h. The obtained extracts were combined, filtered, and analyzed for extractive substances, lipids, and lipophilic compounds.

2.3. Effect of particle size

Unground plant material and fractions with particle sizes of 1.0 and 0.5 mm were used. The samples (500.0 g) were extracted with 96% ethanol under the conditions described above. The combined extracts were analyzed for extractive substances, lipids, and other lipophilic compounds.

2.4. Effect of extraction temperature

Ground plant material (500.0 g; particle size 0.5 mm) was extracted with 96% ethanol at a plant material-to-extractant ratio of 1:4 (w/v) for 8 h at different temperatures. Extraction was performed in a round-bottom flask equipped with a reflux condenser and placed in a thermostatically controlled water bath. The obtained extracts were filtered and analyzed.

2.5. Extraction kinetics

Ground plant material (500.0 g) was extracted with 96% ethanol at 60 °C. Extract samples were collected after 1–7 h at 1-h intervals. Based on the contents of extractive substances, lipids, and lipophilic compounds, the extraction time required to reach equilibrium during the first solid–liquid contact was determined. After the first extract was separated, the residual plant material was contacted with a fresh portion of 96% ethanol for the time determined in the first extraction stage (second contact stage). The same procedure was repeated using another fresh portion of ethanol for the third contact stage. Thus, the first, second, and third contact stages represented three successive extractions of the same plant material with fresh portions of the extractant.

For the determination of lipids and lipophilic compounds, the combined ethanol extracts were concentrated to obtain a thick residue. The residue was subjected to five successive extractions with hexane at a residue-to-extractant ratio of 1:2. The hexane fractions were combined and evaporated to constant weight. The mass of the dry hexane-soluble residue was considered as the content of lipids and lipophilic compounds.

The hexane-soluble fraction was considered the total fraction of lipids and lipophilic compounds. Its qualitative composition has been previously confirmed by GC and GC–MS analyses, which revealed characteristic constituents of *Ocimum* plant material, including fatty acid derivatives, phytosterols, pigments, and other lipophilic compounds [7].

2.6. Statistical analysis

All extraction experiments were performed independently in triplicate (n = 3). The results are expressed as mean ± standard error of the mean (SEM). The obtained data were summarized descriptively using mean values and SEM. No inferential statistical tests or post hoc multiple-comparison analyses were performed. Therefore, the differences between experimental conditions are presented as observed differences in mean values and are not interpreted as statistically significant or non-significant.

3. Results and discussion

Ethanol was selected as the extraction solvent because of its suitability for the present study, owing to its ability to dissolve compounds of different polarities, relatively low toxicity, environmental safety, and its widespread use in the pharmaceutical and food industries. Ethanol at concentrations ranging from 60% to 96% (v/v) was used to extract lipids and lipophilic compounds from air-dried, ground plant material consisting of the flowers and seeds of *O. basilicum* and *O. tenuiflorum* (Table 1).

The analysis of the data presented in Table 1 shows that, as the ethanol concentration increases, the yield of total extractive substances increases up to a certain limit, after which it decreases.

The highest yield of extractive substances was observed when using 70% ethanol, amounting to 26.3% for *O. basilicum* and 25.7% for *O. tenuiflorum*. These results indicate that among the ethanol concentrations investigated, 70% ethanol provided the highest extraction yield of polar and semi-polar compounds

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Table 1. Effect of ethanol concentration on the yield of extractive substances, lipids, and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material.

Ethanol concentration (%)	Yield (%) of raw material mass			
	Extractive substances		Lipids and lipophilic compounds	
	O. basilicum	*O. tenuiflorum*	*O. basilicum*	*O. tenuiflorum*
60	21.6 ± 0.6	20.8 ± 0.5	3.9 ± 0.11	3.8 ± 0.10
70	26.3 ± 0.9	25.7 ± 0.7	5.4 ± 0.16	5.0 ± 0.14
80	25.5 ± 0.7	24.8 ± 0.6	6.5 ± 0.19	6.2 ± 0.17
90	19.4 ± 0.5	18.5 ± 0.4	7.9 ± 0.23	7.5 ± 0.21
96	14.9 ± 0.4	14.2 ± 0.2	8.3 ± 0.24	8.1 ± 0.23

under the tested conditions.

At the same time, the yield of lipids and lipophilic compounds increased progressively with increasing ethanol concentration. The maximum values were recorded when using 96% ethanol, reaching 8.3% for *O. basilicum* and 8.1% for *O. tenuiflorum***.** This can be explained by the higher efficiency of concentrated ethanol in extracting non-polar compounds.

A comparative analysis of the results showed that the patterns of changes in the yield of extractive substances and lipophilic compounds were similar for both *O. basilicum* and *O. tenuiflorum*, with only minor differences observed between the two species. Thus, among the investigated ethanol concentrations, 70% ethanol was selected for subsequent experiments aimed at obtaining total extractive substances, whereas 90–96% ethanol provided the highest lipid and lipophilic compound yields under the tested conditions.

The efficiency of extracting biologically active compounds from plant material, including lipids and lipophilic substances, directly depends on the degree of particle size reduction. A decrease in particle size increases the total surface area and enhances the contact area with the solvent, thereby intensifying the extraction process. This factor is particularly important for the recovery of lipids and lipophilic compounds. However, excessive comminution is also undesirable. Very fine particles may become compacted during extraction, hindering solvent penetration through the plant material bed and slowing down filtration, ultimately reducing the overall efficiency of the process.

O. basilicum and *O. tenuiflorum* raw materials are characterized by specific morphological features, as their seeds have a relatively small and dense structure. During the study, it was established that approximately 70% of the material passed through a sieve with a 2.0 mm mesh size, which was considered sufficient for the initial technological degree of comminution. At the same time, it should be noted that mainly the seed fraction of the material passed through the sieve.

Lipids and lipophilic compounds are predominantly localized in seeds. Therefore, insufficient disruption of the seed coat may lead to incomplete extraction of these compounds during the extraction process. This factor is particularly important for *O. basilicum* and *O. tenuiflorum* raw materials, which are characterized by dense seed structures.

Considering this, an additional degree of comminution was applied in the study. The samples were prepared by passing the material through sieves with mesh sizes of 1.0 mm and 0.5 mm, after which the efficiency of lipid and lipophilic compound extraction using 96% ethanol was investigated (Table 2).

As can be seen from the data presented in Table 2, the degree of particle size reduction affected the yield of extractive substances as well as lipids and lipophilic compounds. The lowest values were obtained for the unground material: the content of lipids and lipophilic substances was 6.2% for *O. basilicum* and 6.0% for *O. tenuiflorum*.

Comminution of the plant material to a particle size of 1.0 mm increased the yield of lipids and lipophilic compounds, reaching 8.2% and 8.1%, respectively. The highest values were recorded at a particle size of 0.5 mm, amounting to 9.1% for *O. basilicum* and 8.9% for *O. tenuiflorum*. Notably, the increase in the yield of lipids and lipophilic compounds is primarily associated with more complete disruption of the seeds, damage to the integrity of the seed coat, and

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Table 2. Dependence of the yield of extractive substances, lipids, and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material on the degree of particle size reduction

Particle size of plant material (mm)	Yield of raw material mass (%)			
	Extractive substances		Lipids and lipophilic compounds	
	O. basilicum	*O. tenuiflorum*	*O. basilicum*	*O. tenuiflorum*
Unground	12.4 ± 0.3	11.9 ± 0.2	6.2 ± 0.2	6.0 ± 0.2
1.0	14.8 ± 0.4	14.1 ± 0.4	8.2 ± 0.2	8.1 ± 0.2
0.5	16.1 ± 0.5	15.5 ± 0.4	9.1 ± 0.3	8.9 ± 0.3

Table 3. Dependence of the yield of extractive substances, lipids, and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material on the extraction temperature.

Process temperature (°C)	Yield of raw material mass (%)			
	Extractive substances		Lipids and lipophilic compounds	
	O. basilicum	*O. tenuiflorum*	*O. basilicum*	*O. tenuiflorum*
20	16.1 ± 0.4	15.5 ± 0.3	9.1 ± 0.2	8.9 ± 0.3
40	18.2 ± 0.5	17.9 ± 0.4	9.7 ± 0.3	9.5 ± 0.3
60	20.4 ± 0.6	19.8 ± 0.5	11.2 ± 0.4	11.4 ± 0.4
80	20.7 ± 0.6	20.1 ± 0.6	11.5 ± 0.4	11.6 ± 0.4

exposure of the internal structure of the plant material.

Overall, the results obtained for *O. basilicum* and *O. tenuiflorum* showed a similar trend indicating that the degree of seed comminution is one of the main factors determining the efficiency of lipid and lipophilic compound extraction. According to the obtained data, the maximum yield of these compounds was observed at a particle size of 0.5 mm. Thus, based on the obtained extraction yields, plant material ground to 0.5 mm was selected for subsequent experiments, as it provided the highest recovery of lipids and lipophilic compounds under the tested conditions.

The efficiency of extracting biologically active compounds, including lipids and lipophilic substances, from plant material consisting of the flowers and seeds of *O. basilicum* and *O. tenuiflorum* depends on several factors, including extraction temperature.

With increasing temperature, molecular motion intensifies, the diffusion capacity of the solvent increases, and the transfer of biologically active compounds from plant tissues into the extracting solvent is facilitated. This leads to an increase in the yield of extractive substances, as well as lipids and lipophilic compounds. However, excessively high temperatures may cause the degradation of certain thermolabile compounds, which can negatively affect the overall yield of target components.

Therefore, the influence of temperature on the extraction of extractive substances, lipids, and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* was investigated (Table 3).

According to the data presented in Table 3, the yield of extractive substances, along with lipids and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material, directly depends on the extraction temperature. At 20 °C, the yield values were relatively low with the content of extractive substances being 16.1% for *O. basilicum* and 15.5% for *O. tenuiflorum*, while the yield of lipids and lipophilic compounds reached 9.1% and 8.9%, respectively.

An increase in all studied parameters was observed with an increase in temperature to 40 °C. At this stage, the lipids and lipophilic compound yields increased to 9.7% for *O. basilicum* and 9.5% for *O. tenuiflorum*. The highest values were obtained at 60 °C and 80 °C, although only minor differences were observed between these temperatures. Thus, at 60 °C, the yield of lipids and lipophilic compounds was 11.2% for *O. basilicum* and 11.4% for *O. tenuiflorum*, whereas at 80°C, these values increased only slightly to 11.5% and 11.6%, respectively.

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Table 4. Kinetics of the yield of lipids and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material in 96% ethanol at 60 °C.

Extraction time (h)	Yield of lipids and lipophilic compounds (%) of raw material mass					
	Ocimum basilicum			*Ocimum tenuiflorum*		
	1st extraction stage	2nd extraction stage	3rd extraction stage	1st extraction stage	2nd extraction stage	3rd extraction stage
1	1.6 ± 0.05	1.2 ± 0.04	0.4 ± 0.01	1.3 ± 0.04	1.0 ± 0.03	0.3 ± 0.01
2	3.2 ± 0.09	3.6 ± 0.10	0.7 ± 0.02	2.9 ± 0.08	3.4 ± 0.09	0.5 ± 0.02
3	7.9 ± 0.21	3.7 ± 0.11	0.7 ± 0.02	7.7 ± 0.22	3.5 ± 0.11	0.5 ± 0.02
4	8.0 ± 0.24	-	-	7.8 ± 0.24	-	-

Since increasing the temperature above 60 °C resulted in only a slight increase in the yield of the target compounds, 60 °C was selected for subsequent experiments, as it provided high extraction efficiency under the investigated conditions. At this temperature, a sufficiently high yield of lipids, lipophilic compounds, and extractive substances was achieved, along with stable process conditions.

Extraction of lipids and lipophilic compounds is primarily based on mass transfer phenomena. During extraction, compounds diffuse from the cellular structures of plant material into the solvent phase. The study of extraction kinetics makes it possible to identify conditions associated with higher recovery of biologically active compounds from *O. basilicum* and *O. tenuiflorum*. Extraction dynamics are characterized by changes in the concentration of extracted substances over time.

In this regard, the extraction kinetics of lipids and lipophilic compounds from the studied plant material were investigated using 96% ethanol at 60 °C as a function of extraction time (Table 4).

According to the data in Table 4, during the initial contact phase for both plant species, a rapid and pronounced increase in the yield of lipids and lipophilic compounds was observed. For example, in *O. basilicum*, the yield increased from 1.6% after 1 hour to 7.9% after 3 hours, while in *O. tenuiflorum*, it increased from 1.3% to 7.7% over the same period. By the 4th h, the process in the first phase had almost reached equilibrium: the yield amounted to 8.0% for *O. basilicum* and 7.8% for *O. tenuiflorum*. Based on the obtained extraction yields, a duration of approximately 3 h was selected for the first extraction stage.

During the second contact phase, the increase in yield proceeded at a slower rate. Within the 2–3 h interval, the values stabilized at 3.6–3.7% for *O. basilicum* and 3.4–3.5% for *O. tenuiflorum*. The transition of the process to a steady-state condition at this stage indicates a deceleration of diffusion processes and a reduced rate of transfer of compounds into the solvent. Accordingly, a duration of 2–3 h was selected for the second extraction stage.

As can be seen from the data in Table 4, during the third contact phase, the yield remains almost unchanged after 2–3 hours, reaching approximately 0.7% for *O. basilicum* and 0.5% for *O. tenuiflorum*. This indicates the practical completion of the extraction process at this stage, making further extension of extraction time unnecessary. Overall, only minor differences were observed between *O. basilicum* and *O. tenuiflorum* throughout the extraction process.

During the second contact phase, the increase in yield proceeded at a slower rate. Within the 2–3 h interval, the values stabilized at 3.6–3.7% for *O. basilicum* and 3.4–3.5% for *O. tenuiflorum*. The transition of the process to a steady-state condition at this stage indicates a deceleration of diffusion processes and a reduced rate of transfer of compounds into the solvent. Accordingly, a duration of 2–3 h was selected for the second extraction stage.

As can be seen from the data in Table 4, during the third contact phase, the yield remains almost unchanged after 2–3 hours, reaching approximately 0.7% for *O. basilicum* and 0.5% for *O. tenuiflorum*. This indicates the practical completion of the extraction process at this stage, making further extension of extraction time unnecessary. Overall, only minor differences were observed between *O. basilicum* and *O. tenuiflorum* throughout the extraction process.

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Thus, based on the obtained results, it was established that efficient extraction of lipids and lipophilic compounds from *O. basilicum* and *O. tenuiflorum* plant material can be achieved using two successive extraction stages with 96% ethanol at 60 °C. The duration of the first stage should be at least 3 h, while the second stage should be at least 2 h.

The obtained ethanolic extracts contained biologically active lipids and lipophilic substances such as unsaturated fatty acids, phytosterols, pigments, and components of the essential oil of basil cultivated in Uzbekistan [7].

4. Conclusions

The present study demonstrated that the yield of lipids and lipophilic compounds from the flowers and seeds of *O. basilicum* and *O. tenuiflorum* in a solid–liquid system is determined by a combination of technological factors, including ethanol concentration, particle size of the plant material, extraction temperature, and extraction time.

Under the investigated conditions, the highest numerical extraction yields were obtained using 90–96% ethanol, plant material ground to 0.5 mm, and extraction at 80 °C. However, because the increase in lipid and lipophilic compound yield at 80 °C compared to 60 °C was small, 60 °C was selected as the preferred extraction temperature. Based on the extraction kinetics, a two-stage extraction scheme consisting of 3 h for the first stage and 2 h for the second stage was selected for the subsequent experiments.

Overall, the extraction patterns for both studied *Ocimum* species (*O. basilicum* and *O. tenuiflorum*) exhibited similar behavior.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, A.D. and B.A.; Methodology, B.R., K.N., A.D. and B.A.; Investigation, B.R., K.N. and Z.Z.; Formal analysis, B.R. and K.N.; Resources, B.A. and R.K.; Writing – Original Draft, B.R. and R.K.; Writing – Review & Editing, A.D., B.A. and R.K.; Supervision, B.A. and R.K.; Project Administration, A.D.; Funding Acquisition, B.A. All authors read and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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