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EFFICIENCY OF OBTAINING DRY EXTRACTS FROM MEDICINAL PLANTS USING CELLULASE ENZYME

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Abstract
This article presents data on the efficiency of obtaining dry extracts from medicinal plants by subjecting the outer layers of various morphological plant organs (seeds, rhizomes, and flowers) to hydrolysis using cellulase enzyme. According to the obtained results, the application of cellulase enzyme increased the yield of extractive substances from plant raw materials by 26.8–35.8%. The results demonstrate that enzymatic treatment of different morphological organs of medicinal plants plays an important role in improving the efficiency of the extraction process.

Keywords: Antioxidant, preparations, cellulase, enzyme, rhizome, hydrolysis, biologically active substance, extraction, Silybum marianum L., Acorus calamus L., Calendula officinalis L., Tanacetum vulgare L., Matricaria chamomilla L.

INTRODUCTION

Medicinal plants have been widely used in traditional medicine for thousands of years, and their bioactive components continue to attract significant attention in modern pharmacological research due to their anti-inflammatory, antioxidant, and hepatoprotective properties [1]. Medicinal plants such as Silybum marianum L.

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contain a rich complex of flavonolignans, and their pharmacological effects—including antimicrobial, antidiabetic, antioxidant, cardioprotective, and hepatoprotective activities—have been confirmed by scientific studies [2]. Recent studies have shown that bioactive components of medicinal plants, such as flavonoids and triterpenoids, exhibit neuroprotective effects and other biological activities, further supporting their clinical therapeutic potential [3]. In addition, the immunomodulatory and anti-inflammatory effects of medicinal plants such as Calendula officinalis and Matricaria chamomilla have been confirmed by several clinical and experimental studies [4]. The seeds of Silybum marianum L. contain flavonolignans—collectively known as the silymarin complex—which have been shown to possess hepatoprotective and antioxidant properties [5]. Silymarin and its main components, silybin and silibinin, exhibit immunomodulatory, anti-inflammatory, and antioxidant effects, and have been found to help reduce symptoms of rheumatoid arthralgia and osteoarthritis [6]. Recent studies confirm that Silybum marianum exerts antitumor, antioxidant, and immunoregulatory effects through the flavonolignans present in silymarin, demonstrating its effectiveness in various diseases [7].

Figure 1. Silybum marianum L. (Milk thistle)

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The essential oil of Acorus calamus L. (sweet flag) rhizome contains phytochemical compounds such as β-asarone and α-asarone, which have demonstrated strong antibacterial activity against both Gram-positive and Gram-negative bacteria [8]. Recent scientific studies have systematically investigated the ethnopharmacological uses, phytochemistry, and pharmacological properties of Acorus calamus var. angustatus Besser, including its toxicology and pharmacokinetics [9]. Clinical studies have shown that preparations of Acorus calamus contribute to the alleviation of symptoms in home-based treatment and lead to improvements in daily quality of life [10].

Figure 2. Acorus calamus L. (Sweet flag)

Calendula officinalis L. (marigold) enhances its pharmaceutical potential due to its antioxidant, anti-inflammatory, and wound-healing properties; the abundance of bioactive compounds in this plant contributes to its wide therapeutic applicability [11]. The flowers of C. officinalis contain chlorogenic acid, rutin, and other components that protect dopaminergic neurons and play a significant role in alleviating symptoms of Parkinson's disease [12]. Extracts of Calendula arvensis have demonstrated antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory properties [13].

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Figure 3. Calendula officinalis L. (Marigold)

Tanacetum vulgare L. (tansy) is a perennial herbaceous plant belonging to the Asteraceae family. In Uzbekistan, two species are found: common tansy (T. vulgare) and mountain tansy (T. pseudoachillea S.). Common tansy is a highly branched plant reaching up to 150 cm in height. Its leaves are pinnate, either petiolate or sessile. The flowers are yellow and arranged in corymbose inflorescences. The fruit is an achene. The inflorescences contain essential oils, alkaloids, flavonoids, and other compounds. Infusions and powders prepared from the inflorescences are used as anthelmintic agents and for the treatment of hepatitis, angiocholitis, and intestinal diseases [14]. Due to the presence of flavonoids, tannins, polyphenols, and other biologically active compounds in its chemical composition, Tanacetum vulgare exhibits anti-inflammatory, antiseptic, and antihistamine properties. This medicinal plant is widely used in both traditional and modern medicine for the treatment of skin diseases, diathesis, dermatitis, allergic reactions, and mild inflammatory conditions. In pharmaceutical practice, it serves as an important raw material for the preparation of infusions, herbal teas, extracts, and various phytopreparations. The biological activity and therapeutic efficacy of this plant make it a promising natural agent for use in phytotherapy.

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Figure 4. Tanacetum vulgare L. (Tansy)

Matricaria chamomilla L. (chamomile) leaf and flower extracts have been shown in in vivo studies to reduce memory loss, enhance antioxidant activity, and exhibit neuroprotective effects [17]. Randomized clinical trials have demonstrated that chamomile possesses anti-inflammatory and antimicrobial properties [18]. The main phytochemical constituents of M. chamomilla L. include α-bisabolol, chamazulene, and flavonoids, whose diverse biochemical effects are associated with a wide range of biological activities [19].

Figure 5. Matricaria chamomilla L. Matricaria chamomilla L. (Chamomile)

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Extraction methods are currently used to efficiently isolate biologically active compounds belonging to different groups from medicinal plant raw materials [20]. Among them, we selected the method we previously applied in practice [21], namely enzymatic treatment of plant raw materials using enzymes. Enzymes are highly active protein compounds that function as specific reaction catalysts. They catalyze millions of chemical transformations in the cells of animals, plants, and microorganisms, and also act on appropriate substrates outside the cell. The concepts of enzymes and enzyme preparations are distinguished. Enzymes are present in almost all living organisms: plants, animals, and microorganisms. Enzyme preparations may consist of mixtures of enzymes or a single type of enzyme, with varying degrees of purification. They can be added to raw materials or products, or immobilized on a carrier (immobilized enzymes). The activity of enzymes can be regulated, as their reaction properties change depending on environmental conditions such as temperature, acidity, and others [22]. Cellulase is a widely used enzyme applied in various industrial sectors and technological processes, including alcohol and beer production, baking, detergent manufacturing, pulp and paper industry, textile industry, biogas production, and others. The acceleration of socio-economic development requires reconsideration of the structure and quality of human dietary intake and the inclusion of vitamins and other biologically active additives (BAAs) in food products [23–25].

Aim of the Study

To increase the yield of dry extracts from medicinal plant materials by softening the outer layers of different morphological organs (seeds, rhizomes, and flower parts) and enhancing the transfer of biologically active compounds into the dry extract.

Experimental Objects

1. Milk thistle seeds (Silybum marianum L.) – 100 g
2. Sweet flag rhizome (Acorus calamus L.) – 100 g
3. Marigold flowers (Calendula officinalis L.) – 100 g
4. Tansy inflorescences (Tanacetum vulgare L.) – 100 g

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5. Chamomile flowers (Matricaria chamomilla L.) – 100 g

MATERIALS AND METHODS (EXPERIMENTAL PROCEDURE)

A liquid cellulase enzyme (Russia), isolated from a selective strain of the fungus Trichoderma reesei, was used. The enzyme had a concentration activity of 4000 units/mL, an optimal pH of 3.5–4.5, and an optimal temperature of 50–65°C.

Preparation of Plant Samples

For the study, seeds, rhizomes, inflorescences, and flowers of Silybum marianum L., Acorus calamus L., Calendula officinalis L., Tanacetum vulgare L., and Matricaria chamomilla L. were selected. The quality and maturity of the samples were verified under laboratory conditions. The plant materials were then cleaned from dirt and dust to ensure uniformity.

Mechanical Treatment

The cleaned plant parts were cut into small pieces, mechanically ground, and passed through a 2 mm sieve. The purpose was to increase the surface area and prepare the material for enzymatic processing. The ground material was divided into two equal parts and placed into ten 2000 mL glass containers. In containers 1–5, distilled water was added to the substrates, while in containers 6–10, a 50% enzyme solution was added until a thick mixture was formed. The containers were placed in a water bath and hydrolyzed at 50°C for 45 minutes. The mixture was continuously stirred to maximize the release of bioactive compounds. After completion, the obtained mass was separated from the liquid by filtration. The enzymatic filtrate was inactivated using 10% ethanol solution to stop enzyme activity and ensure extract stability.

Reagents and Equipment

Enzyme solution (50%), distilled water, laboratory glassware (flasks, test tubes), porcelain dishes, analytical balance, and measuring cylinders.

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Experimental Procedure

Plant materials were placed into containers numbered 1–10:

1. Milk thistle seeds (Silybum marianum L.) – 100 g
2. Sweet flag rhizome (Acorus calamus L.) – 100 g
3. Marigold flowers (Calendula officinalis L.) – 100 g
4. Tansy inflorescences (Tanacetum vulgare L.) – 100 g
5. Chamomile flowers (Matricaria chamomilla L.) – 100 g

The following step involved soaking the samples (1–5) with distilled water:

- Sample 1 – 160 mL distilled water
- Sample 2 – 160 mL distilled water
- Sample 3 – 240 mL distilled water
- Sample 4 – 300 mL distilled water
- Sample 5 – 300 mL distilled water

Incubation with 50% Enzyme Solution

The ground plant materials were then assigned numbers 6–10:

6. Milk thistle seeds (Silybum marianum L.) – 100 g
7. Sweet flag rhizome (Acorus calamus L.) – 100 g
8. Marigold flowers (Calendula officinalis L.) – 100 g
9. Tansy inflorescences (Tanacetum vulgare L.) – 100 g
10. Chamomile flowers (Matricaria chamomilla L.) – 100 g

Enzyme solution was added as follows:

- Sample 6 – 160 mL enzyme solution
- Sample 7 – 160 mL enzyme solution
- Sample 8 – 240 mL enzyme solution
- Sample 9 – 300 mL enzyme solution
- Sample 10 – 300 mL enzyme solution

Each sample containing 50% enzyme solution was incubated in a 50°C water bath for 45 minutes. The enzymatic degradation process was evaluated based on visual and physicochemical parameters.

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RESULTS AND DISCUSSION

Samples 1–10 were kept in a 50°C water bath for 45 minutes. After incubation, samples 6–10 were treated with 10% ethanol for 30 minutes to inactivate enzyme activity. The inactivated substrates were washed with distilled water. After filtration, the obtained material was dried under low-temperature conditions to prevent degradation of biologically active compounds. The dried material was extracted using 40% ethanol by percolation method. The solvent was gradually passed through the plant mass to isolate bioactive compounds. The ethanol was then removed using a rotary evaporator, and the remaining mass was placed in porcelain dishes and evaporated in a water bath for 120 minutes. After completion, the thick mass was dried in a drying oven for 180 minutes. The obtained dry extracts were ground into powder form and weighed using an analytical balance. The extracts were stored under special conditions for further physicochemical and biological analysis. The obtained extracts serve as a reliable source for evaluating the efficiency of medicinal plants. The results showed a significant increase in dry extract yield, especially in Silybum marianum and Matricaria chamomilla. This confirms that enzymatic treatment is an effective technological approach for improving the extraction of biologically active compounds from medicinal plants.

Table 1. Results of dry extract (g) obtained from seeds, rhizomes, inflorescences, and flowers of medicinal plants with and without enzymatic hydrolysis

№	**1**	**2**	**3**	**4**	**5**
Plants	**Silybum marianum L.**	**Acorus calamus L.**	**Calendula officinalis L.**	**Tanacetum vulgare L.**	**Matricaria chamomilla L.**
Control: Dry extract yield of samples without enzymatic hydrolysis	9,46	10,52	8,9	9,66	10,26
Enzymatically hydrolyzed samples: dry extract yield results	12,85	12,64	12,16	12,82	13,87
Difference in obtained dry extract yield	3,39	2,12	3,26	3,16	3,61

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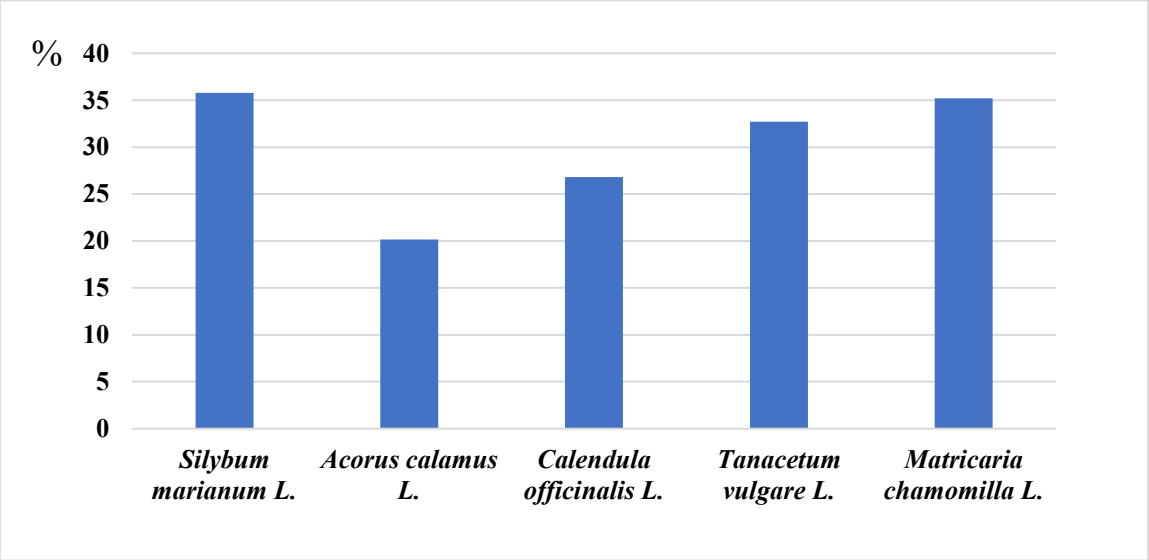
Samples treated with cellulase enzyme hydrolysis showed a 26.8–35.8% higher yield of dry extract compared to samples treated with water. The results confirm that cellulase-assisted enzymatic hydrolysis is an effective method for extracting biologically active compounds from medicinal plant raw materials.



Dry extract yield (%) obtained from enzymatically hydrolyzed seeds, rhizomes, inflorescences, and flowers of medicinal plants

RESULTS AND DISCUSSION

Improving the efficiency of extracting biologically active compounds from medicinal plants is one of the important directions in modern biotechnology and pharmaceutical science. In this study, the effect of enzymatic treatment on the yield of dry extract from different morphological organs of Silybum marianum L., Acorus calamus L., Calendula officinalis L., Tanacetum vulgare L., and Matricaria chamomilla L. was investigated.The dry extract mass obtained from each plant was determined, and the quantitative results were compared. The obtained results showed that enzymatically treated samples had a higher dry extract yield compared to the control variants. In particular, a significant increase in extract yield was observed in Silybum marianum L., reaching up to 35.8%. This was considerably

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higher compared to the non-enzymatically treated samples. Similarly, in Matricaria chamomilla L., the dry extract yield increased by 35.19%. Such results can be explained by the effect of enzymes on plant cell walls. It is known that plant cell walls have a complex polysaccharide structure, mainly consisting of cellulose, hemicellulose, and pectin. During enzymatic hydrolysis, these compounds are partially degraded, resulting in loosening of the cell wall structure and softening of plant tissues. This facilitates the transfer of intracellular biologically active compounds—flavonoids, phenolic compounds, essential oils, and other metabolites—into the solvent medium.

CONCLUSION

The results of the experiments demonstrated that enzymatic treatment of different morphological organs of medicinal plants plays an important role in improving the efficiency of the extraction process. Seeds, rhizomes, and flowers of Silybum marianum L., Acorus calamus L., Calendula officinalis L., Tanacetum vulgare L., and Matricaria chamomilla L. were subjected to enzymatic hydrolysis, and its effect on dry extract yield was studied. The obtained results showed that the amount of dry extract in enzymatically treated samples significantly increased compared to the control samples without enzymatic treatment. This phenomenon can be explained by the ability of enzymes to degrade polysaccharides forming plant cell walls, including cellulose, hemicellulose, and pectin. As a result, the structure of the cell wall becomes loosened, facilitating the release of biologically active compounds into the solvent medium. During enzymatic hydrolysis, partial disruption of plant tissue structure was observed, which increased the transfer of bioactive components—flavonoids, phenolic compounds, essential oils, and other biologically active substances—into the extract. This enhances both the biological value and pharmacological significance of the obtained extracts. At the same time, enzymatic treatment improves the technological efficiency of the extraction process by softening plant raw material structure and ensuring maximum release of biologically active compounds. This increases the efficiency of raw material utilization in extract production. In general, enzymatic treatment is considered one

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of the promising and effective methods for improving technologies of biologically active compound extraction from medicinal plants. The use of this method has significant scientific and practical importance in obtaining high-quality extracts in the pharmaceutical, biotechnology, and food industries.

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