

**ФУНДАМЕНТАЛ ВА
КЛИНИК ТИББИЁТ
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**BULLETIN OF FUNDAMENTAL
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**ВЕСТНИК ФУНДАМЕНТАЛЬНОЙ И
КЛИНИЧЕСКОЙ МЕДИЦИНЫ**

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ISOLATION OF ALKALOIDS AND VITAMINS FROM DESERT PLANTS AND INVESTIGATION OF BIOETHANOL PRODUCTION POTENTIAL FROM THE REMAINING BIOMASS**Muminbayev D.J.**

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Resume. This article investigates the technology for isolating alkaloids and vitamins from the biomass of *Peganum harmala* L. (*Harmala*) and *Climacoptera lanata* (*Climacoptera*), as well as the effect of separation parameters (extractant concentration, time, and temperature) on the yield of biologically active substances (BAS). During the study, the relative amounts of alkaloids and vitamins extracted from the vegetative and generative parts of the biomass were compared. As a result of the analysis, it was determined that extraction using 70% ethanol solution at 78.3 °C for 180 minutes provided the highest yield: the alkaloid content reached up to 6.12% in the generative parts of *Peganum harmala*, and the vitamin content reached up to 9.32% in the vegetative parts of *Climacoptera lanata*. The number of alkaloids and vitamins isolated was determined using the thin-layer chromatography (TLC) method, and their dependence on plant parts was confirmed. The residual cellulosic biomass after separation was processed via two-stage acid hydrolysis, fermented using microorganisms, and successfully directed toward bioethanol production. It was shown that these plants are important not only as sources of biologically active substances but also as renewable energy resources.

Keywords: *Peganum harmala*, *Climacoptera lanata*, alkaloid isolation, vitamin analysis, thin-layer chromatography (TLC), bioactive compound extraction, sulfuric acid hydrolysis, fermentation with *Saccharomyces cerevisiae*, lignocellulosic biomass, bioethanol production.

ЧЎЛ ЎСИМЛИКЛАРИДАН АЛКАЛОИДЛАР ВА ВИТАМИНЛАРНИ АЖРАТИБ ОЛИШ ВА БИОМАССАДАН БИОЭТАНОЛ ИШЛАБ ЧИҚАРИШ ИМКОНИЯТЛАРИНИ ЎРГАНИШ**Мўминбаев Д.Ж.**

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Резюме. Ушбу мақолада *Peganum harmala* L. (*гармала*) ва *Climacoptera lanata* (*климакоптера*) биомассасидан алкалоидлар ва витаминларни ажратиб олиш технологияси ҳамда ажратилиш шaroитлари (экстрагент концентрацияси, вақт ва ҳарорат) биологик фаол моддалар (БФМ) чиқишига қандай таъсир кўрсатиши ўрганилган. Тадқиқот давомида биомассанинг вегетатив ва генератив қисмларидан ажратилган алкалоидлар ва витаминлар нисбати таққосланган. Таҳлил натижаларига кўра, 70% этанол эритмаси билан 78,3 °C ҳароратда 180 дақиқа давом этган экстракция энг юқори самарани бергани аниқланди: *Peganum harmala* нинг генератив қисмларида алкалоидлар 6,12% гача, *Climacoptera lanata* нинг вегетатив қисмларида эса витаминлар 9,32% гача бўлган. Ажратилган алкалоидлар ва витаминлар миқдори юққа қатламли хроматография (ЮҚХ) усули орқали аниқланиб, уларнинг ўсимлик морфологик қисмларига боғлиқлиги тасдиқланди. Экстракцидан қолган целлюлозали биомасса икки босқичли кислотали гидролизга учратилиб, микроорганизмлар ёрдамида ферментациядан ўтказилди ва натижада биоэтанол олишга эришилди. Ушбу ўсимликлар нафақат биологик фаол моддаларнинг манбаи, балки қайта тикланувчи энергия ресурси сифатида ҳам катта аҳамиятга эга экани кўрсатилди.

Калим сўзлар: *Peganum harmala*, *Climacoptera lanata*, алкалоид ажратилиш, витамин таҳлили, юққа қатламли хроматография (ЮҚХ), БФМ ажратилиш, сульфат кислота гидролизи, *Saccharomyces cerevisiae* билан ферментация, лигноцеллюлозали биомасса, биоэтанол ишлаб чиқариш.

ВЫДЕЛЕНИЕ АЛКАЛОИДОВ И ВИТАМИНОВ ИЗ ПУСТЫННЫХ РАСТЕНИЙ И ИССЛЕДОВАНИЕ ПОТЕНЦИАЛА ПРОИЗВОДСТВА БИОЭТАНОЛА ИЗ БИОМАССЫ**Муминбаев Д.Ж.**

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Резюме. В статье рассматривается технология выделения алкалоидов и витаминов из биомассы *Peganum harmala* L. (*гармала*) и *Climacoptera lanata* (*климакоптера*), а также влияние параметров экстракции (концентрация экстрагента, время и температура) на выход биологически активных веществ (БАВ). В ходе исследования проведено сравнение относительного содержания алкалоидов и витаминов, извлечённых из вегетативных и генеративных частей растений.

Установлено, что наивысший выход достигается при экстракции 70% раствором этанола при температуре 78,3 °C в течение 180 минут: содержание алкалоидов в генеративных частях *Peganum harmala* достигло 6,12%, а содержание витаминов в вегетативных частях *Climacoptera lanata* — 9,32%. Количество выделенных веществ определяли методом тонкослойной хроматографии (ТСХ), при этом была подтверждена зависимость состава от морфологических частей растений. Оставшаяся после экстракции целлюлозная биомасса подвергалась двухступенчатому кислотному гидролизу, последующей ферментации с применением микроорганизмов и была эффективно использована для производства биоэтанола. Доказано, что данные растения представляют интерес как источники биологически активных веществ и как возобновляемые источники энергии.

Ключевые слова: *Peganum harmala*, *Climacoptera lanata*, выделение алкалоидов, анализ витаминов, тонкослойная хроматография (ТСХ), экстракция БАВ, сернокислотный гидролиз, ферментация *Saccharomyces cerevisiae*, лигноцеллюлозная биомасса, производство биоэтанола.

Introduction. In recent years, the production of biofuels has become one of the key areas in the development of sustainable energy systems, allowing for the reduction of dependence on conventional petroleum products, the mitigation of global warming, and the alleviation of environmental problems. In particular, the prospect of producing bioethanol from non-food, secondary raw materials—specifically the biomass of desert plants—has become a globally relevant issue. Although *Peganum harmala* L. (Harmala) and *Climacoptera lanata* (Climacoptera), which are widespread in the arid zones of Uzbekistan, currently have little economic significance, they possess high potential in terms of the biologically active compounds they contain (alkaloids and vitamins). Moreover, their residues may serve as an efficient raw material for biofuel production. While previous scientific studies have confirmed the presence of individual compounds in these plants, the modern approaches to their extraction, evaluation, and the technological basis for producing bioethanol from the remaining biomass have not been thoroughly explored. In particular, the effects of extraction parameters (temperature, time, and solvent concentration) on the isolation process, as well as the fermentability of the biomass, have not been sufficiently addressed at the international level. In this study, the isolation of alkaloids and vitamins from *Peganum harmala* and *Climacoptera lanata* was carried out, their quantitative analysis was performed using thin-layer chromatography (TLC), and the biotechnological basis of bioethanol production from the residual biomass was investigated. The novelty of this research lies in the fact that, for the first time, an integrated biotechnological approach was applied to the processing of *Peganum harmala* and *Climacoptera lanata*, assessing not only the extraction of biologically active substances, but also the potential for energy generation from their residues. The scientific and practical significance of this study lies in revealing the potential to produce environmentally friendly and economically viable products by comprehensively utilizing wild plants that grow in ecologically unfavorable regions of Uzbekistan.

Materials and Methods. The objects of the study were the desert plants *Peganum harmala* and *Climacoptera lanata*. The generative and vegetative parts of their biomass were collected separately and processed as fractions rich in biologically active compounds. For the isolation of vitamins and alkaloids, 0.1 g of crystalline substances was placed into a 100 ml volumetric flask, followed by the addition of 10 ml of 95% ethanol. The solution was heated in a boiling water bath for 60 minutes. After cooling to room temperature, the volume was adjusted to 100 ml. From the resulting extract, 0.03 ml was applied in drop form onto chromatographic plates with silica gel layers of 5×12 cm and 9×12 cm using a micropipette. The prepared plates were air-dried for 5 minutes, then placed into a pre-saturated chromatographic chamber for 1 hour. The following solvent systems were used: hexane–ethanol (8:2; 6:4), chloroform–ethanol (8:2), ethyl acetate–ethanol–water (5:1:4), and ethyl acetate–acetonitrile–water (5:1:4). Once the solvent front reached 12 cm, the plates were removed and air-dried for 10 minutes. The analysis was carried out under UV light (365 nm) and after treatment with 5% AlCl_3 solution. For alkaloid detection, Dragendorff's reagent was used: 1.7 g of $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ was dissolved in 100 ml of 20% acetic acid and mixed with a solution of 40 g of KI in 100 ml of water. The number and color of the separated spots were used to qualitatively and quantitatively assess the presence of alkaloids and vitamins. To obtain bioethanol from the biomass residues, a two-stage hydrolysis method was employed. Initially, the raw material was hydrolyzed using a diluted sulfuric acid solution (0.5–3%). Then, deep hydrolysis was carried out using concentrated H_2SO_4 . After saccharification, fermentation was conducted under anaerobic conditions at 27°C and pH 4.5 for 72 hours using industrial strains of *Saccharomyces cerevisiae*. Based on the obtained data, the effects of extraction parameters, types and numbers of chromatographic spots, and bioethanol yield were studied. The residual sugar content and ethanol output after fermentation were determined under laboratory conditions, and the results were presented in the form of graphs and tables.

Results and Discussion.

Isolation of Alkaloids and Vitamins

The content of alkaloids and vitamins extracted from the vegetative and generative parts of *Peganum harmala* and *Climacoptera lanata* was analyzed using the Thin-Layer Chromatography (TLC) method.

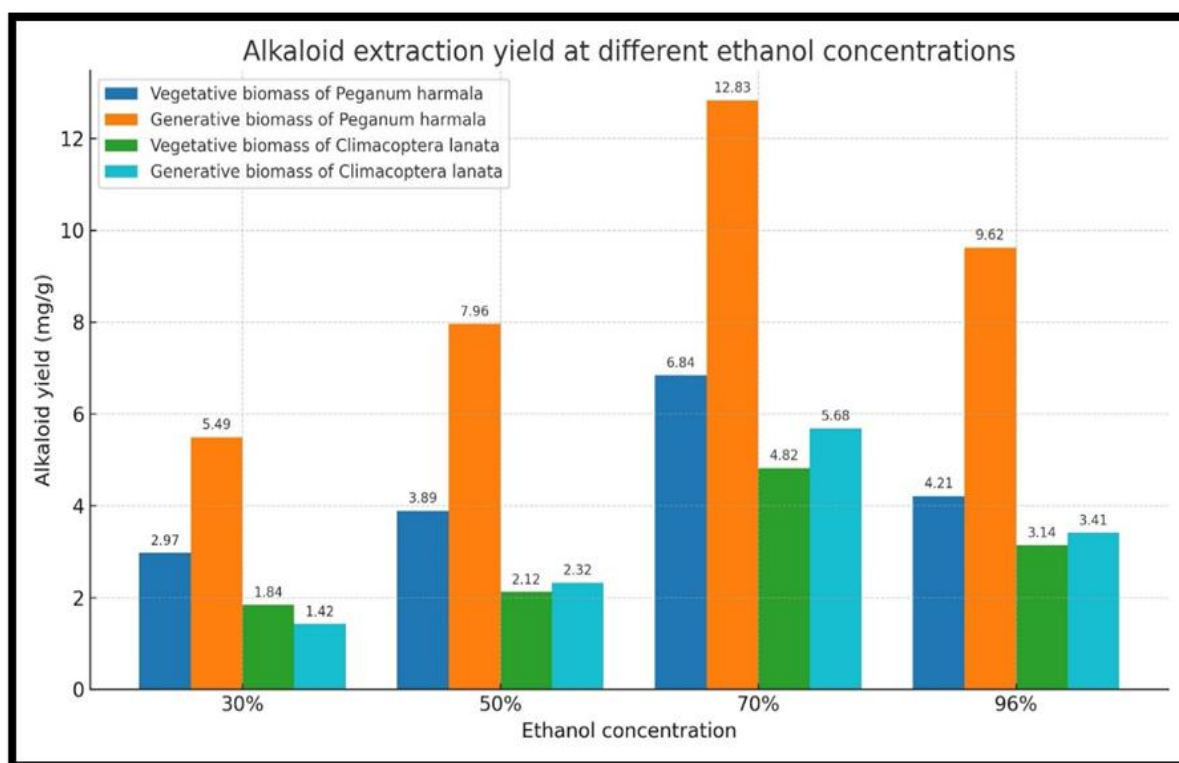
In the generative parts of *Peganum harmala*, 9–10 alkaloid spots were identified, while 6–7 spots were observed in the vegetative parts. The spots were visualized using Dragendorff's reagent, appearing as dark orange, confirming the presence of alkaloids. These findings demonstrate the high alkaloid potential of *Peganum harmala*.

In *Climacoptera lanata*, 3–4 alkaloid spots were detected in the generative parts and 2–3 in the vegetative parts, indicating that this species contains significantly fewer alkaloids compared to *Peganum harmala*.

As for vitamins, *Peganum harmala* showed 2–3 spots in the generative parts and 4–5 in the vegetative parts. These spots appeared greenish-yellow under visible light and blue-green under UV-365 nm, turning blue-brown fluorescent after treatment with 5% ethanolic AlCl_3 , a typical behavior for flavonoid-like vitamins. *Climacoptera lanata* demonstrated 4–5 vitamin spots in its generative parts and 6–7 in the vegetative parts, suggesting a richer vitamin profile compared to *Peganum harmala*.

Quantitative Analysis of Extracts

Experimental findings indicated that the optimal extraction condition was 70% ethanol at 180 minutes. Under these conditions, the vitamin yield from the vegetative parts of *Peganum harmala* was 9.32%, while from the generative parts it was 6.98%. Identical yields were obtained from *Climacoptera lanata* under the same extraction conditions.



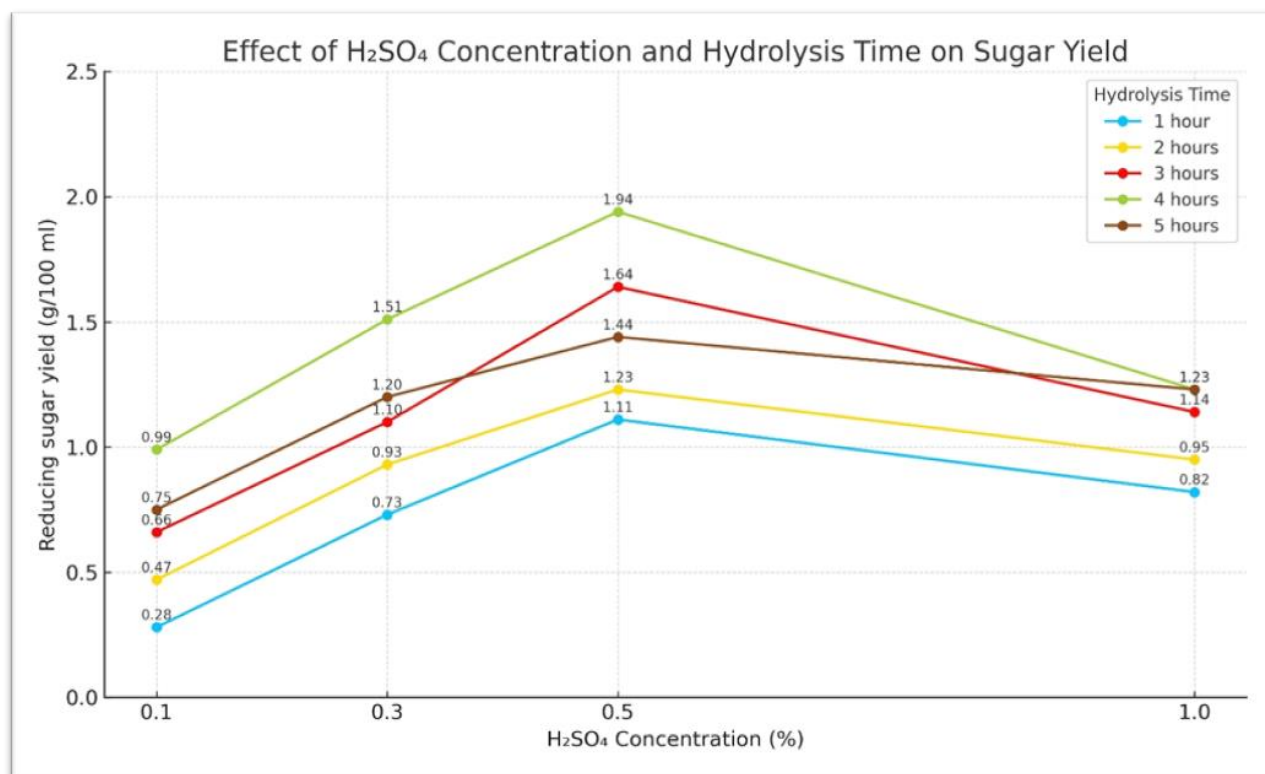
In contrast, the lowest vitamin yields were observed when 30% ethanol was used for 60 minutes — 0.32% for *Peganum harmala* and 3.04% for *Climacoptera lanata*, particularly in the generative biomass.

Hydrolysis and Bioethanol Production Potential

The residual biomass remaining after the extraction of alkaloids and vitamins was subjected to acid hydrolysis. The yield of reducing substances (RS) from *Peganum harmala* biomass was assessed as a function of sulfuric acid concentration and hydrolysis duration. Maximum RS yield was observed after 3 hours using 2% H_2SO_4 . A similar trend was recorded for *Climacoptera lanata*.

In all cases, RS yield increased with acid concentration and duration up to 3 hours, after which it plateaued. These findings suggest that hydrolyzing the biomass for 3 hours using 2–4% H_2SO_4 is optimal for maximizing RS production.

Concentration of H ₂ SO ₄ , %	Duration of time (hours)				
	1	2	3	4	5
0,1	0,29	0,45	0,68	0,21	0,09
0,3	0,41	0,49	0,77	0,93	0,65
0,5	0,45	0,96	1,31	1,75	1,23
1	0,34	0,57	1,03	1,24	0,97



Fermentation Process

After the extraction of alkaloids and vitamins, the remaining biomass of *Peganum harmala* and *Climacoptera lanata* was hydrolyzed and subsequently fermented using industrial strains of *Saccharomyces cerevisiae*. The yeast cultures were cultivated on wort-agar nutrient media for 48 hours at 28 °C.

Following the assessment of yeast viability, 5 g of yeast suspension was added to each type of plant hydrolysate. Fermentation was conducted under anaerobic conditions at 27 °C, pH 4.5, for 72 hours.

According to the experimental results, active sugar consumption by the yeast occurred primarily during the first 48 hours of fermentation. After this period, the residual sugar content in the fermented broth of *Peganum harmala* decreased to 1.87%, and in that of *Climacoptera lanata* to 1.49%, as presented in Table 3.2.1 and Figure 3.2.3.

These findings confirm that the hydrolysates from both desert plants provide a suitable medium for yeast metabolism. The observed decrease in sugar levels demonstrates the effective utilization of fermentable sugars and the potential of these biomasses for bioethanol production.

Plant name	Fermentation duration (hours)				
	0	12	24	36	48
Peganum harmala	7,52±0,19	5,32±0,15	4,28±0,18	3,15±0,17	1,87±0,32
Climacoptera lanata	8,36±0,28	4,83±0,17	4,54±0,17	3,28±0,25	1,49±0,29

Conclusion. The conducted research demonstrated that *Peganum harmala* and *Climacoptera lanata* contain significant amounts of biologically active compounds—alkaloids and vitamins. Optimization of extraction conditions (70% ethanol concentration, temperature of 78.3°C, and 180 minutes of extraction) resulted in the maximum yield of these compounds. Using thin-layer chromatography (TLC), the quantity and variety of alkaloids and vitamins were identified in both the vegetative and generative parts of the plants. The remaining biomass was subjected to two-stage acid hydrolysis, yielding hydrolysates suitable for fermentation. During the fermentation process with *Saccharomyces cerevisiae*, a notable reduction in sugar content and the formation of bioethanol were observed. These findings indicate that *Peganum harmala* and *Climacoptera lanata* are promising not only as sources of biologically active compounds, but also for the production of biofuels from their secondary biomass.

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