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| Research Article

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Obtaining a Nano-Silver Drug with Peganum Harmala Extract

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Annotation

Preliminary phytochemical analyses of the composition of the Peganum harmala plant were carried out. The sum of the extracts was obtained from different parts of the plant. Colloidal solutions containing nanosilver were synthesized in the presence of glucose and sodium citrate reducers, using PVA as a stabilizer. Based on the tobacco extract and colloidal solutions containing nanosilver, a composition with bactericidal and fungicidal properties was obtained.

Keywords: Peganum harmala, alkaloid, flavonoid, tannin, anthraquinone, saponin, carbohydrate, bactericide, fungicide, nanosilver, plant.

Introduction. P. harmala L. - belongs to the Zygophyllaceae (in some sources Nitrariaceae) family. A plant called "isiriq" by our people and used for various purposes since ancient times. P. harmala is mainly found in the Middle East, North Africa and Central Asia. The plant is usually not grazed by animals due to its bitter taste. P. harmala is a perennial herbaceous plant with a strong characteristic odor, flowering in May-July, growing in the steppes, 40-70 cm high. The root is multi-headed, woody, thick (diameter 3-5 cm. The stem is branched, curved, bare, densely leafy. The leaves are pubescent, alternate, 4-5 cm long, 5-6 cm wide, three-edged at the base, and the lobes are also divided into linear segments. The flowers are white, elliptical, usually 10-13 mm long. The fruit is a three-celled capsule with a diameter of 0.6-1 cm. The fruits ripen from late June to August. The seeds are dark black, 1-2 mm long (Figure 1).


a)


b)


c)

d)

Figure 1. Peganum harmala L.: a) leaf, b) flower, c) fruit, d) seed.

In folk medicine, Peganum harmala L. is used as a central nervous system sedative, analgesic, anti-inflammatory, anthelmintic, antiseptic and diuretic in the form of vapors and decoctions. Alkaloids contained in Peganum harmala, as well as extracts of all parts, have shown antimicrobial activity [1-3].

The fungicidal effect of P. harmala has been demonstrated on various pathogenic fungi. 16 fungi were tested on Sabouraud dextrose agar (Oxoid) plates with harmine, harmaline and their derivatives at concentrations of 50-500 μg/ml and the inhibition zones were recorded [4-9].

The inhibitory effect of P. harmala on various animal viruses and plant viruses has been reported. The anti-TMV activity of β-carboline, dihydro-β-carboline and tetrahydro-β-carboline alkaloids and their derivatives was tested using the Ishida method. All alkaloids and some of their derivatives were found to exhibit higher anti-TMV activity in vitro and in vivo than ribavirin. In particular, harmaline (60.3%) and tetrahydroharmanine (59.5%) were shown to have significantly higher in vitro and in vivo activity than ribavirin (38.5%) at 500 μg/ml [10, 11].

To date, more than 390 secondary metabolites have been identified from various parts of P. harmala, including alkaloids, flavonoids, triterpenoids, phenolic acids, anthraquinones, fatty acids, carbohydrates, amino acids, hydrocarbons, and essential oils. The plant and its important biologically active compounds, mainly β-carboline alkaloids, have numerous pharmacological effects, including antimicrobial (bacteria, parasites, fungi, and viruses), anticancer, antiatherogenic, antidiabetic, anti-inflammatory, antioxidant, neuropsychological, analgesic, gastroprotective, diuretic, and hypothermic effects. P. harmala exerts antimicrobial effects by interfering with the uptake of microorganisms and host cells, and regulates genetic transcription. The anticancer effects of P. harmala and its β-carboline alkaloids are associated with a complex network of signaling pathways involved in cell cycle arrest, autophagy, and apoptotic death. exhibit diverse pharmacological activities. However, excessive consumption of high doses of P. harmala extract can lead to serious hepatic, nephritic, and neuropathic toxicities, and therefore, additional caution is required in its use [12-20].

Literature review. Based on the above, we aimed to isolate the biologically active substances contained in P. harmala by extraction and prepare a drug with insecticidal-bactericidal properties containing nanosilver.

General separation methods are based on the following principle: according to the difference in solubility of the substance to be separated (at different temperatures for recrystallization or in different solvents for sequential precipitation); or according to the difference in solubility of the substance in two-phase solvents for separation (extraction, liquid-liquid countercurrent distribution, adsorption of the substance with activated carbon or other solid phase for separation of alkaloids) [21].

The following methods are available for the isolation of alkaloids [22]:

1) Acid extraction. The method involves the isolation of alkaloids by immersing the plant material in prepared acid solutions. Typically, 0.1 to 1% concentrated solutions of sulfuric, hydrochloric, acetic, oxalic or tartaric acids are used as solvents.

2) Alcohol extraction. Methanol, ethanol, isopropanol or other alcohols are used as extractants.

3) Extraction with a lipophilic organic solvent. Due to the lipophilicity of most free alkaloids, chloroform, benzene, ethoxyethyl, ethyl acetate or dichloromethane are used to isolate alkaloids. Typically, before extraction, the plant material is moistened with a small amount of sodium, potassium or ammonium hydroxide solution in order to completely extract the bound alkaloid by converting it into the main alkaloid.

4) Adsorption column chromatography. Al2O3 or silica gel (SiO2) is commonly used as an adsorbent. Sometimes the main adsorbent is additionally mixed with cellulose, polyamide, etc. Benzene, chloroform, ethers or other lipophilic organic solvents, as well as their mixtures, are used as eluents.

5) Column separation chromatography. Silica gel is used as the stationary phase, and a chloroform solution saturated with a pH=5.0 buffer is used as an auxiliary medium.

6) High-performance liquid chromatography (HPLC). To isolate alkaloids, plant raw materials are treated with alkaline solutions to convert the salt form of the alkaloids into the basic form. After that, the alkaloid bases are separated with non-polar or weakly polar organic solvents, for example, 1,2-dichloroethane, chloroform, diethyl ether, benzene, etc. To remove impurities, the resulting extract is treated with a weak acid solution.

The choice of a method for isolating physiologically active compounds from plant raw materials is based on the tasks set for the researcher. It is known that there are many methods for isolating physiologically active compounds from plants - extraction, pressing, chromatography, distillation, etc. Among these methods, extraction is widespread and of great importance in industry.

Extraction is the process of separating the compound using an extractant, in which the extracted substance is better soluble in one of two practically immiscible solvents. Extraction is widely used in the extraction of complex compounds from natural and technical solutions, in the separation and concentration of substances in analytical chemistry, etc. The dissolved substance can be extracted several times with small portions of the extract or once with the same total amount of extractant. A number of requirements are imposed on the organic solvents used for extraction [23, 24].

1. The organic solvent should separate the studied substance well from the aqueous phase.

2. It is desirable that the solvent used be selective. It should separate only one substance or a group of related compounds from the solutions.

3. The solvent should have a very low solubility in water and water should not be significantly soluble in this solvent. For extraction, the organic solvent should be saturated with water and the water with the organic solvent.

4. The organic solvent should not boil at a low temperature, if possible, because the loss of solvents increases due to rapid evaporation. At the same time, the low boiling point of organic solvents is desirable from the point of view of their recovery after extraction. Too high a boiling point of the solvent leads to increased energy losses during recovery of the solvent and an increase in the duration of the process.

5. The density of organic solvents should, if possible, differ from the density of water and aqueous solutions. The process is facilitated by the fact that a large difference in the density of the liquids accelerates the separation of phases.

6. Solvents should be as non-flammable or non-toxic as possible.

In addition, there are other requirements for solvents. The specificity of the extraction of natural raw materials is the complexity of their composition and the thermolability of some substances.

Research methodology. Preparation of raw materials. Peganum harmala was collected from the Namangan region in June-July 2024. The plant was dried in the shade, separated into components - florets, body parts and fruits. The necessary part for extraction was crushed to a size of 1-10 mm.

Obtaining Peganum harmala extract. Peganum harmala extraction was carried out in an acidic medium with the participation of water. The mass, kept at a certain time and temperature, was filtered through a “blue ribbon” filter and separated into extract and residue. A new portion of extractant was added to the residue and the process was repeated 2 more times. All the obtained extracts were summarized and sent for further processing.

Conducting phytochemical analyses. Alkaloids 5 mL of P. harmala plant extract was heated in the presence of 2% H_2SO_4, then mixed with Dragendorff's reagent. The appearance of an orange-red precipitate indicated the presence of alkaloids.

Qualitative reaction for flavonoids. 5 mL of P. harmala extract was added dropwise with a little Mg powder and 30% HCl solution. The appearance of a pink-red color indicated the presence of flavonoids.

Qualitative reaction for tannins. 2 mL of P. harmala plant extract was added dropwise with 5% iron(III) chloride solution. The appearance of a green-blue color indicated the presence of tannins.

Qualitative reaction for anthraquinones. 10 mL of P. harmala extract was heated briefly in 10% HCl. After cooling, the solution was filtered and chloroform was added. 10% NH3 was added to the mixture and heated. The appearance of a pink color indicated the presence of anthraquinones.

Qualitative reaction for saponins. 20 mL of P. harmala extract was mixed with distilled water, then boiled and shaken vigorously. The formation of foam indicated the presence of saponins.

Qualitative reaction for carbohydrates. 5 mL of P. harmala extract was added to α-naphthol and 2 mL of concentrated sulfuric acid was added. The formation of a reddish-purple color indicated the presence of carbohydrates.

Qualitative reaction for coumarin. 2 mL of plant extract was added to 3 mL of 10% NaOH solution. The appearance of a yellow color indicated the presence of coumarin.

Synthesis of nanosilver (AgNP). Polyvinyl alcohol as a stabilizer was dissolved in the $AgNO_3$ solution in the required stoichiometric ratios, and the reducing agents (sodium citrate or glucose) were slowly added dropwise with constant stirring.

Preparation of a bactericidal-fungicidal drug. The resulting Peganum harmala extract and nanosilver colloidal solution were mixed in the required ratios and directed to check the stability of the preparation.

Results and discussions. The extraction process was carried out as follows. For extraction, 10 grams of samples were taken from each component and placed in 4 numbered beakers. 50 ml of 1% acetic acid solution was poured onto each sample and the extraction process was carried out at 60°C for 30 minutes with constant stirring using a magnetic stirrer. After the specified time, the mixtures were filtered and separated into filtrate and residue. The above process was repeated twice with the residue. All the filtrates obtained were combined and filtered, and the extracts were finally filtered through blue ribbon filter paper.

Table 1. Phytochemical analysis of Pegnum harmala extracts

№	Identified compound class	Qualitative reaction	Result	Change
1	Alkaloids	Dragendorf reagent Wagner reagent	+ +	Orange precipitate formed Brown precipitate formed
2	Flavonoids	Synod reaction	+	Pinkish purple color formed
3	Tannins	5% $FeCl_3$ solution	+	Blue-black color formed
4	Anthraquinones	Reaction of HCl and NH_3	+	Red color formed
5	Saponins	Extract shaking	+	Foam formed
6	Carbohydrates	Mulish reaction	+	Violet color
7	Coumarins	Filter paper moistened with diluted NaOH	+	Purple color formed on filter paper

The color of the extracts obtained was different. The extract from the leaves of Peganum harmala was dark brown, the extract from the seeds was brown, the extract from the stem was light yellow, and the extract from the seed coat was cloudy yellow.

Table 2. Efficiency of extracting extracts from parts of the Peganum harmala plant

№	Sample	Extract volume, ml	Dry residue, g	Yield, %
1	Leaf	75	0.65	6.5
2	Branch	90	1.60	16
3	Seed	98	1.10	11
4	Seed coat	90	0,32	3,2

Determination of the extraction yield. In order to determine the extraction yield, 10 ml of each extract was taken and its mass was measured on an analytical balance with an accuracy of 4 digits after the comma. Then it was dried in a drying cabinet at 100°C until constant mass was reached, and the dry residue was re-measured on an analytical balance under the above conditions. Dry mass and yield were calculated as a percentage.

The total volume of the extracts and the yield in terms of dry matter were calculated using the following formula:

$$C_{\%}=\frac{m_1}{m_2} * 100\%$$

where: m1 is the mass of the dry residue, m2 is the mass of the extract.

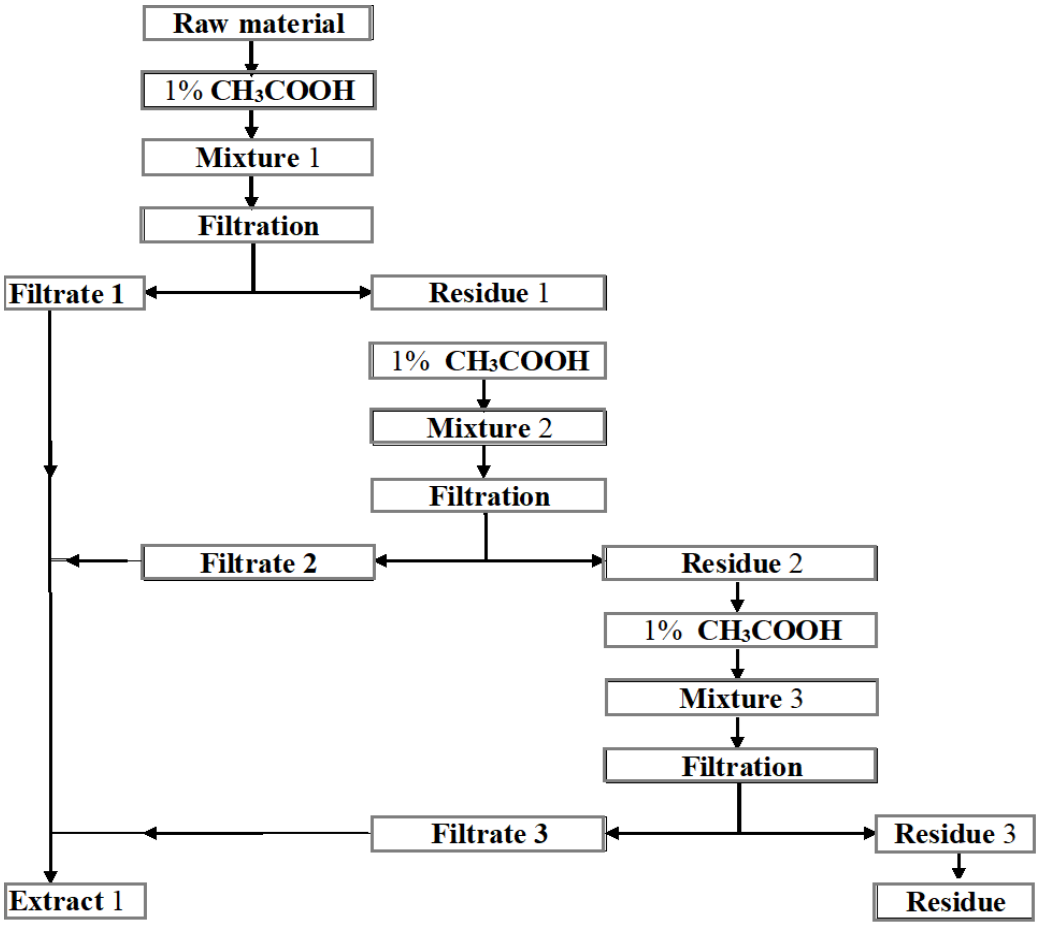

Figure 2. Scheme of extracting Peganum harmala at room temperature (3 times, 30 minutes each, raw material:extractant=1:5 mass ratio).

These are the sum of extractive compounds. It is known from the literature that the Peganum harmala plant contains extractive groups of natural compounds - salts of inorganic and organic substances, alkaloids, proteins, carbohydrates, amino acids, water-soluble vitamins, hydrolyzable tannins, glycosidic forms of flavonoids, and coumarins.

In the second stage of experiments, experiments were carried out to obtain a colloidal solution containing nanosilver based on the sum of Peganum harmala extracts. $AgNO_3$ was used as a source of silver nanoparticles (AgNP), citric acid (CA) as a reducing agent, polyvinyl alcohol (PVA) and carboxylmethylcellulose sodium salt (KMS-Na) as stabilizers.

Biostimulant nanosilver is widely used in agriculture to increase crop yield and protect plants. It can be used for pre-sowing seed treatment, soil cultivation, foliar spraying and plant nutrition. Biostimulants containing nanosilver promote the absorption of nutrients, activate physiological processes in plants, and increase resistance to stress and diseases. Studies conducted by scientists show the effectiveness of using nanosilver biostimulants in various crops and their effect on yield. Nanosilver-containing biostimulants are a promising method in agriculture that helps to increase crop yield and protect plants.

Methods for obtaining, properties and application of nanosilver-containing biostimulants have been studied in a number of studies, which allows them to be used to improve agricultural production. To prepare the AgNP system in a “green” way, as described in the literature, a 0.1 M solution of AgNO3 was added directly to the Peganum harmala extract. However, turbidity was observed as soon as the AgNO3 solution was added to the extract. In this case, the extract became turbid, as in the case of tobacco extract [25-27].

This once again confirmed that some compounds in the extracts containing alkaloids react with Ag+ ions and form a precipitate.

It is known from the literature that although the most abundant element in the mineral composition of Peganum harmala is potassium, halogens are also found in small quantities, in particular chlorine [28-30]. Therefore, the turbidity and color change of precipitates when $AgNO_3$ solution is added to the extract can be explained by the above. Of course, since our extract is a mixture of various substances, we can assume that reactions also took place with the participation of reducing compounds.

In subsequent experiments, work was carried out to obtain a stable colloidal system containing AgNP with bactericidal properties by first preparing a colloidal solution of AgNP and then adding it to the Peganum harmala solution.

For this, silver nitrate was used as the source of AgNP, polyvinyl alcohol as a stabilizer, and solutions of sodium citrate in the 1st reaction and glucose in the 2nd reaction as reducing agents. The necessary stoichiometric amounts of reagents were calculated and dissolved in distilled water. By changing the reaction time, stirring intensity, concentration of reagents and stabilizers, optimal conditions for obtaining nanosilver were found. The reactions were carried out at room temperature, adding equal volumes of reducing solutions to the stabilizer and silver nitrate mixture solution, and stirring the solutions continuously.

Reaction 1:

$12AgNO_3 + 4Na_3C_6H_5O_7 + 6H_2O \rightarrow 12\ Ag\downarrow + 12NaNO_3 + 4C_6H_8O_7 + 3O_2$

Reaction 2:

$2AgNO_3 + C_6H_{12}O_6 + NaOH \rightarrow 2Ag\downarrow + C_6H_{12}O_7 + NaNO_3$

Table 3. Nanosilver composites obtained by reducing AgNO3 in the presence of various reducing agents and polyvinyl alcohol (20°C)

№	Reducing agents	Change	Rasmi
1	Glucose	After 24 hours, the solution turned light brown and remained transparent.	
2	Sodium Citrate	After 24 hours, the solution turned gray-black and became cloudy.	

Initially, solutions were prepared at 20°C. Then, a silver nitrate solution with a concentration of 500 mg/ml was prepared. Nanosilver samples were prepared by mixing the stabilizer and silver nitrate solutions and adding an equal volume of reducing solution to the resulting mixtures with constant stirring.

For example, to obtain nanosilver of the desired size, 20 ml of an aqueous solution of sodium citrate (0.1 M) was slowly added to a mixture of 20 ml of PVA (2.04%) and silver nitrate (0.0463 M) to initiate a reaction between the solutions. The reaction of the solution with Ag+ ions was carried out for 5 minutes at 20°C with constant stirring. The color change of the solution provides visual information about the completion of the reaction, as well as the formation of a colloidal solution of nanosilver (Table 3).

Conclusion. Preliminary phytochemical analyses were conducted on the composition of Peganum harmala and showed the presence of alkaloids, flavonoids, tannins, anthraquinones, saponins,

carbohydrates, and coumarins. The sum of aqueous extracts from various parts of the plant in an acidic medium was obtained. A nanosilver-containing preparation could not be synthesized by directly treating the extracts with $AgNO_3$. When $AgNO_3$ solution was added, the extract became cloudy and precipitates formed. Then, colloidal solutions containing nanosilver were synthesized in the presence of reducing agents glucose and sodium citrate, using PVA as a stabilizer. A composition with bactericidal-fungicidal properties was obtained by combining the extract of the plant and colloidal solutions containing nanosilver. Field tests are planned to study the insecticidal, fungicidal, and biostimulatory properties of the resulting compositions.

The development of effective broad-spectrum insecticide, fungicide and antimicrobial drugs based on the compounds contained in Peganum harmala is an important research direction, and the study is an attempt in this direction. These results indicate the bright prospects of Peganum harmala as a natural source of new generation drugs, and also serve to further illuminate its therapeutic mechanisms.

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