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# OBTAINING COLLOID COMPOSITIONS BASED ON PEGANUM HARMALA EXTRACTS AND NANOSILVER

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**Abstract:** The article provides information on the compounds contained in the Peganum harmala plant, the use of plant parts and individual compounds. The sum of extractive substances was isolated and their yields were determined by extracting different parts of the Peganum harmala plant with water. Nanosilver compositions of the extracts were prepared and their IR spectra were obtained.
**Keywords:** Peganum harmala, harmaline, harmine, harmalol, peganin, extraction, nanosilver, reducing agent, stabilizer, composition, IR spectrum, yield.

**Introduction.** Peganum harmala is rich in resources, widely distributed in the world, and has a medicinal history of over 2000 years. Traditional use in disinfectants and mosquito control has led to its successful application in the treatment and prevention of diseases in humans, animals, and plants. The plant Peganum harmala, popularly known as Peganum harmala, contains a large number of biologically active substances, especially alkaloids. Peganum harmala alkaloids are also psychoactive compounds in humans. Plant extracts have insecticidal properties against pests of agricultural plants. Infectious diseases cause great harm to the health of humans, animals, and plants, and they occur worldwide due to the diversity of pathogens and their high infectivity. Effective prevention and treatment of infectious diseases are major medical problems. Antimicrobial drugs play an important role in the prevention and treatment of infectious diseases [1, 2].

Despite the continuous development and progress in medical science, many pathogens have developed drug resistance to antimicrobial drugs due to genetic changes, reducing the effectiveness of antibiotics and greatly limiting their clinical application. In addition, the development of antibiotics has almost come to a standstill in the past decade due to the long R&D cycles of antibiotics, high R&D costs and low commercial profits [3]. Therefore, there is an urgent need for new antimicrobial drugs to eliminate diseases caused by drug-resistant microorganisms. P. harmala L. - belongs to the family Zygophyllaceae (in some sources Nitrariaceae). In Uzbekistan, it is called "isiriq" and is a plant that has been used for various purposes since ancient times. P. harmala is a plant distributed mainly in the Middle East, North Africa and Central Asia. The plant is usually not grazed by animals due to its bitter taste.

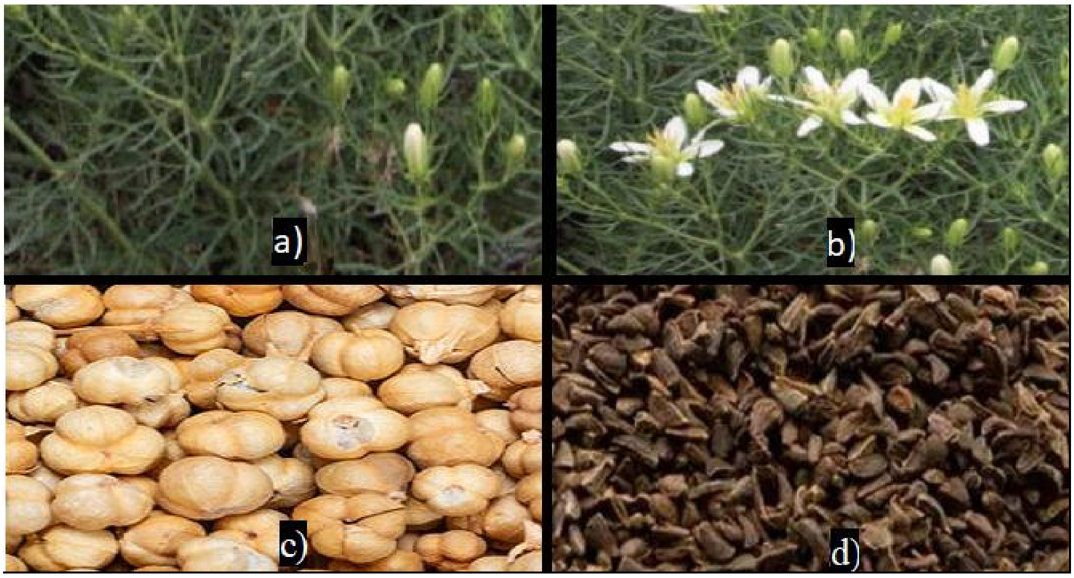


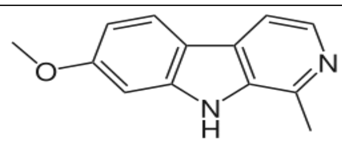

**Figure 1. Peganum harmala L.: a) leaf, b) flower, c) fruit, d) seed.**

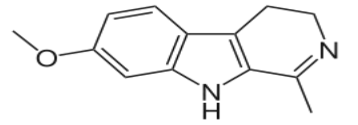
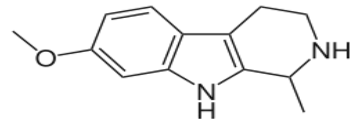
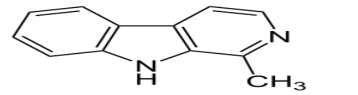
P. harmala is a perennial herb with a strong odor, blooming in May-July, growing in steppes and hills, 40-70 cm high. The root is multi-headed, woody, thick, 3-5 cm in diameter. The stem is branched, bare, densely leafy. The leaves are alternate, 4-5 cm long, 5-6 cm wide, three-edged at the base, and the segments are also divided into linear segments. The flowers are white, the fruit is a three-celled capsule with a diameter of 0.6-1 cm, the fruits ripen from late June to August, 1-2 mm long (Figure 1).

In recent years, many studies have shown that more and more natural products of plants have special biological activities as natural antibiotics, which play an important role in the prevention, treatment and reduction of the spread of disease. Peganum harmala L., a perennial plant belonging to the Zygophyllaceae family. Phytochemical studies have shown that the main chemical components in the plant are alkaloids, flavonoids, esters and trace elements [4-7]. However, there are few studies on the production and practical application of nanosilver-containing alkaloid extracts, and the content is limited to the synthesis of nanosilver using the extract using the “Green” method. Based on this, we aimed to obtain a drug with bactericidal and fungicidal properties from the extract of the Peganum harmala plant containing nanosilver.

**Literature review.** The pharmacological, chemical and biological activity of Peganum harmala has been studied for more than 100 years. Peganum harmala contains a wide variety of compounds. More than 308 compounds have been isolated from Peganum harmala, of which 97 are alkaloids, 24 are flavonoids, 10 are triterpenoids, 3 are anthraquinones, 2 are phenylpropanoids, 18 are carbohydrates, 17 are amino acids, 99 are volatile oils, 26 are fatty acids, 26 are linoleic acids, 3 are carotenes, and 6 other trace elements. Among these compounds, the highest content is the β-carboline alkaloids (βC). The alkaloid content is up to 10% in the seeds, followed by the roots, and the lowest in the leaves. Their main compounds include harmine, harmaline, harmalol, harman, and harmol; several new compounds have been reported in recent years (Table 1) [8].

**Table 1.**
**Formula and structure of the most abundant alkaloids of Peganum harmala**

| Name | Formula | IUPAC name | Structure |
|---|---|---|---|
| Garmin | $C_{13}H_{12}N_2O$ | 7-Methoxy-1-methyl-9H-pyrido[3,4-b]indole |  |

| | | | |
|---|---|---|---|
| Garmalin | $C_{13}H_{14}N_2O$ | 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-b]indole |  |
| Tetragidrogarmin | $C_{13}H_{16}N_2O$ | 7-Methoxy-1,2,3,4-tetrahydro-garmine |  |
| Garman | $C_{12}H_{10}N_2$ | 1-methyl-9H-β-carboline |  |

There are a large number of alkaloids, quinazoline and indole derivatives. Of the total number of alkaloids, harmaline, harmine (banisterine), harmalol and L-peganine (vazizine) were isolated for the first time in pure form, while pegamine, peganol, deoxypeganine, peganidin and others were isolated in recent years. It is known that 50% of the total amount of alkaloids in the seeds is harmine, pregamines, harminates, and up to 78% of the total amount of alkaloids in the grass is peganine. It has also been found that young roots contain twice as many alkaloids as old ones, with harmine prevailing. As the above-ground part of the plant develops, the amount of alkaloids and the proportion of peganine in it decreases, while the amount of harmine increases. The qualitative composition of alkaloids largely depends on the place where the plant grows. In addition to alkaloids, red dye and drying oily oil are isolated from the seeds of the plant. The herb contains protein (24%), fatty oil (4%) and extractives (31%). The raw material is used to obtain the drug deoxypeganine hydrochloride, which has anticholinesterase activity. The drug is used for lesions of the peripheral nervous system.

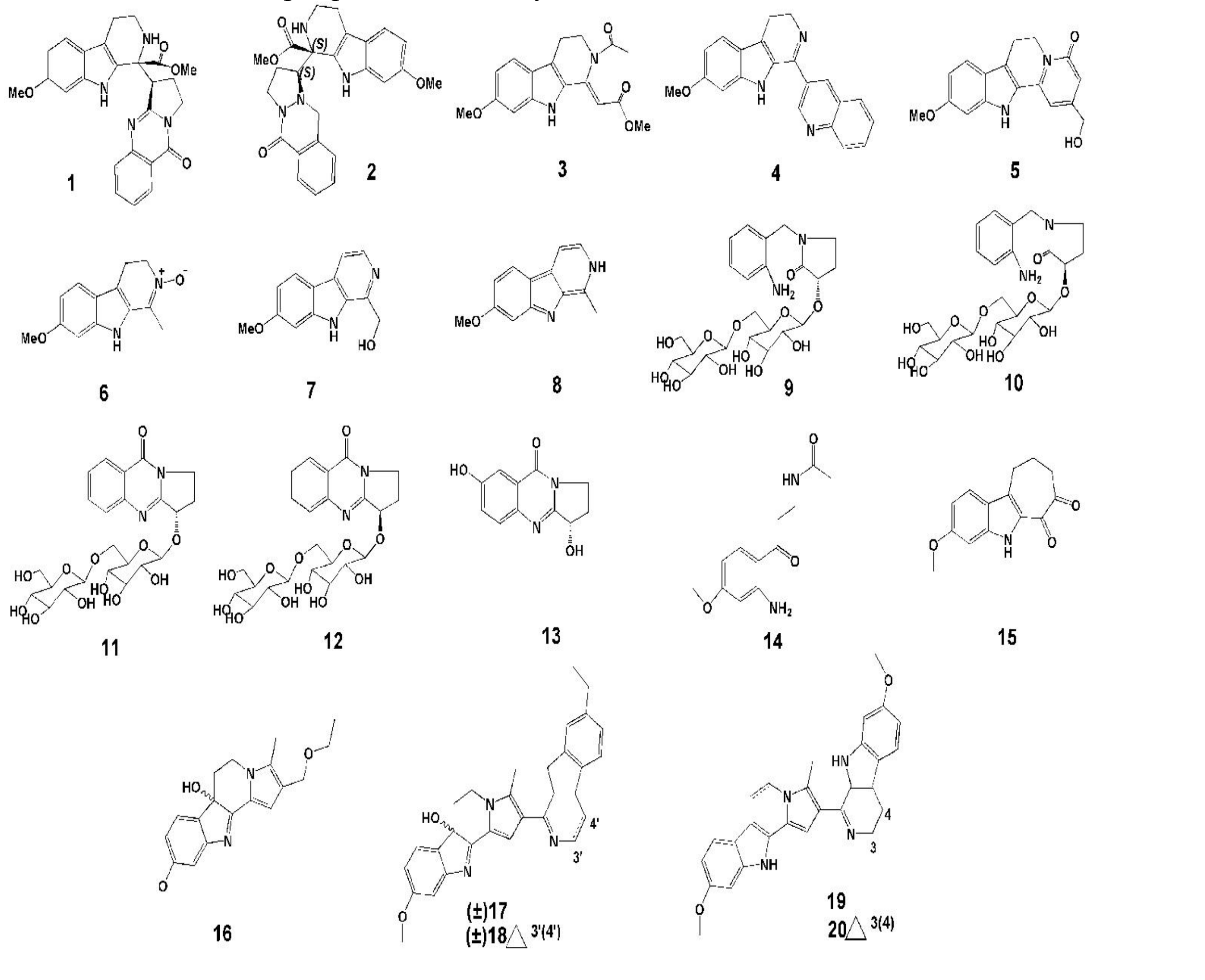


**Figure 2. Structures of the last 20 new compounds isolated from Peganum harmala.**

In addition to the above, other alkaloids such as harminic acid (methyl 7-methoxy-β-carboline-1-carboxylate), harmalic acid (7-methoxy-3,4-dihydro-β-carboline-1-carboxylic acid), harmanamide (1-carbamoyl-7-methoxy-β-carboline), and acetylnorgarnine (1-acetyl-7-methoxy-β-carboline) are also found in large quantities. The latest novel compounds from Peganum harmala have been named as follows: (−)-pegarmalin A (1), (+)-pegarmalin A (2), pegaharmines F–K (3–8), (S)- and (R)-1-(2-aminobenzyl)-3-hydroxypyrrolidin-2-one β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (9, 10), (S)- and (R)-vasicinone β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (11, 12), N-[3-(2-amino-4-methoxyphenyl)-3-oxopropyl] acetamide (14), pegagarines A–F (15–20) (Figure 2)[9].

The various compounds are the material basis for its broad-spectrum antimicrobial activity. Due to the diversity of secondary metabolites of Peganum harmala and the development of technology, new compounds have been discovered from plant extracts. The antimicrobial activity of these new compounds has been poorly studied. The antibacterial effect of Peganum harmala has been demonstrated in many gram-positive and gram-negative bacteria. It has also been found that seed and root extracts have a synergistic effect when used together with drugs such as neomycin, colistin and carbenicillin. In conclusion, it has been shown that the harmine alkaloid can be used in the development of drugs for the treatment of infectious diseases [10-12].

Smoke from Peganum harmala seeds can act as an air disinfectant to reduce the concentration of bacteria in the air [40]. In residential areas, the rate of destruction of airborne bacteria after 5 g of seeds was as high as 71.4%, with smoke generation for 5 minutes. In an educational setting, the elimination rate of airborne bacteria after 10 minutes of fumigation from 10 g of seeds reached 92.8% [13].

Peganum harmala has been shown to have fungicidal effects on various pathogenic fungi. As can be seen from the current studies, there are many fungicidal studies on Peganum harmala, which show good fungicidal activity of Peganum harmala. The active component is mainly the extract of the seeds. However, studies on screening fungicidal compounds and optimizing the structure of highly antibacterial compounds are rare. It is an urgent need to identify the active compounds and optimize the lead compounds in future studies [14, 15].

The inhibitory effects of Peganum harmala on various animal viruses and plant viruses have been reported. Current studies have reported the antiviral activity of various components of Peganum harmala. Most of these studies have only reported on the antiviral effects of Peganum harmala extracts, but studies on screening for antiviral compounds in extracts are rare, and studies on the antiviral mechanism of Peganum harmala are even less. Therefore, more research is still needed in these aspects [16-18].

Peganum harmala has been shown to have significant antiparasitic and acaricidal activities. At present, Peganum harmala seed extract has good inhibitory effects on pathogens. Therefore, further studies using modern molecular biology and other tools are needed [19-21].

The inhibitory activity of Peganum harmala against a wide range of microorganisms may account for its therapeutic effects on many pathogen-related diseases, such as wound healing, skin inflammation, hemorrhoids, and cough. Peganum harmala is a potential source for the prevention or treatment of infectious diseases of plants such as rust and wilt due to its insecticidal effect and inhibition of the activity of various plant pathogens [22, 23]. Peganum harmala has antibacterial, anti-inflammatory, anti-leukemia and psoriasis effects, and has many clinical pharmacological effects such as memory enhancement, and is clinically used to treat cough, hypertension, diabetes, jaundice, colic, malaria, Alzheimer's disease and other diseases. Reports on the bactericidal, fungicidal, antiviral and antiparasitic effects of Peganum harmala are increasing year by year. However, the antifungal and antimicrobial effects and mechanisms of Peganum harmala have not been described in detail.

The isolation of these substances and the development of biologically active systems based on them are of great importance in the fields of medicine, agriculture and nanotechnology. In particular, colloidal systems enriched with nanosilver particles are widely used due to their antibacterial and antioxidant properties. In this regard, this work is devoted to the isolation of extracts from various parts of the Peganum harmala plant and the synthesis of colloidal systems containing nanosilver based on these extracts.

**Methods.** The plant was harvested and dried in June-July. Extractions from plant parts were carried out at a temperature of 60oC, in acidic and neutral media, in a mass ratio of raw material:solvent = 1:10. Nanosilver synthesis was carried out chemically, in the presence of AgNO3 reducing agents sodium citrate and glucose. Distilled water was used as a solvent in all reactions. The IR spectra of the samples were obtained on a Shimadzu FTIR spectrophotometer.

**Results and analysis.** Isolation of alkaloids from different parts of the Peganum harmala plant - leaves, stems, seeds and seed coat - by acetic acid extraction method, determination of the mass yield of substances in each part, synthesis of nanosilver and synthesis of nanosilver-containing compositions and study of IR spectra. Preparation of the plant for extraction. The dried Peganum harmala plant was separated into structural vegetative parts - leaves, stems, seeds and seed coat. The structural parts were ground separately to a size of 1 mm (Figure 3).

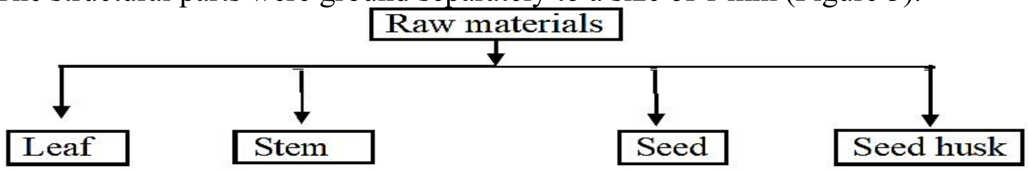



**Figure 3. Scheme of separation of raw materials into components.**

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 with constant stirring using a magnetic stirrer for 30 minutes at a temperature of 60°C. After the specified time, the mixtures were filtered, the filtrate and residue were separated. The above process was repeated twice more with the residue. All the filtrates obtained were combined and concentrated, and the extracts were finally filtered through blue ribbon filter paper. The colors of the obtained extracts were different. The extract obtained from Peganum harmala leaves was dark brown, the extract from seeds was brown, the extract from stems was light yellow, and the extract from seed coat was cloudy yellow (Figure 4).

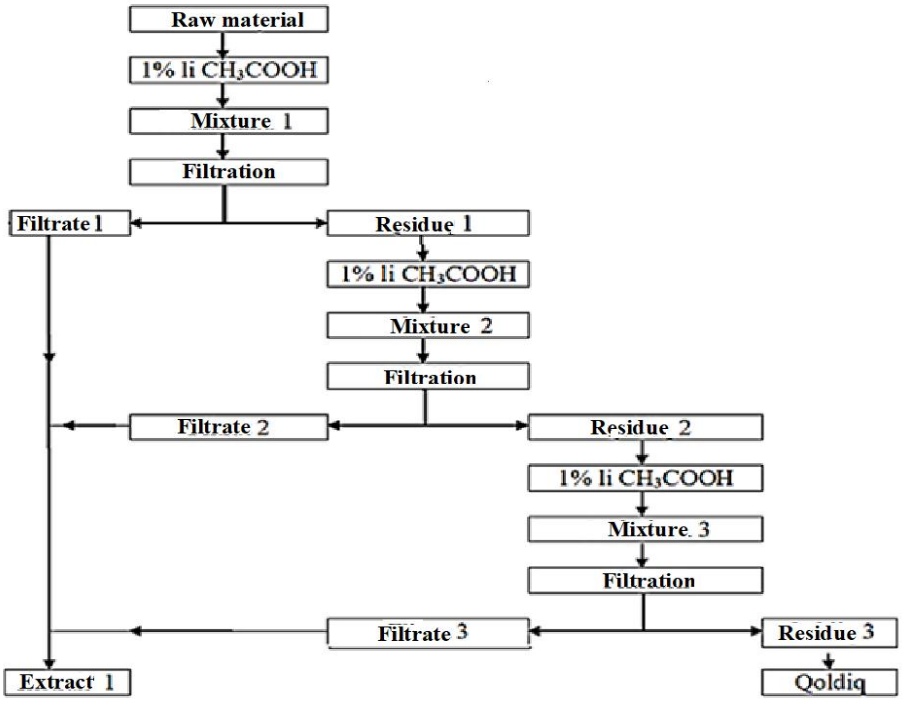



**Figure 4. Scheme of extracting Peganum harmala at 60$^o$C temperature (**extraction time - 30

minutes, extraction - 3 times, raw material:extractant = 1:5 mass ratio).

Determination of extraction yield. In order to determine the extraction yield, 10 ml of each extract was weighed on an analytical balance to the nearest 4 decimal places. Then, it was dried in a drying cabinet at 100°C until constant mass was reached, and the dry residue was reweighed on an analytical balance under the above conditions. Dry mass and yield were calculated as a percentage (Table 2). The total volume of extracts and the yield in terms of dry matter were calculated using the following formula:

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

Where: $m_1$ is the mass of the dry residue, $m_2$ is the mass of the extract.

**Table 2.**

**Yield of extract sums from parts of the Peganum harmala plant**

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

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, glycosidated forms of flavonoids, 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), sodium citrate and glucose as reducing agents, polyvinyl alcohol (PVA) and carboxylmethylcellulose sodium salt (CMS-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. Nanosilver-containing biostimulants promote nutrient absorption, activate physiological processes in plants, and increase resistance to stress and diseases. Studies conducted by scientists show the effectiveness of using nanosilver-containing biostimulants in various crops and their effect on yield. Nanosilver-containing biostimulants are a promising method for increasing crop yield and protecting plants in agriculture. Methods for obtaining, properties, and applications 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 $AgNO_3$ was added directly to the Peganum harmala extract. However, turbidity was observed immediately after the $AgNO_3$ solution was added to the extract. In this case, as in the case of tobacco extract, the extract became turbid [24-27].

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

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 a solution of Peganum harmala.

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

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

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).

**Table 3**

**Nanosilver composites obtained by reducing $AgNO_3$ in the presence of various reducing agents and polyvinyl alcohol (20°C).**

| № | Reducing agents | Change | Photo |
|---|---|---|---|
| 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. |   |

The IR spectrum of Peganum harmala leaf extract revealed the following information. The absorption region around 3309 $cm^{-1}$ is a characteristic broad peak associated with the stretching vibrations of the -OH or 0NH groups corresponding to hydrogen bonds or amine groups. These peaks indicate the presence of phenolic and alkaloid compounds. The region 3000-2800 $cm^{-1}$ is a characteristic peak corresponding to the -CH and -NH groups. This indicates the presence of harmine and harmine-like alkaloids. The absorption region around 2378 $cm^{-1}$ and 2308 $cm^{-1}$ are peaks associated with the asymmetric vibrations of carbonyl or carboxyl groups. The absorption at 1636 $cm^{-1}$ is characteristic of the vibrations of carbonyl (C=O) or amide groups.

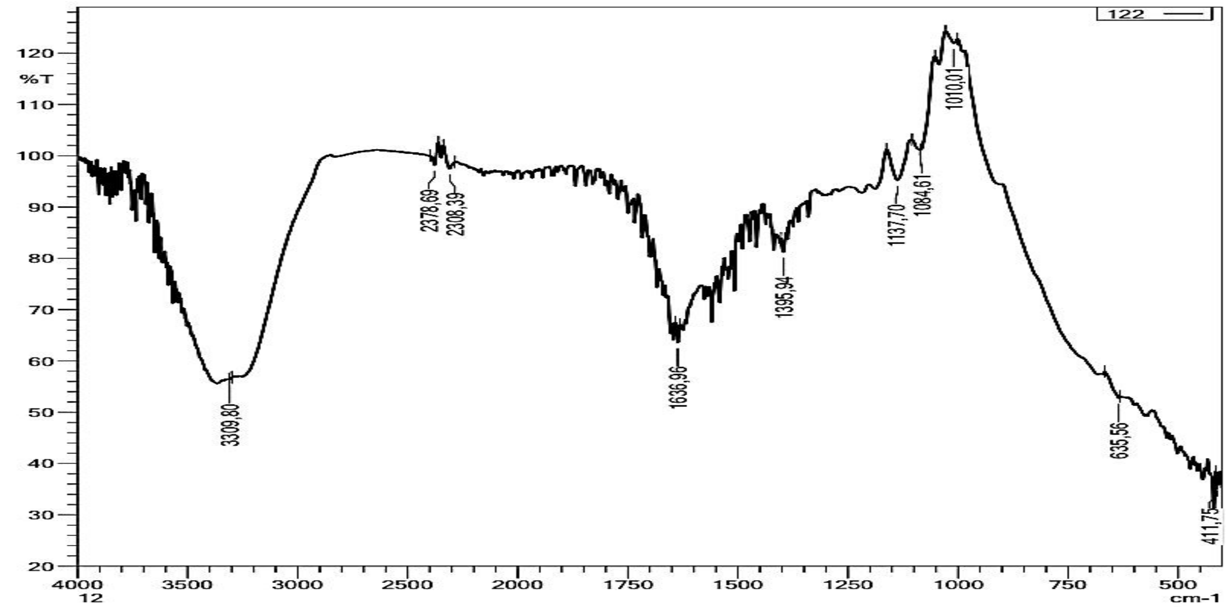


**Figure 5. IR spectrum of Peganum harmala leaf extract.**

The absorption region around 1396 cm⁻¹ is due to stretching vibrations of N-O or C-N groups. The absorptions around 1137 cm⁻¹ and 1010 cm⁻¹ are due to vibrations of the CO group, which are characteristic of ether or glycoside structures. This indicates the presence of carbohydrates or other compounds containing oxygen. The peaks in the absorption region 635-411 cm⁻¹ are due to deformation vibrations of various bonds of the aromatic ring (Figure 5).

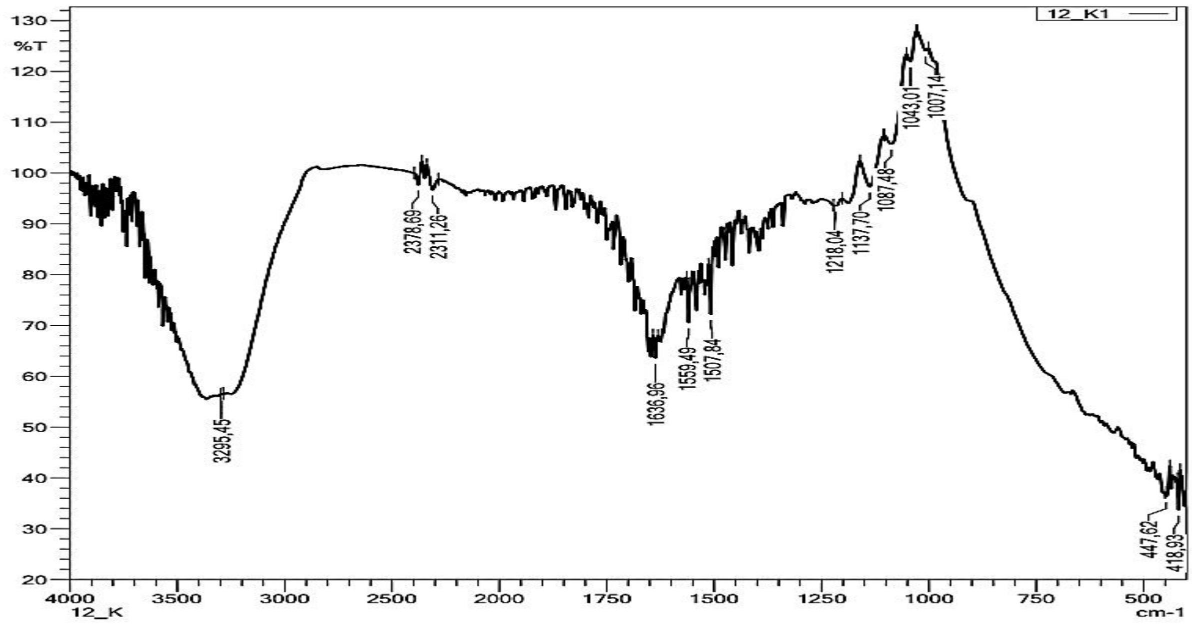


**Figure 6. IR spectrum of Peganum harmala leaf extract containing nanosilver.**

The IR spectrum of Peganum harmala leaf extract containing nanosilver showed the following: the absorptions around 3295 cm⁻¹ differ from the previous spectrum by shifting to the higher frequency region. This broad peak indicates the presence of -OH or -NH groups, which indicates the presence of phenols, phenolic glycosides, amines and hydrogen bonds. The absorption region at 2378–2311 cm⁻¹ is almost the same as the previous spectrum and is associated with carbonyl groups. The region around 1636 cm⁻¹ is characteristic of the C=O stretching vibrations of carbonyl groups in amides or carbonyl compounds of aromatic systems. The region around 1559 cm⁻¹ indicates the deformation vibrations of the C-H, N-H groups of aromatic compounds and is characteristic of phenolic and aromatic amines present in the extract. The absorption regions at

1218 and 1043 cm⁻¹ shifted to higher frequencies compared to the previous spectrum. They are associated with C-O or C-N groups characteristic of esters and glycosides present in the extract. The absorption region at 447 cm⁻¹ also shifted to higher frequencies by 36 cm⁻¹ compared to the previous spectrum. This low-frequency vibration indicates the presence of silver complexes or silver nanoparticles (Figure 6).

**Conclusion:** The yield of extract sums from different parts of Peganum harmala plant ranged from a minimum of 3% in the seed coat to a maximum of 16% in the stem. It was impossible to obtain the sums of nanosilver-containing extracts by directly treating the extracts with $AgNO_3$, because the extract became cloudy and precipitates formed when $AgNO_3$ solution was added. Then, colloidal solutions containing nanosilver were synthesized in the presence of reducing agents glucose and sodium citrate, and polyvinyl alcohol was used as a stabilizer. A composition with bactericidal-fungicidal properties was obtained by combining the extracts of the plant and the colloidal solutions containing nanosilver. The IR spectra of the obtained extracts showed that the extracts contained groups characteristic of alkaloids, carbohydrates, aromatic compounds, and absorption frequencies characteristic of nanosilver.

The development of broad-spectrum insecticide, fungicide, and antimicrobial drugs based on the compounds contained in Peganum harmala is an important research direction, and the research conducted is an attempt in this direction. These results indicate the bright prospects of Peganum harmala as a natural source of next-generation drugs, and also serve to further elucidate its therapeutic mechanisms. Field tests are planned to study the insecticidal, fungicidal, and biostimulatory properties of the obtained compositions.

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