

PAPER • OPEN ACCESS

Evaluation of Phytochemical Constituents by GC-MS, HPLC and Biological Activity of *Peganum Harmala* L. Seeds Extract

To cite this article: Mohammed R. Najm and Fatimah I. Sultan 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **1060** 012097

View the [article online](#) for updates and enhancements.

You may also like

- [The Effect of some Plant Extracts Against TYLCV on Tomato Plants](#)
A K Jabar, H A Salim and G M Dagher
- [Fabrication of folic acid-conjugated chitosan-coated PLGA nanoparticles for targeted delivery of *Peganum harmala* smoke extract to breast cancer cells](#)
Masoud Homayouni Tabrizi
- [The Long Tails of the Pegasus–Pisces Arch Intermediate Velocity Cloud](#)
R. L. Shelton, M. E. Williams, Matthew C. Parker et al.

Evaluation of Phytochemical Constituents by GC-MS, HPLC and Biological Activity of *Peganum Harmala L.* Seeds Extract

Mohammed R. Najm¹ and Fatimah I. Sultan²

^{1,2} Technical Agricultural College, Northern Technical University, Mosul, Iraq.

¹E-mail: mohammed.na.student@ntuedu.iq

²E-mail: drfatimah@ntu.edu.iq

Abstract. The chemical compounds known as the active compounds separated from the seeds of *Peganum harmala* are characterized by their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Agrobacterium rhizogenes*. A successive solvent system was used to obtain the petroleum ether and methanolic extract according to polarity.

The *Peganum harmala* seeds using Soxhlet continuous extraction device separated the fatty acids from the crude petroleum ether extract after soapification and diagnosed by gas chromatography-mass spectrometry (GC-MS). The results showed the presence of Pentadecanoic acid, octadecadienoate, Octadecenoic acid, Hexadecanoic acid, Cyclobutanecarboxylic acid. While the alkaloids were separated from the crude methanol extract and diagnosed by HPLC technology, the results indicated the presence of Harmine and Harmalol. The above-mentioned compounds are considered to have a high inhibitory activity against the bacteria used under study using a disk susceptibility test compared to standard antibiotics.

Keywords. *Peganum*, GC-MS, HPLC, Fatty acids, Harmine, Harmalol.

1. Introduction

Medicinal plants, especially *Harmal*, are rich in natural active compounds resulting from secondary metabolism within the plant. Mostly, it is rich in alkaloids, the most important of which are Harmine, Harmalol. It is used in various fields, including as defense materials against microorganisms and fungi, pain reliever, diabetes treatment and heart rate regulation [1].

Peganum harmala it belongs to the family *Nitrariaceae* and is found in the Mediterranean basin, North Africa, Iraq and the Arabian Peninsula. The plant is distinguished by its foul smell. It is considered one of the perennial herbaceous plants, it has a very branched stem that reaches a height of about 30-120 cm. The leaves are seated, many lobed, long oval, dark green, and its white flowers are star-shaped, seated or petioled. They appear in the armpits of the branches. It includes 5-4 sepals, tapering and thin, longer than the petals. Fruits on the shape of a spherical box and its seeds are dark brown in color. Medicinal plants have many benefits, including intervention in the treatment of many diseases. Attention has recently been drawn to extracting effective compounds and using them as antibiotics in a safe manner without side effects, unlike the manufactured chemical compounds that are used and used as anesthetics. It is an anthelmintic, and leaves may be burned and fumes inhaled to heal headaches and nerve pain. A few roots are eaten to treat chest crises. Oil is extracted from the seeds for the treatment of eyes and skin diseases [2].



2. Materials and Methods

It includes two axes, the axis of the separation of the active compounds, and the second axis, the inhibitory activity against pathogenic bacteria.

2.1. Active Compound Separation Axis

The plant *Peganum harmala* was classified by taxonomists and collected from the markets of the city of Mosul from and taken from reliable sources [3].

2.2. How to Prepare Plant Extracts

The extracts were prepared using a continuous extraction device based on the method of the researcher [4]. The extraction process was carried out in three different solvents starting from petroleum ether and methanol, 100 gm of *peganum harmala* seed powder was taken after grinding and then placed in a continuous extraction device with 500 ml of solvent, and the extraction process continued at a rate of 8 per day until the used solvent turns colorless, the same process is repeated for the rest of the solvents according to polarity, and then the raw plant extract is transferred to the RVE rotary evaporator at a temperature of 40°C, and it is placed in opaque glass bottles and kept in the refrigerator until use [5].

2.3. Separation of Fatty Acids by Coalification Process

The separation process was carried out by taking about 10 g of crude petroleum ether extract and adding 100 ml of a solution (KOH 7.5 molar), and then heating it at a temperature of 100°C for a period of 90 minutes, and then cooling the solution to its natural temperature (room) and adding distilled water by 100 ml to give the shape of the emulsion solution the emulsified solution was placed in a separating funnel and 2×25 ml ether was added to remove the insoluble fat. The dissolved layer was taken and acidified by 20% of concentrated sulfuric acid H₂SO₄ Until the hydrogen reached 2=(PH), then the saponified fat was taken and the addition of ether to it in a separating funnel of 25 ml, the upper two layers of ether form with humic acids and the lower aqueous, which are neglected and kept inside sterilized and opaque glass bottles in the refrigerator until they are diagnosed by GC-MS technology [6,7].

2.4. Separation of the Alkaloids from the Crude Methanol Extract

Take 1mg of crude methanolic extract and add sulfuric acid at a concentration of 5% (5 ml of sulfuric acid + 95 ml of distilled water) with ether at a ratio of 50/50 (v/v) and the solution is placed in a separating funnel with shaking for 10 minutes two layers will be the upper layer is ether, while the lower layer is the alkaloids are taken and the upper layer is neglected and then sodium hydroxide is added 10 N, and then 30 ml of chloroform is added and placed in a separating funnel with shaking for 10 minutes the upper two layers will be neglected and the lower one will be chloroform with alkaloids as shown in the fig. (1) and stored in the refrigerator until use and diagnosis using HPLC technology [8].

2.5. Instruments Used to Diagnose Active Compounds

2.5.1. Gas Chromatography-Mass Spectrometer (GC-MS)

Identified fatty acids of petroleum ether extract from harmala in Food Research and Consumer Protection Laboratory - University of Basra, GC MS QP210 Ultra GC-MS QP210 Ultra from SHIMADZU of Japanese origin The separation process was carried out according to MS-GC thermal system at 40°C for one minute and start increasing to 150 m for a minute at a rate of 5 m/min until it reaches 280 m at a rate of 5 m/min and perform the auto-injection process by injecting (1 microliter) in the Gas device, from the upper layer containing fatty acids after the esterification process of the oil samples that were studied, type AOC-AHIMADZU, 20i+5, and the separation conditions, after obtaining the results, were processed by GC-MS solutions program and looking at the schematics [9].

2.5.2. HPLC High Performance Liquid Chromatograph

The diagnosis of alkaloid compounds was carried out in Baghdad after acid hydrolysis of phenols using HPLC device Shimadzo, LC-2010 AHT type with membrane filters of 0.1 μm diameter, wavelengths 320 nm, flow rate 1 ml/min and mobile phase (Methanol: H_2O) (85:15). For the separation column C18 with dimensions of 240*4 mm at a temperature of 30°C, supplied by an American company, Macherey – Naged.

2.6. Bio-Efficiency Hub

2.6.1. The Bacteria Used in the Study

Three bacterial isolates were used in this study, which were *Staph. aureus*, *E. coli*, *Agro. rhizogenes*.

2.6.2. Equipped Agricultural Circles

Prepare the nutritious agar medium according to the manufacturer's instructions.

2.7. Antibiotic

The sensitivity of bacterial isolates to antibiotics was tested according to the standard Kirby-Bauer method as shown in Table (1) [10].

Table 1. Shows the types, concentrations and symbols of antibiotics used in the study (mg/cm^3).

Antibiotics	Counter symbol	Focus/disc
Cefixime	CFM	5
Amoxicillin	AMC	30
Ciprodar	CIP	10
Trim Methbrin	TMP	10
Azithromycin	AZM	15
Nalidixic acid	NA	30

2.8. Antibiotics Sensitivity Test

The inhibitory activity against the bacteria used under study was tested using the method of diffusion by digging the sensitivity test at a rate of three replicates for each bacteria, as holes were prepared in the nutrient medium with a diameter of 6 mm and the bacterial suspension was prepared at the age of 16-14 hours and then the bacterial suspension was transferred to the nutrient medium in comparison with the standard MacFarland solution (108cfu). /ml $\times$ 1.5) by relying on a sterile cotton swab to spread the suspension on the surface in a consistent manner, then the dishes were placed in the incubator at a temperature of 37°C for a time of 30 minutes to be imbibed. Concentrations (100, 200, 400) mg/cm^3 , if a concentration of 400 mg/cm^3 of separated active compounds was prepared By dissolving 1 g of each extract in 2.5 cm^3 of DMSO (Dimethyl sulfoxide) and ethylene glycol for volatile oil, then sterilizing the mixture by pasteurization method at a temperature of 62°C for a period of 10-15 minutes and by means of membrane filters 0.22 μm for volatile oil to prevent the passage of germs from Through this, standard and approved concentrations were obtained in preparing the diluted sequential concentrations of the extracts An amount of 0.5 microliter was taken by micropipet from each prepared extract and placed inside the holes that were perforated with a drill hole and in the middle of the nutrient acre or Mulrenton at a rate of three replicates and incubated at a temperature of 37°C for 16-14 hours [11,12].

The results were compared with the selected antibiotics under study as a control sample for bacteria, based on what is used in public health laboratories and based on the tests of the World Health Organization [13].

3. Results and Discussion

3.1. The Active Compounds are Identified in Groups

3.1.1. Diagnosis of some Fatty Acids Using GC-MS Technology

The use of GC-MS technology to diagnose the fatty acids present in the petroleum ether extract of the seeds of the Harmal plant after the soapification process the retention time of petroleum ether extract was compared with the standard retention time as the graphs indicated the presence of fatty acids, octadecadienoate, Octadecenoic acid, Hexadecanoic acid, Cyclobutanecarboxylic acid the results of the diagnosis after the soapification process showed that the presence of Octadecadienoic acid is the highest at a retention time of 17.306/min, followed by the rest of the acids, while docosanoic acid has the lowest concentration of 0.09% with a retention time of 21,244/min compared with the rest of the fatty acids mentioned above, as shown in the figures from (1) to (6) and table (2).

The results of the researcher's study (4), which revealed some effective chemical compounds produced from the methanolic extract of the seeds of *peganum harmala*, based GC-MS technology, revealed eight compounds, including harmine, harmaline, leptalorine, it is also used as an antibacterial specifically on pathogenic bacteria and therefore it is likely to be developed further as an alternative to chemical and antibacterial agents, as well as for therapeutic activities against cancer [14].

The current study was similar to the researcher's study [15], as the results of the petroleum ether extract of the seeds of the harmala plant when diagnosed using GC-MS technology indicated the presence of alkaloids, fatty acids and oily compounds and the researcher referred to treating depression and reaching a normal state.

Table 2. Diagnosing the terpene compounds separated from the seeds of the harmala plant by GC-MS.

NO	Peak	R time	Area	Area%	Name	Type
1	1	15.386	17943316	4.09	Pentadecanoic acid, 14-methyl-, methyl	Ester
2	2	17.333	320441496	73.04	Methyl 10-trans,12-cis-octadecadienoate	Ester
3	3	17.386	66063640	15.06	Trans-13-Octadecenoic acid, methyl	Ester
4	4	17.544	5544836	1.26	Hexadecanoic acid, 15-methyl-, methyl	Ester
5	6	23.908	15076657	3.44	Cyclobutanecarboxylic acid, tridec-2-ynyl	Ester

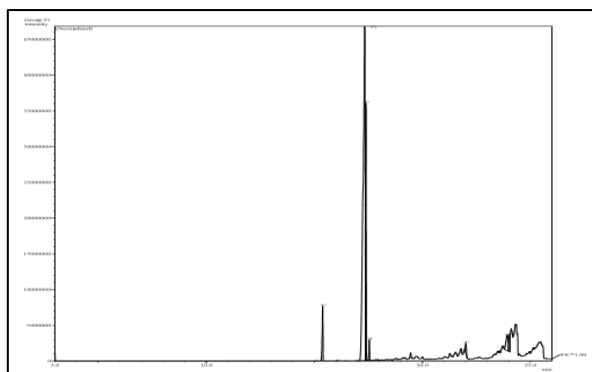


Figure 1. The schematic diagram shows the fatty acids of harmala plant that were diagnosed by (GC-MS).

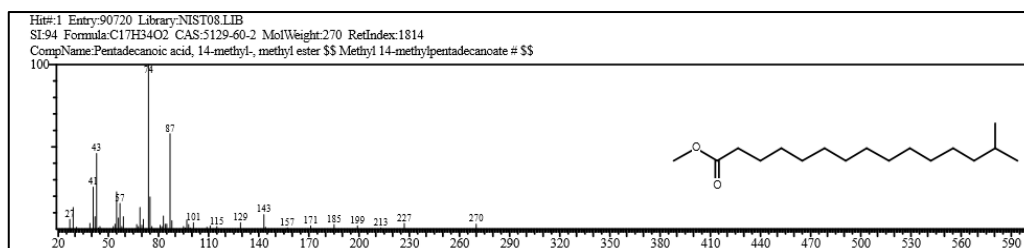


Figure 2. Curve of Pentadecanoic acid with GC-MS technology.

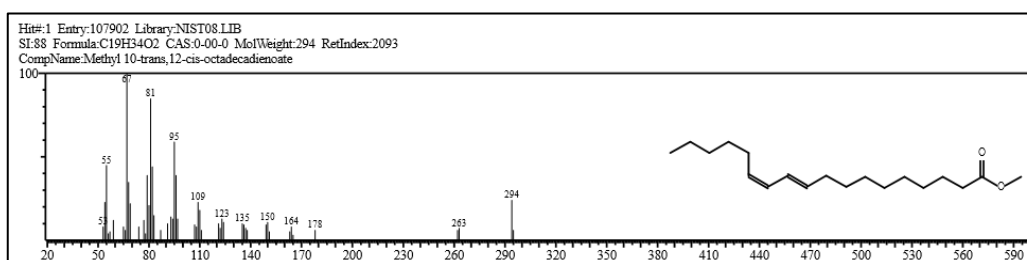


Figure 3. Curve of octadecadienoate with GC-MS technology.

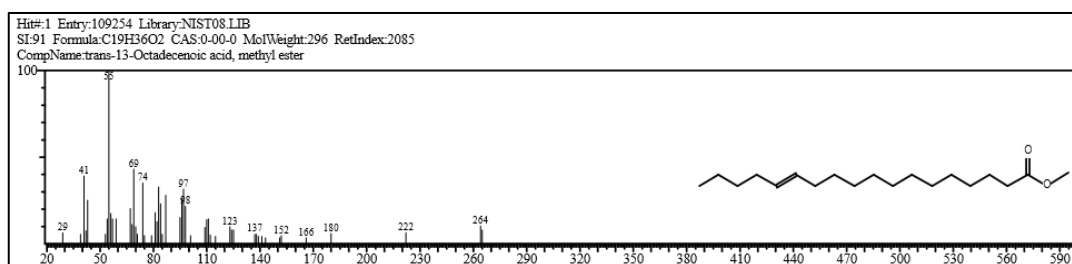


Figure 4. Octadecenoic acid curve with GC-MS Technology.

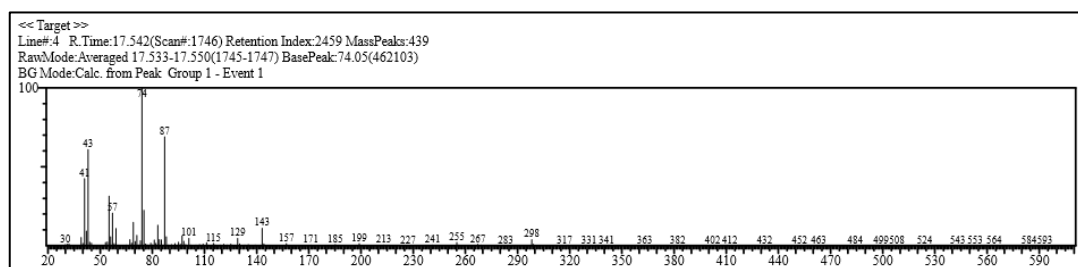


Figure 5. Curve Hexadecanoic acid with GC-MS technology.

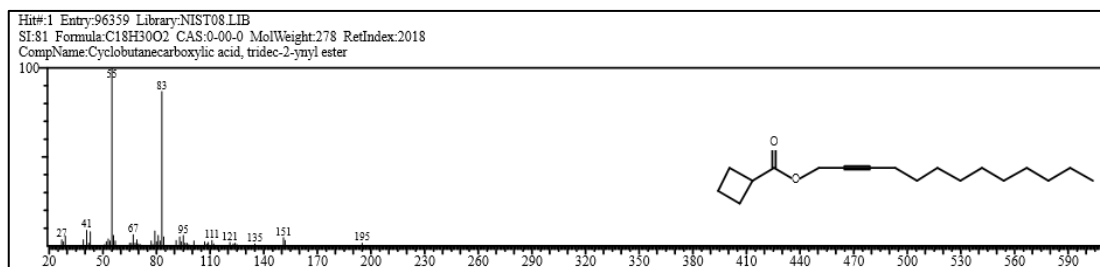


Figure 6. Curve of cyclobutanecarboxylic acid with GC-MS technique.

3.1.2. Diagnosis of Alkaloid Compounds by HPLC

As for the separated alkaloids from the methanolic extract of harmal seeds, the results showed the Harmine alkaloid at a retention time of 3.827 / min and a higher concentration of 8.500 mg/g compared with Harmalol compound with a retention time / 6.920 minutes at a concentration of 2.700 mg/g as shown in figure from (7) to (8) and Table (3).

The above results are in agreement with many studies, as the researcher (16) proved that the amount of alkaloids differs greatly between the natural compounds found inside the harmala plant, which are important sources for medical and industrial purposes in terms of their biological activity. It has anti-tumor and anti-inflammatory properties and plays an important role in the inhibitory activity and particularly affects the enzymatic activity, and this depends on the stages of growth. Harmine, Harmalol are often stored in high concentrations in the ripe seeds. These results are also similar to the study of researchers [16,17], if it gives an inhibitory effect against pathogenic bacteria.

Table 3. Alkaloid compounds diagnosed using HPLC technology after the acid hydrolysis process.

Alkaloid compounds	Harmine		Harmalol	
Vehicle retention time standard/mi	3.93		6.78	
Cumin plant	Hold time (minutes)	Concentration (mg/g)	Hold time (minutes)	Concentration (mg/g)
Crude Methanolic Extract	3.827	8.500	6.920	2.700

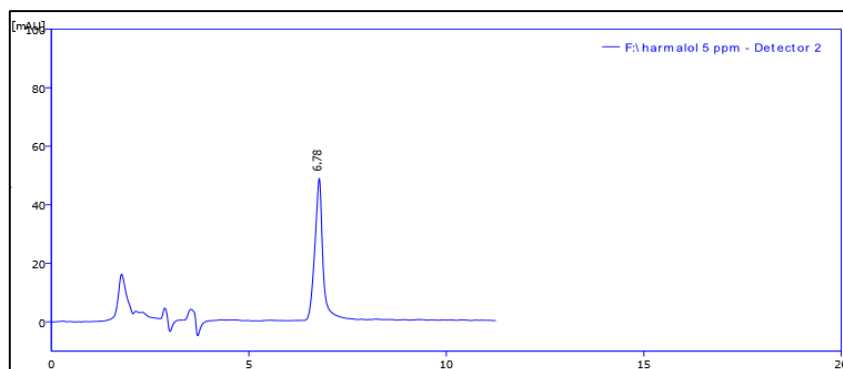


Figure 7. Curve of harmalol with HPLC technique.

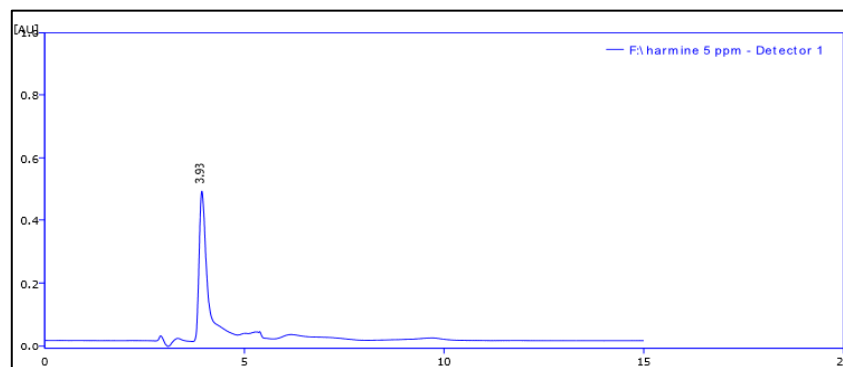


Figure 8. Curve of harmine with HPLC technique.

3.1.3. Inhibitory Effect of Fatty Acids and Alkaloids Separated from Harmala Seeds Against the Bacteria Used Under Study.

The results of the petroleum ether extract of harmala seeds showed the highest areas of inhibition against *Staph.aureus* at a concentration of 400 mg/cm³ with an effect of 20 mm, while the effect of the concentration was 200 mg/cm³ at a diameter of 16 mm inhibition, as for the concentration of 100

mg/cm³ and all concentrations of *E. coli* bacteria did not. There is no significant inhibitory effect, while the petroleum ether extract had an effect against *Agro. rhizogenes* bacteria at the highest concentration of 400 mg/cm³ by 11 mm, but the effect of the concentration of 200 mg/cm³ at 10 mm as shown in Table (4) and (6), Pictures (1) [18].

The results showed through the researcher's study [19], that the extract of petroleum ether, chloroform and methanol of the seeds of harmala showed an inhibitory effect against pathogenic bacteria, but the crude methanol extract was the most effective and active against *Staphylococcus aureus* bacteria compared to the rest of the extracts.

Table 4. Shows the effect of the crude petroleum ether extract separated from the seeds of the harmal plant against the bacteria used under study with different concentrations (400,200,100).

Concentration Mg/c ³	Types of bacteria		
	<i>Staph</i>	<i>E.coli</i>	<i>Agro.</i>
400	20a ±2.0	---	11c ±1.1
200	16d ±0.5	---	10 ±1.5
100	---	---	9c ±0.5

Average diameter of the circle using three isolates for each bacterium.

Different letters horizontally mean that there are significant differences at the level of significant difference ≤ 0.01P.

The diameter of the damping circle is measured in mm.

(--) indicates lack of inhibitory activity.

The numbers after (±) indicate experimental error.

3.1.4. Inhibitory Effect of Alkaloids Separated from the Crude Methanolic Extract of Harmel Seeds Against the Bacteria Used Under Study.

The results indicated that the alkaloid compounds separated from the crude methanol extract harmala seed showed an effect on *Staph.aureus* bacteria, as it gave the highest concentration of the alkaloid compounds is 400 mg/cm³ at 31 mm, while the two concentrations recorded 100, 200 mg/cm³ inhibitory diameter by 26,30 mm. *E.coli*, the highest concentrations of the alkaloid compounds were 400 mg/cm³ at a diameter of 30 mm inhibition, as for the rest of the concentrations, they had an inhibitory effect, but with a lower percentage, with an inhibitory diameter of 29, 26 mm for both concentrations, 200 and 100 mg/cm³, while the alkaloid compounds affected *Agro. rhizogenes* and had an effective role in their inhibitory effect and gave the following concentrations 200, 400 100 mg/cm³ with a diameter of 20, 22, 25 mm, respectively, and the highest percentage of inhibitory effect was at a concentration of 100% compared with the standard antibiotics used under study as shown in the table (5) and (6), fig. (2). The results of the researcher's study [20], on the antibacterial effect of harmala extract against the pathogens of urinary tract infection, and the results of the phytochemical examination revealed the presence of flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, and the absence of anthraquinones, while the results of the extract showed The seeds revealed the absence of flavonoids and the presence of alkaloids, saponins, tannins, glycosides, anthraquinones, terpenoids and steroids. The main alkaloids that were discovered are harmin, harmalin, harmalol, and harmol. The results of this study indicate that the alkaloids separated from the crude methanol extract have a beneficial effect. In the treatment of bacteria that cause urinary diseases.

Table 5. Shows the effect of the separated alkaloids from the methanolic extract of the seeds of the harmala plant against the bacteria *Staph.aureus*, *E.coli*, *Agro. rhizogenes* in different concentrations.

Concentration Mg/c ³	Types of Bacteria		
	<i>Staph</i>	<i>E.coli</i>	<i>Agro.</i>
100	26b ±1.5	26b ±0.5	24b ±1.1
200	30a ±2	29a ±1	20c ±1
400	31a ±2.5	30a ±2	21c ±1

Average diameter of the circle using three isolates for each bacterium.

Different letters horizontally mean that there are significant differences at the level of significant difference $\leq 0.01P$.

The diameter of the damping circle is measured in mm.

(--) indicates lack of inhibitory activity.

The numbers after ($\pm$) indicate experimental error.

The effective activity resulting from the seeds of the harmala plant depends on the various chemical compounds present inside the plant under laboratory conditions that mainly affect the physiological and biological properties of the cell, and the solvent succession system has a major role on the separated compounds from the raw extracts. Which prevents the growth and reproduction of bacteria but it also has unique properties that prevent the survival of bacteria effectively, while the alkaloid compounds give their effect as defensive materials because they contain Harmine, Harmalol, which play their defensive role and their inhibitory effect and thus play their role in maintaining human health, as well as in fatty acids. There are many possible reasons that make them powerful antioxidant [8,21]. Different physiological activities appear, and all the compounds extracted from the seeds of harmala lead to disruption of the structure of the lipid bilayer, which is a secondary membrane layer that the fatty acid compounds destroy and then lead to cell death [22,23].

Table 6. Shows the effect of antibiotics against the bacteria andur study.

	Bacteria	NA	AZM	TMP	CFM	AMC	CIP
1	<i>Staph.aureus</i>	14	14	0	0	0	12
2	<i>E. coli</i>	10	10	0	0	12	14
3	<i>Agro. rhizogenes</i>	11	11	12	0	13	10

Bacterial number: 1st tube of McFarland standard solution.

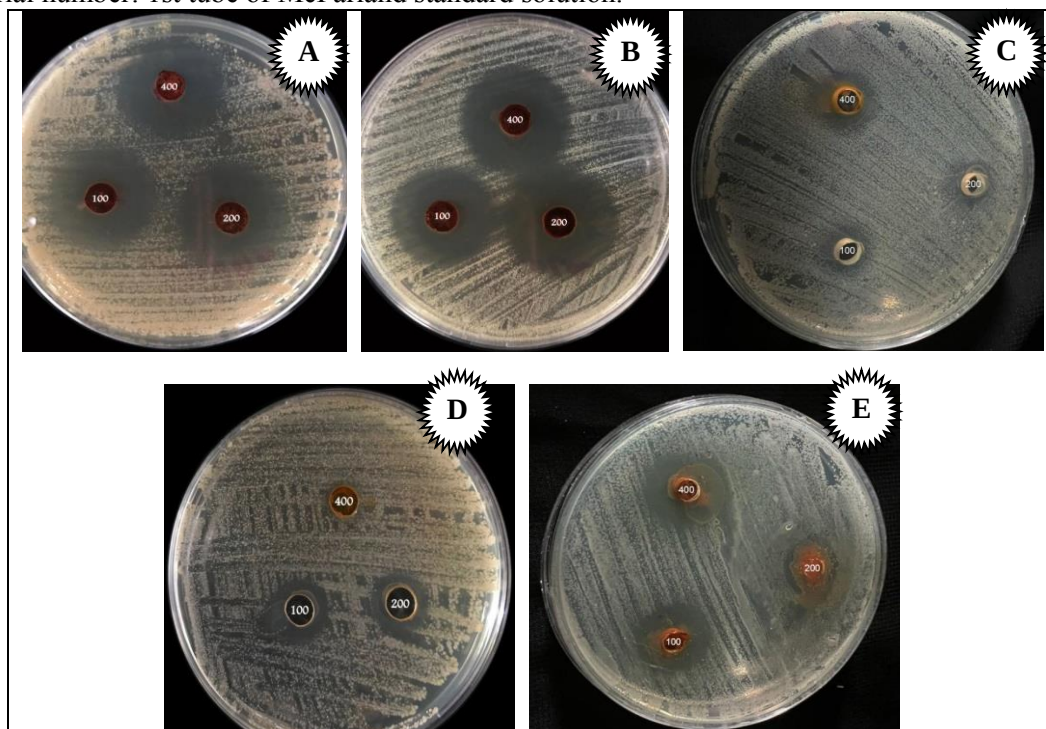


Figure 9. Show the inhibitory effect of the compounds separated from the seeds of *peganum harmala*.

A. Effect of alkaloids separated from the methanolic extract of harmala seeds on *Staph. aureus* in different concentrations (400,200,100), **B.** Effect of alkaloids separated from the methanolic extract of the seeds of the harem plant on *E. coli* at different concentrations (400,200,100), **C.** Effect of alkaloids separated from the methanolic extract of harmala seeds on, *Agro. rhizogenes* in different concentrations (400,200,100), **D.** Effect of petroleum ether extract of harmala plant seeds on *Staph. aureus* in different concentrations (400,200,100), **F.** Effect of petroleum ether extract of harmala plant seeds on, *Agro. rhizogenes* in different concentrations (400,200,100).

References

- [1] Ataee, Z.; Dadpour, B.; Najari, F.; Rahimpour, M. and Najari, D. (2018). Acute Poisoning With *Peganum Harmala*, Esfand: A Rare Case Report. *Int. J. Med. Toxicol. Forensic Med*, 8, 119-121.
- [2] Moloudizargari, M.; Mikaili, P.; Aghajanshakeri, S.; Asghari, M. H. and Shayegh; J.(2013). Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Pharmacognosy reviews*, 7(14), 199.
- [3] Sultan, F.I. (2020). Phytochemical analysis and antibacterial activities of Frankincense of *Boswellia serrate*. *Plant Archives*, 20(2): 5219-5226.
- [4] Al-Daody, A.Ch. (1998). "*Chemical Study on some Iraqi Plants*". Ph.D. Thesis, College of Science, University of Mosul, 112-113.
- [5] Sultan, F.I.; Al-Farha, A.A. and Shaaban, I. (2020A). Separation and identification of some fatty acids and phenolic compounds from *Portulaca oleracea* L. and study their biological effect on two types of pathogenic bacteria. *Asian J. Agric & Biol. AJAB*. 8(3): 1-1.
- [6] AL-Serhan, D.N.A.S. (2021). Separation and identification of a number of natural products in stem shrub of *Tamarix articulata* vahl. and their effect as antioxidants in mosul city. Ph.D.Thesis, college of agriculture and forestry.
- [7] Sultan, F.I.; Khorsheed, A.Ch and Khalel, A.M.S. (2020B). Separation of four fatty acids and two phenolic compounds from *Camellia sinensis* using chromatographic techniques and evaluated their antibacterial activity. *Eur Asian J. Bio. Sci.*, 14(1): 2123-2129.
- [8] Azimi, A., Hashemloian, B., & Majd, H. E. (2008). High in vitro production of anti-canceric indole alkaloids from periwinkle (*Catharanthus roseus*) tissue culture. *African J. of Biotechnology*, 7(16): 2834-2839.
- [9] Chabir, N., Ibrahim, H., Romdhane, M., Valentin, A., Moukarzel, B., Mars, M., Bouajila, J. (2015). Seeds of *Peganum harmala* L. chemical analysis, antimalarial and antioxidant activities, and cytotoxicity against human breast cancer cells. *Medicinal Chemistry*, 11(1), 94-101.
- [10] Lepp, P.W. (2010). General microbiology Laboratory manual, . 2nd ed, BIOL.142:72.
- [11] Rajeshkumar, S. and Malarkodi, C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. *Bioinorganic chemistry and applications*.
- [12] Sultan, F.I. (2018). Chromatographic Separation and Identification of Many Fatty acids and Phenolic Compounds from Flowers of *Celosia cristata* L. and Its Inhibitory Effect on Some Pathogenic Bacteria. *Australian Journal of Basic and Applied Sciences* 12(7): 25-31.
- [13] Al-Dulayymi, F.I.S. (2014). Separation and Identification of Many Natural Products from Some Flowers of Medicinal Plants and Their Biological and Synergistic effects with Isolated Phenazine from *Pseudomonas aeruginosa* Against Some Pathogenic Microorganism. Ph.D. Thesis, College of Education for Pure Science, University of Mosul.
- [14] Adeoye, I.M.O.; Olajuyigbe, O.O. ;Jonathan, S.G.; Coopoosamy, R.M. (2018). Bioactive compounds in ethanol extract of *Lentinus squarrosulus* Mont-a Nigerian medicinal macrofungus. *African Journal of Traditional, Complementary and Alternative Medicines*. 15(2):42-50.
- [15] Sassoui, S.; Seridi, R.; Azin, K. and Usai, M. (2015). Evaluation of phytochemical constituents by GC-MS and antidepressant activity of *Peganum harmala* L. seeds extract. *Asian Pacific Journal of Tropical Disease* 5(12).
- [16] Fahmy, S.A.; Issa, M.Y.; Saleh, B.M.; Meselhy, M.R. and Azzazy, H.M.E. (2021). *Peganum harmala* Alkaloids Self-Assembled Supramolecular Nanocapsules with Enhanced Antioxidant and Cytotoxic Activities. *CrossRef. ACS Omega*, 6, 11954-11963.
- [17] Jassim, S. S. M. H. (2021). antibacterial activity Of alkaloids extract from *peganum harmala* L. seeds against staphylococcus aureus and klebsitlla pneumonia isolated from burns. *Plant Archives*, 21(1), 951-957.

- [18] Sultan, F.I. and Khorsheed, A.Ch. (2018). Investigation of oleanolic acid from flowers of *eugenlia caryophyllus*, using of modern spectroscopy. International Agricultural, Biological & Life Science Conference.
- [19] Prashanth, D.and John, S. (1999). Antibacterial activity of *Peganum harmala*. *Fitoterapia*, 70(4), 438-439.
- [20] Shaheen,H.A.and Issa, M. Y. (2020). In vitro and in vivo activity of *Peganum harmala L.* alkaloids against phytopathogenic bacteria. *Scientia Horticulturae*, 264, 108940.
- [21] Senhaji, S.; Lamchouri, F.; Boulfia, M.; Lachkar, N.; Bouabid, K. and Toufik, H. (2022). Mineral composition, content of phenolic compounds and in vitro antioxidant and antibacterial activities of aqueous and organic extracts of the seeds of *Peganum harmala L.* *South African Journal of Botany*, 147, 697-712.
- [22] Qunfang, W. E. N. G., and Guohua, Z. H. O. N. G. (2005). Bioactivities and physiological effects of extracts of *Peganum harmala* against *Bursaphelenchus xylophilus*. *Scientia Agricultura Sinica*.
- [23] Damej, M.; Skal, S.; Aslam, J.; Zouarhi, M.; Erramli, H.; Alrashdi, A. A.and Lgaz, H. (2022). An environmentally friendly formulation based on *Cannabis Sativa L.* seed oil for corrosion inhibition of E24 steel in HCl medium: Experimental and theoretical study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 128745.