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ANTIBACTERIAL ACTIVITY OF ALKALOIDS EXTRACT FROM *PEGANUM HARMALA* L. SEEDS AGAINST *STAPHYLOCOCCUS AUREUS* AND *KLEBSILLA PNEUMONIA* ISOLATED FROM BURNS.

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ABSTRACT

Medicinal plants are source for a wide variety of natural antioxidants and are used for the treatment of diseases throughout the world. *Peganum harmala* L. widely planted all over Iraq and has different secondary metabolites, such as alkaloids, flavonoids and oils which have been used in treatment of anemia, cancer, fever, diarrhea**,** antibacterial activity and to stimulate the nervous system .The seeds of *harmal* collection from the local markets in Baghdad the alkaloid compounds in the seeds of *P. harmala* was extract than detriment by HPLC The results showed that the extract contains five alkaloids compound (Harmol, Harmalol, Harmane, Harmaline and Harmine). The antibacterial activity assessed against *Staphylococcus aureus* and *Klebsilla Pneumonia* collected from patients with burn infection. The selected bacteria was significant sensitive to the Ciprofloxacin which reached 30 mm for both *S. aureus* and *K. pneumonia*. While antibacterial effect was seen on *Staph. aureus and K. Pneumonia* resistant to the antibiotics it belong to 4,3 respectively from 10 antibiotics, otherwise The best effect of the alkaloids extract on the selected bacteria at 200 mg /ml with inhibition zone are (19, 20 mm) for *S. aureus* and *K. pneumonia,* respectively. The efficacy of extract in terms of minimum inhibitory concentration (MIC) on the concentration was 4 mg /ml for *Staph* and the 8 mg /ml for *Klebsilla* the efficacy of extract in terms of minimum bactericidal concentration (MBC) on the concentration is (8,16) mg /ml to *Staph* and *Klebsilla* respectively.

Keywords: *Peganum harmala* L, Alkaloids, *S. aureus, K. pneumonia*

Introduction

The use of natural products for therapeutic purposes is known since the beginning of civilizations, as the first man knew the use of plants as drugs thousands of years ago, and peoples used plants and herbs according to their primitive cultures with treatments (Naceradska, 2016) where a large part of the world's population, especially in developing countries, benefit from herbal medicines. It is reliable in treating many diseases, in addition to that it is a source of bioactive compounds, and it contributes to the development of new treatment strategies (Firmo *et al*., 2018). With the advancement of industrial organic chemistry, new drugs appeared from industrial sources, and antibiotics appeared as the main therapeutic agents (Wettberg and Khoury, 2020), but these industrial antibiotics showed resistance to some bacterial strains as a result of the random and unexamined uses of these antibiotics, so the use of chemotherapy became no it achieves the required results and has side effects (Juhasz, 2018). Therefore, researchers have tended to find new and alternative therapeutic methods by using natural sources such as medicinal herbs that contain many chemical compounds with high effectiveness in treating many diseases as well as being non-toxic to cells. Natural. (Gao, 2016) *Peganum harmala* L. is one of the medicinally important plants, belonging to the family Zygophyllaceae, a herbaceous plant widely spread in North Africa and the Middle East (Kulkarni, 2017), As the Harmal plant has been known since ancient times for its medicinal importance, its seeds have been used to reduce heat and as a commercial menstrual stimulant medicine (Bellantuono, 2018) and used as a stimulant for the central nervous system (Saganuwan, 2017). The seeds were also used in the treatment of cancer, as the extracts of the seeds of this plant showed great effectiveness against various malignant tumors, whether in vivo or in vitro (Raghavan, 2017) *P. harmala* possesses an anti-microorganism activity due to its high concentration of alkaloids (Moradi *et al.*, 2017) which have biological activity in addition to contain many substances, such as fatty oils, fibers, and some flavonoids, (Ahmad and Pathak, 2016) The reason for treating for many diseases is due to the presence alkaloids of Harmol, Harmalol, Harmane, Harmaline and Harmine. (Pavel *et al*., 2017) in view of the lack of studies available in Iraq on isolating the group of alkaloid compounds from the seed to be recommended as a drug, an alternative to antibiotics in inhibiting *K. pneumonia* and *S. aureus* bacteria, isolated from burn injuries in hospitals. In which patients lie in burn departments, causing infections that may expose a person to losing his life.

Materials and Methods

Plant material

The seeds of the *Peganum harmala* L. were obtained from the local markets in Baghdad - Iraq. A sample was classified by Biology Department, College of Science, University of Baghdad.

Alkaloids extraction and HPLC

Taking 1 g from the seeds of *P. harmala* L., the seeds were ground and soaked three times with 25 ml of methanol for an hour. Then filtered and evaporated the extract with a rotary evaporator device under pressure and a temperature of 45°C, the remaining after that was treated with 25 ml of hydrochloric acid solution 2% volume to volume (v/v). The petroleum ether was filtered three times with 10 ml to remove fats, dyes and impurities. The acid aqueous layer was then amended with ammonia to reach pH 10 and were extracted three times with 25 ml of chloroform. The organic solvents were then evaporated and the total alkaloids extract was obtained, and the remaining obtained was dissolved in 10 ml of methanol, and then filtered through a filter of 0.45 µ ml of polypropylene and 20 µl was injected into an High Performance Liquid Chromatography (HPLC) column under ideal separation conditions compared to the original stand. After that, a comparison was determined between the standard extract area with the sample area under the same separation conditions (Esfahani *et al*., 2008; Frye and Haustein, 2007) for calculating the concentration of a sample as shown in the following formula:

Concentration of sample (µg/ml) = [Area of the sample/Area of the standard] × Standard Conc. × Dilution factor.

Antibacterial assays

Microorganism isolates

Staphylococcus aureus and *Klebsilla pneumonia* had Been obtained from burn patients, the isolates were preserved by culture on the surface of the slanted nutrient medium, incubated at 37 °C for 24 hours and kept at 4 °C until use, the two isolates were diagnosed with VITEK-2 System.

Preparation of microbiology stuck (McFarland)

The microorganisms were prepared by taking 2-4 colonies of the microorganisms under study and placed in sterile physiological saline at a concentration of 0.85%. It was compared to a McFarland tube No. 0.5 containing 1.5 x 108 cells/mL, and it was used in the sensitivity test and the inhibitory activity test of the alkaloid of *P. harmala*

Antibiotic sensitivity test

Kirby-Bauer method was used to perform an antibiotic susceptibility test for 10 different antibiotics (Azithromycin, Amoxicillin, Cefotaxime, Gentamycin, Ceftazidme, Tetracycline, Cefepime, Erythromycin, Ciprofloxacin and Doxycycline) as described by WHO (2003). The bacterial suspension was prepared by selecting 1-2 isolated colonies of bacteria from the original culture and inserting them into a test tube containing 4 ml of normal saline to produce a bacterial suspension of moderate turbidity compared to McFarland tube, this is approximately equal to 1.5×10^8 CFU/ml. A portion of the bacterial suspension was carefully transferred and spread with a sterile cotton swab over Mueller-Hinton agar, then left for 10 minutes. The antibiotics were placed on the agar with sterile forceps pressed firmly to ensure contact with the agar. After that the petri dishes were inverted and incubated at 37 °C for 18-24 hours. The areas of inhibition zones developed around the discs were measured in millimeters (mm) by using the metric ruler according to the Medical Laboratory Standards Institute (CLSI, 2018).

Agar well diffusion for the alkaloid of *P. harmala* to some microorganisms

The Agar well diffusion method was used to observe the effect of the alkaloid extract of the *P. harmala* on the growth of microorganisms by pouring 20 ml of the fed media to each Petridish. The plate was incubated after being cooled in the incubator for 24 hours at a temperature of 37 °C to ensure non-contamination. Inoculating the medium with (0.1) mL of previously prepared microbiological suspension containing (1.5 x 108 cells / mL) spread evenly on the surface of the culture media using a sterile swap, a drill was made on the surface of the culture media by the cork borer The extracts concentrations were (200, 100, 50, 25 mg /ml) prepared by dissolving (0.2, 0.1, 0.05 and 0.025 gm) in water and the volume was supplemented to 1 ml of water and 10% dimethyl sulfoxide (DMSO), in the potency test. Inhibition against the microorganisms under study, with an amount of 0.1 ml /well, the plates were incubated at a temperature of 37 °C for 24 hours. The effectiveness of the extract was determined by measuring the diameter of the inhibition area around each well in millimeters (Valgas *et al*., 2007).

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the for the alkaloid of *P. harmala*

A series dilution of the alkaloid extract with a value (2, 4, 8, 16, 32, 64, 128 mg / ml) was prepared in Mueller Hinton broth, as (MIC) was determined by dilution method and then (MBC) was determined by taking 0.1 ml of media in tubes. And spread it on the surface of Mueller Hinton Agar by a sterile glass diffuser (Spreader). The plates were incubated at 37 °C for 24 hours, and the results were recorded on the basis of the presence of growth (+) with the least number of CFU colonies or lack there of (-) and the results were compared with the positive control tubes and negative. While the MBC value is known as the lowest concentration of the antimatter that reduces the number of colonies by 99.9% of the original culture or its absence (-) (Wan *et al*., 1998).

Statistical Analysis

The Statistical Analysis System SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study.

Results and Discussion

HPLC estimate to the alkaloid compounds

Group of alkaloids had Been extracted and five alkaloid compounds were detected, Harmol, Harmalol, Harmane, Harmaline and Harmine in the alcoholic extract of the seeds of *p. harmala* by using High Performance Liquid Chromatography (HPLC) and the alkaloids are among the main compounds in amounts up to 10% by weight of dry seeds (2003 *et al*. Kartal) .It is noted from Table (1) and both figures (1) (2) the types and quantities of alkaloid active substances in the seeds of *p. harmala* as the HPLC device recorded the active substances studied and the amount of harmine compound was the highest value amounted to 458.624 µg per 1 gram of seeds compared to harmaline, which recorded 393.676 µg l /1g. With the rest of the other compounds shown in the same table.

Table 1 : Estimated production of alkaloid compounds from *p. harmala* seeds.

Al keloid compounds (µg)	Per 1 gram Seed of *P. harmala*
harmol	75.639
harmalol	80.410
harmane	114.311
harmaline	393.676
harmine	458.624

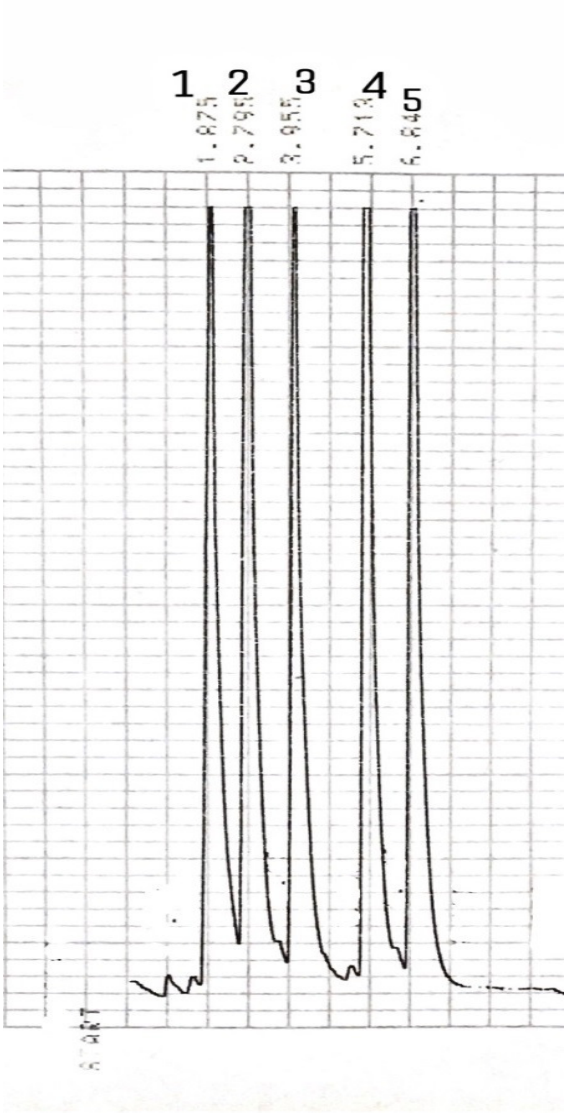

Fig. 1 : Standard curve of alkaloid compounds in seeds of *P. harmala*.

Active compound	Seq. 0f compound	Retention Time (minute)	Area
harmol	1	1.875	248690
harmalol	2	2.795	245458
harmane	3	3.955	224419
harmaline	4	5.713	224418
harmine	5	6.84	212500

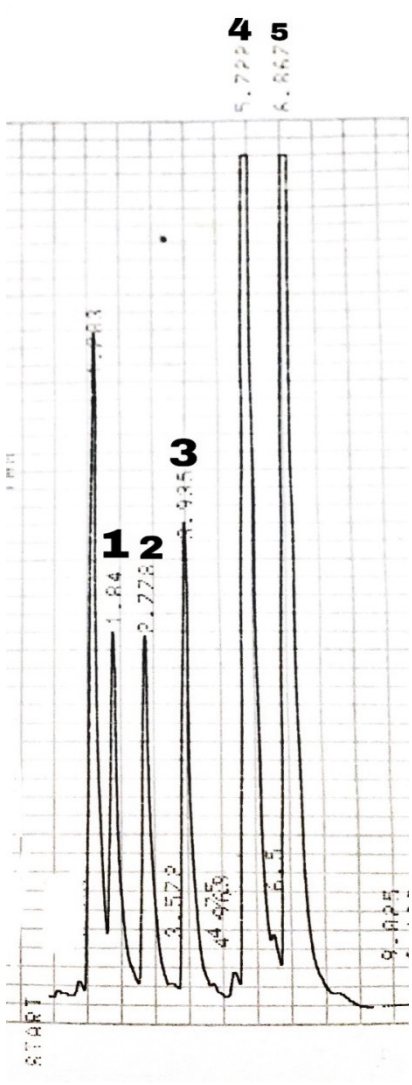

Fig. 2 : Amount of alkaloid compounds in seeds of *P. harmala*.

Active compound	Seq. 0f compound	Retention Time (minute)	Area
harmol	1	1.845	62702
harmalol	2	2.778	65791
harmane	3	3.935	85512
harmaline	4	5.722	294493
harmine	5	6.867	324859

Moloudizargari *et al*. (2013), when studying the chemical composition of the plant, concluded that one of the most important and most quantitative compounds in the *P. harmala* are the beta-carboline alkaloids such as harmalol, harmaline and harmine after performing the HPLC test and in 2014, Tehrani *et al* was able to identify two alkaloid compounds harmaline and harmine 4.3% and 5.6% respectively in *P. harmala* seed extract when performing an HPLC assay. Elgubbi *et al*. (2017), found after conducting a thin layer paper and chromatography examination on haramel seed extract, that it contained alkaloid substances with a high percentage such as harmine. In addition to Iranshahy *et al*. (2019) when studying the alcoholic extracts of the flowers, fruits and seeds of the *P. harmala*, they contain alkaloid compound: harmine in the extract prepared from flowers and

harmaline in the extract prepared from the fruits and seeds in high concentrations. our study are similar to the findings of the above researchers with different quantities and concentrations of compounds due to different environmental conditions, climatic conditions and soil type when planting.

Bacterial activity

Antibiotic Sensitivity test

The sensitivity to antibiotics was tested by Kirby Bauer using Mueller - Hinton agar medium. The sensitivity of the two isolates was tested for ten antibiotics and the effect of antibiotics was significant. As result displayed in Table (2) and Fig. (3) and (4) showed that *S. aureus* bacteria were significantly sensitive to both Ceftazidme and Ciprofloxacin compared to the rest of the antibiotics, as it recorded 23,30 mm, respectively, while the bacteria were resistant to Tetracycline, Azithromycin, Amoxicillin and Erythromycin, While both the antibiotics Ciprofloxacin and Cefotaxime caused the highest inhibition towards *K. Pneumonia* bacteria, it was 30,28 mm, respectively, while *K. Pneumonia* showed. Resistant to Tetracycline, Amoxicillin and Erythromycin.

Table 2 : The sensitivity of bacteria to antibiotics.

Antibiotic	*S. aureus*	*K. Pneumonia*	L.S.D. 0.05
Gentamicin	S 18	S 16	3.27 NS
Tetracycline	R 0	R 0	0.00 NS
Cefotaxime	I 22	S 28	4.67 *
Azithromycin	R14	S 14	2.56 NS
Amoxicillin	R 0	R 0	0.00 NS
Ciprofloxacin	S 30	S 30	3.18 NS
Doxycycline	S 17	S 17	2.52 NS
Ceftazidme	S 23	S 20	3.46 NS
Cefepine	I 17	S 18	2.06 NS
Erythromycin	R 0	R 0	0.00 NS
L.S.D. 0.05	5.96 *	6.79 *	---

(S) Sensitive, (I) Intermediate, (R) Resistant

It is evident through the results that there is a variation in the sensitivity and resistance of bacterial isolates to antibiotics, and this may be due to the fact that some of these antibiotics have been in common use for a long time, which led to the resistant strains, and the manufacture of new specialized anti-drugs that work on sensitive sites in the bacteria Raising the resistance of bacteria to them is unlikely, so the bacteria are sensitive to such antibiotics (WHO, 2003).

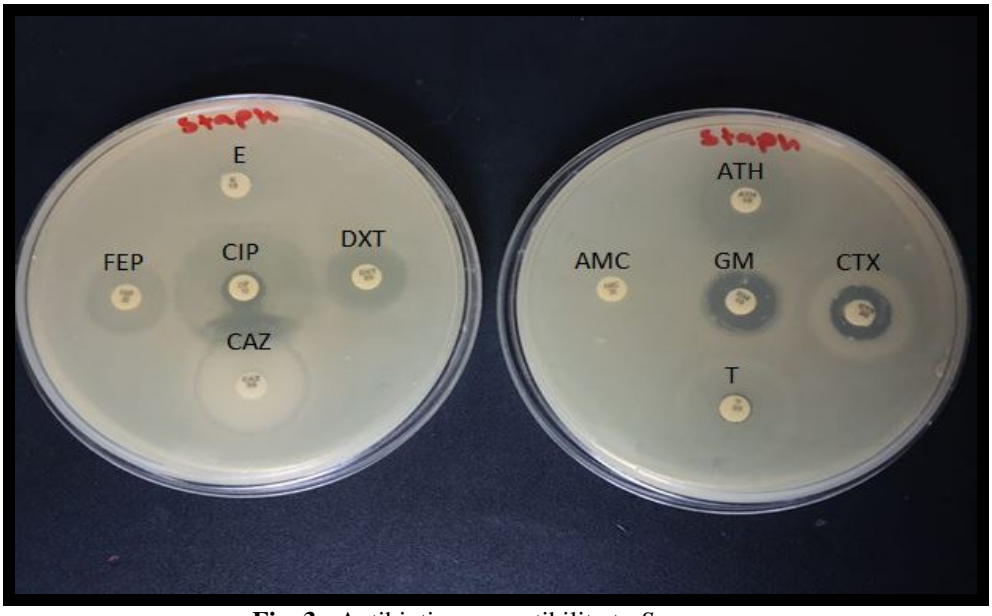

Fig. 3 : Antibiotics susceptibility to *S. aureus*

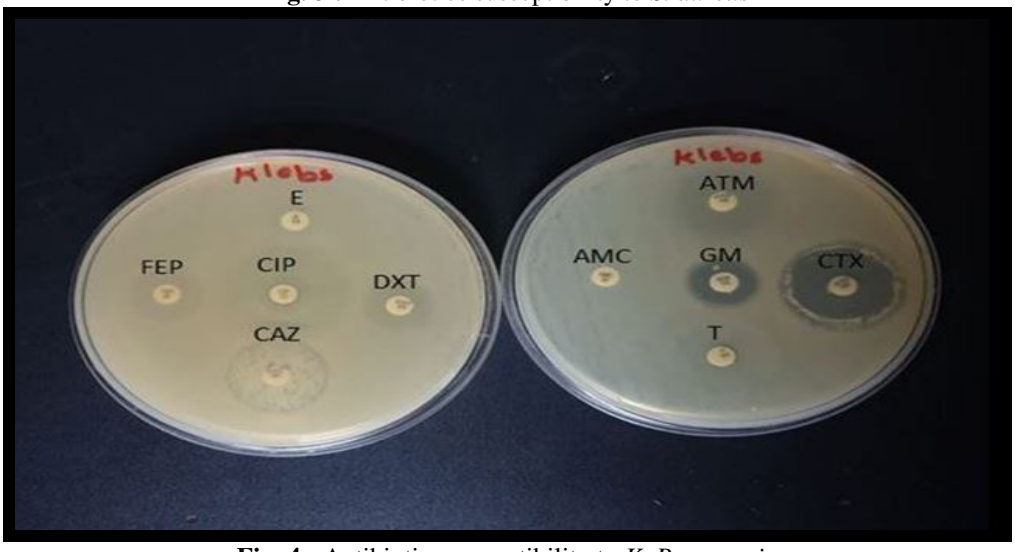

Fig. 4 : Antibiotics susceptibility to *K. Pneumonia*

Well-diffusion method

The results in table (3) indicates that there was a significant gradual increase in the rate of inhibition diameters of the two isolates with increase in the concentration of the Alkaloid extract, as shown in both Fig.(5) and (6). The highest rate of inhibition zone to the Alkaloid extract of *P. harmala*. Seed was recorded at a concentration of 200 mg / ml, as it was (19 and 20 mm) for *S. aureus* and *K. Pneumonia*, respectively, significantly compared to the rest of the other treatments, while the lowest inhibition of the extract was at a concentration 25 mg / ml. The diameter of the inhibition zone was 9 mm for *K. Pneumonia*, whereas *S. aureus* did not show any sensitivity to the concentration of 25 mg / ml.

Table 3 : The Inhibitory activity of alkaloid extract of *P. harmala*. seeds on bacteria.

Conc.	Isolates		L.S.D. 0.05
	S. aureus	*K. Pneumonia*	
0 Control	0	0	0.00 NS
25	0	9	1.603 *
50	11	11	3.584 NS
100	15	16	2.267 NS
200	19	20	2.267 NS
L.S.D 0.05	1.992 *	1.627 *	---

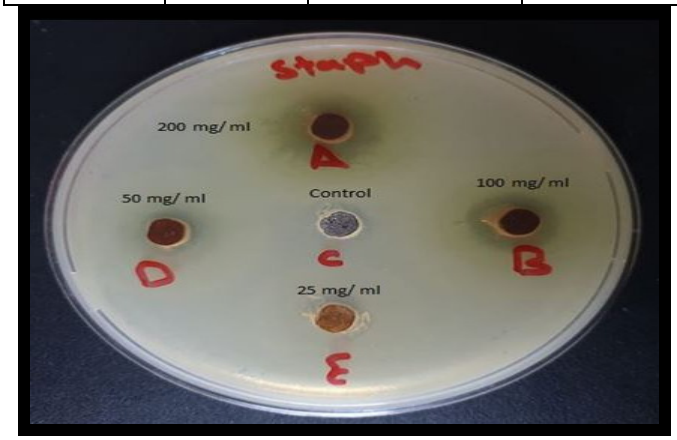

Fig. 5 : Efficacy of the Alkaloid extract against S. aureus

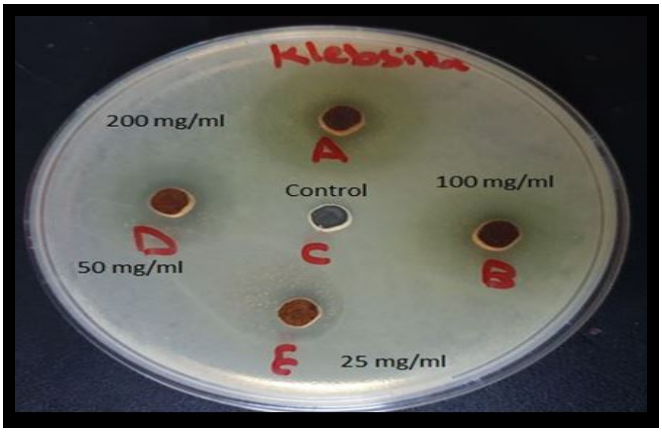

Fig. 6 : Efficacy of the Alkaloid extract against *K. Pneumonia*

When comparing our results with previous studies, we note that they agree with the findings of Benbott *et al*., (2012) where they observed the effect of alkaloid substances present in the seeds on Gram-negative bacteria such *as E. coli, K. pneumoniae, P. aeruginosa, Proteus. mirabilis and Serratia spp*. in addition to Gram positive bacteria such as *S. aureus and S. saprophyticus*, where the inhibitory diameters ranged from 11-22 mm. In 2016, Khorsandi and Moghanian concluded that the alkaloid compound Harmalin found in the *P. harmala* is one of the compounds that have the most anti-bacterial effect of *Staphylococcus aureus*. *Pseudomonas aeruginosa, Acinetobacter*. Abdulridha *et al*., 2019 confirmed that the anti-bacterial efficacy of the *P. harmala* extract is due to the presence of alkaloids in a high percentage in the extract as it had good effect against *Staphylococcus, Aeromonas, E.coli and Acinetobacter*. Perhaps the reason for this is that alkaloid substances have a broad-spectrum and effective effect in inhibiting or killing bacterial isolates, even at low concentrations, and this is a clear indication that the seed extract of the *P. harmala* includes alkaloids that have the anti-bacterial activity, as there is a nitrogen atom in its chemical composition For alkaloids, which have the ability to acquire a proton, in addition to amine groups that have the ability to gain or lose a hydrogen atom, which is due to the process of losing or gaining a proton (Sobhani *et al*., 2002). The alkaloids are more lethal than bacteriostatic, as they kill all bacteria by 99.99% within 1-2 hours (Alhanout *et al*., 2010). The alkaloids generally inhibit the enzyme dehaydrofolate reductase, which leads to inhibition of nucleic acid synthesis (Rao *et al*., 2000). The effectiveness of alkaloids that kill bacteria varies Depending on their types, for example, B-carboline alkaloids such as Harmine work to program the cell to die by changing or disrupting DNA division, altering the activity of mitochondria, inhibiting protein synthesis, disrupting the formation of microtubules, and disrupting the perfusion of bacterial cell membranes (Rosenkranz *et al*., 2008) and thus these are good properties in limiting proliferation. Human pathogen microorganisms.

Determination of (MIC) and (MBC)

The Broth dilution method was followed to find out the value of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against the selected bacteria. The results in Table (4) showed that MIC and MBC of *S. aureu*s were (4, 8) mg / ml respectively Figure (7) while the concentration of MIC and MB were (8,16) mg / mL for *K. Pneumonia* respectively Fig. (8).

Table 4 : The results of (MIC) and (MBC) for Alkaloid extract of *P. harmala*. seeds

Concentrations of Alkaloid extract mg/ mL							Isolates
2	4	8	16	32	64	128	
-	MIC	MBC	-	-	-	-	*S. aureus*
-	-	MIC	MBC	-	-	-	*K. Pneumonia*

The results were in agreement with the findings of Goudarzi and Azimi in 2017 that the best value (MIC) of *P. harmala* extract when the concentration (12.5 mg / mL) against *S. areus* bacteria. also Jasim (2018) found that the best value (MIC) of the *P. harmala* extract was at concentration (5 mg/mL) against *S. aureus* and *E. coli.*

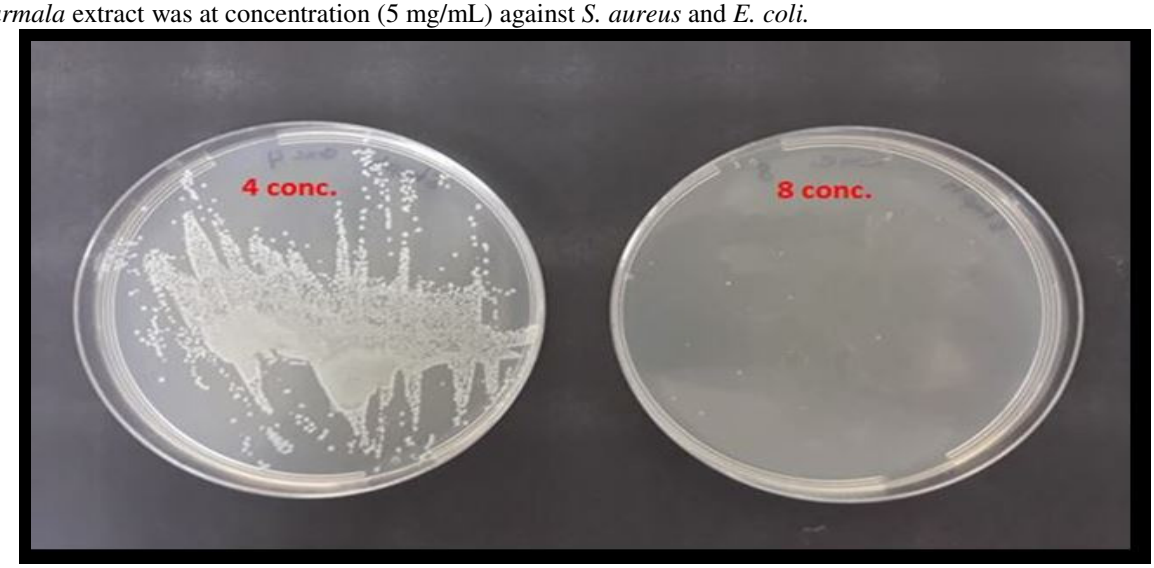

Fig. 7 : MIC and MBC ratio of *S. aureus*

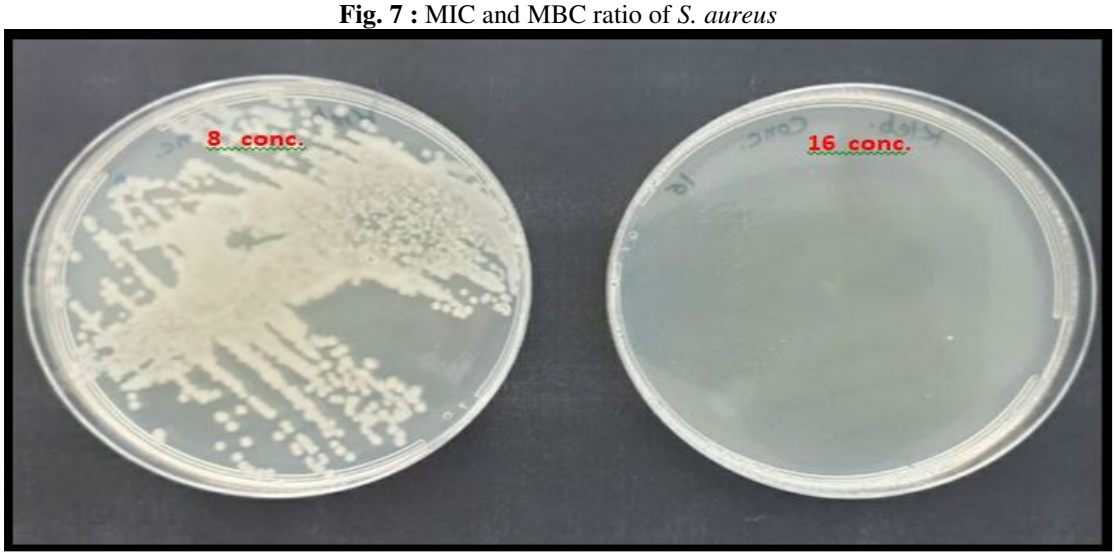

Fig. 8 : MIC and MBC ratio of *K. Pneumonia*

Conclusion

In the present work, the agar diffusion test showed that alkaloid extract of *P. harmala*. Seeds have high antibacterial property and have the potential to inhibit multi drug resistance strains against gram positive and negative bacteria.

References

Abdulridha, M.M.; Abdulhussein, H.S.; Alyaseen, F.F. and Hassan, B.A. (2019). Phytochemical and Antibacterial Activity of the *Peganum harmala* Seeds and its Alkaloids. Plant Archives, 19 (1): 1439-1444.

Ahmad, L. and Pathak, N. (2016). Antifungal Potential of Plant Extracts against Seed-borne Fungi Isolated from *Barley* Seeds *(Hordeum vulgare L.).* Journal of Plant Pathology and Microbiology, 7(5): 1- 4.

Alhanout, K.; Malesinki, S.; Vidal, N.; Peyrot, V.; Rolain, J.M. and Brunel, J.M. (2010). New insights into the antibacterial mechanism of action of squalamine. Journal of Antimicrobial Chemotherapy, 65(8): 1688–1693.

Bellantuono, I. (2018). Find drugs that delay many diseases of old age. *Nature*, 554(7692): 293-295.

Benbott, A.; Yahyia, A. and Belaidi, A. (2012). Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant *Peganum harmala L. Journal of Natural Product and Plant Resources*, 2 (5):568- 573.

Clinical Laboratory Standards Institute (CLSI) (2018). Performance Standards for Antimicrobial Susceptibility Testing; CLSI Supplement, CLSI M100-28thed., Clinical and Laboratory Standards Institute Wayne, PA, U.S.A.

Diba, K.; Shoar, G.M.; Shabatkhori, M. and Khorshivand, Z. (2011). Anti-fungal activity of alcoholic extract of *Peganum harmala* seeds. Journal of Medicinal Plants Research, 5(23): 5550-5554.

Elgubbi, H.; Haith, G.S.G. and Balto, M. (2017). Harmine Isolation and Purification by a New Design of Silica Gel Column Chromatography. EC Nutrition, 10(2): 51-56.

Firmo, W.; Menezes, V.; Passos, C.; Dias, C.; Alves, L. and Dias, I. (2018). Historical context, popular use and scientific conception on medicinal plants. *Cadernode Pesquisa,* 18:90-95.

Frye, A. and Haustein, C. (2007). Extraction, Identification, and Quantification of *Harmala* Alkaloids in Three Species of Passiflora. American Journal of Undergraduate Research, 6: (3) 19-26.

Gao, S. (2016). Medicinal Compounds form Traditional Herbs. Natural Products Chemistry and Research, 4(4). 117

Goudarzi, M. and Azimi, H. (2017). Antimicrobial Activity of *Peganum harmala* Against Methicillin-Resistant *Staphylococcus aureus* Strains and Assessment of Its Cytotoxicity Effect on HEK-293 Cells. International Journal of Infection, 4(4):.2-5

Iranshahy, M.; Bazzaz S.F.; Haririzadeh, G.; Abootorabi, B.Z.; Mohamadi, A.M. and Khashyarmanesh, Z. (2019). Chemical composition and antibacterial properties of *Peganum harmala L.*. Avicenna Journal of Phytomedicine ., 9(6): 530-537.

Jasim, T.M. (2018). Antibacterial, muscle relaxant, and hypnotic effects of seeds of *Peganum harmala* on mice. African Journal of Microbiology Research, 13(21):353-356.

Juhasz, K. (2018). Pollution for cleanliness Lessons from an ethnographic research on the rural use of water for hygienic purposes. "*Historia Provinciae* – The Journal of Regional History" (Eng.), 2(1): 29-54.

Kartal, M.; Altun, M.L. and Kurucu, S. (2003). HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of *Peganum harmala* L. *Journal of Pharmcological and Biomedical Analysis*, 31: 263-269.

Khorsandi, D. and Moghanian, A. (2016). Personalized Medicine: Regulation of Genes in Human Skin. Ageing Journal of Allergy Therapy, 7(6): 2-9.

Kulkarni, P. (2017). Phytoestrogens Medicinal Herbs - Safe and Effective Alternative to Hormone Replacement Therapy in Menopausal Syndrome. Journal of Research in Traditional Medicine, 2(5): 147-150.

Moloudizargari, M.; Mikaili, P.; Aghajanshakeri, S.; Asghari, M.H. and Shayegh, J. (2013). Pharmacological and therapeutic effects of *Peganum harmala* and its main alkaloids. *Journal of Phcognosy Reviews*, 7(14): 199-212.

Moradi, M.T.; Karimi, A.; Rafieian-Kopaei, M. and Fotouhi, F. (2017). *In-vitro* antiviral effects of *Peganum harmala* seed extract and its total alkaloids against Influenza virus. *Microbial Pathogenesis*, 110:42–49.

Naceradska, M. (2016). Use of herbs in veterinary practice. *Medicinal and AromaticPlants*, 5(5): 27.

Pavel, P.; Premysl, S.; Katerina, P.; Jaroslav, S. And Jan, V. (2017). Effects of biologically active substances used in soybean seed treatment on oil, protein and fibre content of harvested seeds. Plant, Soil and Environment, 63(12): 564-568.

Raghavan, P.R. (2017). Metadichol and infectious diseases: One processes many diseases, many possible cures. *Virology and Mycology*, 6(2): 18.

Rao, K.N. and Venkatachalam, S.R. (2000). Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the *in-vitro* cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. *Toxicol In-Vitro,* 14:53–59.

Esfahani, H.R.M.; Faramarzi, M.A.; Mortezaee, V.; Amini, M. and Rouini, M.R. (2008). Determination of Harmine and Harmaline in *Peganum harmala* Seeds by High-Performance Liquid Chromatography. Journal of Applied Sciences, 8(9): 1761-1765.

Rosenkranz, V. and Wink, M. (2008). Alkaloids induce programmed cell death in bloodstream forms of trypanosomes [Trypanosoma b. brucei]. Molecules, 13(10): 2462-2473.

Saganuwan, S. (2017). Piroxicam: Source for Synthesis of Central Nervous System (CNS) Acting Drugs. *Central Nervous System Agents in Medicinal Chemistry,* 17(2):135-140.

SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sobhani, A.M.; Ebrahimi, S.A. and Mahmoudian, M. (2002). An *in-vitro* evaluation of human DNA topoisomerase I inhibition by *Peganum harmala L.* seeds extract and its beta-carboline alkaloids. Journal of Pharmacy and Pharmaceutical Sciences, 5(1):19–23.

Tehrani, S.S.H.; Shabani, S.H.S.; Enferadi, S.T. and Rabiei, Z. (2014). Growth Inhibitory Impact of *Peganum harmala L.* on Two Breast Cancer Cell Lines. Iran Journal of Biotechnology, 12(1): 9-14.

Valgas, C.; de Souza, S.M.; Smania, E.F.A. and Smania, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, 38: 369–380.

Wan, J.; Wilcock, A. and Coventry, M.J. (1998). The effect of essential oil of Basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. Journal of Applied Microbiology, 84 (2):152-158.

Wettberg, E. and Khoury, C. (2020). Access to crop digital information and the sharing of benefits derived from its use: Background and perspectives. *Plants, People, Planet,* 2(3): 178-180.

World Health Organization (WHO) (2003). Basic laboratory procedures in clinical bacteriology. 2nd ed. Geneva, Switzerland.