Evaluation of Antimicrobial Activity of Aqueous and Methanolic Extracts of *Peganum harmala* against Reference and multidrug resistance Clinical Isolates organisms

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ABSTRACT

Background: Down the ages, extracts of plants have evoked interest as sources of natural products as alternative remedies for treating many infectious diseases. Due to the emergence of resistance to antimicrobial drugs. This issue has prompted the need for effective and efficient of herbal treatment. **Objectives:** To assess the antimicrobial activity of *Peganum harmala* against certain type of pathogenic microorganism. **Materials and Methods:** This is an experimental in*vitro* study was conducted on ten organisms. The coarsely seeds powdered 200g of *Peganum harmala*was exhaustively extracted with 80% Methanol and water. Antibacterial activity of the extracts were tested using the cup-plate agar diffusion method (25). The broth microdilution method was used to determine the Minimum inhibition concentration (MIC) and Minimum bactericidal concentration (MBC). **Results:** The extracts of *Peganum harmala* have broad antimicrobial activity against most of the organisms, with high methanolic extract activity compared with water extracts, it was showed thatall tested microorganisms were sensitive to the Methanolic extract at the concentration of 100 mg/ml(Zone of inhibition >18 mm), with high antimicrobial activity against *S. aureus* ATCC 6538, *E.coli* ATCC 8739 and *Proteus mirabilis* under all concentrations, The highest inhibition zone reached 32 mm to ward *E. coli* ATCC 8739 at concentration 100 mg/ml and smallest inhibition zone zero mm toward *Pseudomonas aeruginosa* ATCC 9027 at concentration 12.5 mg/ml. The Minimum inhibition concentration (MIC) revealed high value was 1.25 in methanolic extract against (*Ps. aeruginosa*ATCC 9027, *C. albicans* ATCC 10231,*Proteus mirabilis).*

keywords: Peganum harmala, MDR, ESBL, MBC, MIC.

INTRODUCTION :

Bacterial contaminations are around the world, this present circumstance is more basic in the underdeveloped nations where much of the time absence of satisfactory disinfection and essential medical care programs makes it troublesome and costly to battle sicknesses of bacterial beginning, along these lines, a few underdeveloped nations are starting to understand that logical assessment of conventional medication might enhance current clinical benefits. The higher plants are chosen based on writing information and data provided by customary healers, the technique for extraction of the plant and testing is fundamental assuming that tantamount outcomes are to be gotten. Various higher plants have been utilized for nations as solutions for human infections. This has urged the researchers to evaluate higher plantfor different natural exercises including antibacterial and antifungal effects(1)

As a matter of fact, it can accurately be contended that the improvement of current drugs depends on the old information on restorative plants and customary clinical practice,thus around 40% of drugs are gotten from regular sources (plants, creatures, microbes and growths) in addition a few normal items got from therapeutic plant prompted the advancement of different drug analogs or derivatives(2). In Africa and the non-industrial nations, conventional prescriptions from plants kept on framing the premise of provincial clinical consideration this is because of the way that these regular meds are effectively accessible and cheap(3). Be that as it may, the utilization of such meds in their unrefined structures without laying out deductively their viability and security could in brief time or long run, be adverse to very strength of humankind in this way, there is a pressing need to complete efficient logical assessment of these meds to check their guaranteed natural action and to disconnect and distinguish the bioactive agents(4). One of these normal medications

Peganum harmala Wild Syrian regret otherwise called Harmal, "Espand" in Iran,"Harmel" in North Africa and "African regret," "Mexican mourn" or "Turkish lament" in the US which is a restoratively significant glabrous plant and enduring spice of family Zygophyllaceae(5) . Different pieces of this plant including, its seeds, bark, and root have been utilized as people medication. Late long stretches of exploration have exhibited different pharmacological and remedial impacts of *Peganumharmala* and its dynamic alkaloids, particularly harmine and harmaline. Insightful examinations on the substance arrangement of the plant show that the main constituents of this plant are beta‑carboline alkaloids, for example, harmalol, harmaline, and harmine. Harmine is the most contemplated among these normally happening alkaloids. There are a few reports which demonstrate the incredible assortment of

pharmacological and natural exercises of *Peganumharmala*, for example, angiogenic inhibitor and to treat hypertension and cardiovascular sickness likewise has bactericidal movement, anticancer,against protozoal, antibacterial, antifungal, calming properties (6)

Traditional medicine and superstition: -

has been customarily used to treat diabetes in people medication of certain pieces of the world(7). likewise, to treat colic in man and animals (8). most often used to treat hypertension and cardiovascular infection around the world, it has been utilized as pain relieving, emmenagogue, and abortifacient agent(5). In Moroccan seeds of *Peganum harmala* were utilized as powder, decoction, maceration or mixture for fever, loose bowels, early termination and subcutaneous cancers and is broadly utilized as a solution for distressed occasions (rheumatic torment, excruciating joint and digestive pain(9). it is significant therapeutic plant in Iranian customary medication, which has different advantages, for example, hostile to contamination, against irritation, hostile to cancer and hostile to parasite(10).

Botanical description: -

grow up to 30-100 cms in level. the leaves are palmatisected into 3-5 liner curves which are 3-6 cms in length and 1.5-3.0 mm wide blossoms emerge by 1-3 on peaks of branches which bear whitish - yellow petals in variety. The organic products are globular case with 3 chamber ,0.9-1.3 cm in width and containing 35-45 rakish blackish seeds,the plant has unpleasant taste so generally not brushed by animals(11).

Pharmacological uses: -

Is notable and compelling restorative plant in Turkey, Iran and China, particularly in Xinjiang and Mongolia (12) The seed and the entire plant gangs therapeutic properties (Uighur Medication Standard of the Service of General Wellbeing) and different reports propose that the plant can be utilized to treat illness like stiffness ,hypertension , diabetes , asthma and jaundice additionally groups stimulating and hypothermic properties and are utilized as a clinical cure, incense, fixing with necrotic ,narcotic , love potion , energizer and emetic properties and it is one of the most often utilized restorative plants to treat hypertension and cardiovascular sickness worldwide(13). It has additionally been displayed in different pharmacological examinations that P. harmala separate or its vitally dynamic alkaloids, harmine, harmaline, Harman and harmalol, have different cardiovascular impacts, for example, bradycardia, diminishing fundamental blood vessel circulatory strain and absolute

fringe vascular opposition, expanding beat pressure, top aortic stream and heart contractile force(5). antitumor and pain-relieving impacts, antimicrobial properties, and solid inhibitors of monoamine oxidase (14). It was uncovered in a review that harmine is a strong angiogenic inhibitor This substance can fundamentally diminish the multiplication of vascular endothelial cells and lessen articulation of various pro‑ angiogenic factors, for example, vascular endothelial development factor(15).

Toxicity: -

While this plant has customarily been utilized in Bedouin medication as an emmenagogue and as abortifacient specialist and to treat hypertension and cardiovascular sickness around the world, The excess ingestion of *Peganum harmala* can cause human poisonous impacts and condition, and a few instances of harmfulness have been as of now detailed. It causes cerebral pain, dazedness, queasiness seizure, fantasy, loss of motion, happiness, stomach related issues, bronchodilator, hypothermia and bradycardia, Numerous pharmacological investigations. Recommend a cell reinforcement and free revolutionary rummaging impact of *Peganum harmala* (16).

PHYTOCHEMISTRY: -

Several alkaloids have been isolated from Peganum harmala, including - carbolines such as harmine, harmaline (identical to harmidine), harmalol, and harman, and quinazoline derivatives such as vasicine and vasicinone (20).The total alkaloid content ranges from 2 to 5%.Alkaloids are most abundant in seeds and roots, with low levels in stems and leaves and none in flowers.The roots contain 2.0 and 1.4% (w/w) harmine and harmol, respectively (18).Harmaline (harmidine) (harmidine).C13H15ON2 was first isolated from the plant's seeds and roots, and it is the plant's main alkaloid (1).Is nearly twice as toxic as harmine, causing tremors and clonic convulsions in moderate doses but no increase in spinal reflex excitability (21)Harmine (banisterine) (banisterine).C13H12ON2 is found in *Peganum.harmonium* and and in some species of *Banisteia*, viz., *B.caapi, Spruce., B. lutea* and *B. metallicolor*,the alkaloidis optically inactive is pharmacologically resembles harmaline in its action, but is less toxic (22)Harmalol.C12H12ON2 - Which occurs in *Peganumharmala* crystallizes from water as the trihydrate.Harman.C12H10N2. This alkaloid is inactive.Vasicinone the potential quinazoline alkaloids and were first discovered in flowers and stems , It is an active bronchodilator ,The authors reported that these alkaloids were mainly investigated in seeds by using different methods (%yield, retention factor values, melting point , Ultraviolet and

infrared spectra)for their identification and isolation(17**)**. Confirmed harmaline, harmine, harmol and tetrahydroharmine and quantified as the mainalkaloids in *Peganumharmala* extracts(18).

Materials and methods

Ethical approval:

This study was approved by ethical committee of the Sudan International University (SIU), Facultymedical Laboratory Sciences.

Collection of plant and identification:

Deride mature seeds of*Peganum harmala*were collected from different herbal medicine shops of Khartoum state Sudan in February 2020. seeds were identified and authenticated in Medicinal and Aromatic plants Research Institute (MAPRI). seeds were then ground to obtain fine powderThe powdered material was weighed properly 200g and stored in tight polythene bags for further usage.

Preparation of Crude Extracts:

For the methanolic extract thecoarsely powdered material (100 g) was exhaustively extracted by macerated in 500ml Methanol(80%)for 3daysat room temperature, Occasional shaking themethanolic extract was filtered and evaporated under reduced pressure using Rota-vapor apparatus, then air-dried, repacked in the Soxhlet and exhaustively extracted,each residue was weighed and the yield percentage was determined,the methanol residue (3.5g) was dissolved in methanol 8.75 ml (con. 400mg/ml), and kept in refrigerator until used.

For aqueous extract 100 g of the plant sample was infused in 500 ml hot water for 4 hours then filtered with Whatman filter paper. Extracts kept in Deep freezer for 48 hours, then introduced in freeze dryer till completely dried. The residue was weighed and the yield percentage was determined. The aqueous residue (4g) was dissolved in sterile distilled water 10 ml (con. 400mg/ml), and kept in refrigerator until used.

Percent recovery of the crude extracts of plants was calculated by weighing the dried extracts of pants. Following formula was used for calculating percent extract recovery.

% Extract recovery = (a / b) × 100

Where; a = Total weight of dried extract obtained after drying, and b = Total weight of ground plant material taken for each extraction.

Microorganisms used:

A total of tenorganisms were selected for this study. Five reference standard microorganisms from American Type Culture Collection (ATCC) One Gram positive bacteria *Staphylococcus aureus*(ATCC 3889),three Gram negative bacteria *Eschericia coli*(ATCC 3887), *salmonella entrica* (ATCC 14028) and *Pseudomonas aeruginosa*(ATCC 9027), as well as one fungi *Candida albicans* (ATCC 10231).

And five Multidrug resistanceclinical isolated organisms*Proteus mirabilis*and *Pseudomonas aeruginosa*, *Escherichia coli*and *Klebsiella pneumoniae* (ESBL makers), and Methicillin resistance *staphylococcus aureus* (MRSA), These species were originally isolated from clinical specimens and identified by standard biochemical reactions based on morphological and biochemical characters according to the methods described in **(19)**.

Different characteristics were Gram stain, pigmentation, motility and utilization of different carbon and nitrogen sources and antibiotic sensitivity tests.

Identification of MRSA and ESBL isolates:

Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected using the disc diffusion method with Cefoxitin and oxacillin disc, as recommended by the Clinical and Laboratory Standards Institute (CLSI).

The identification of *Escherichia coli* and *Klebsiella pneumoniae*Extended Spectrum Beta Lactamse (ESBL) makers isolates were achieved by disc diffusion method for screening and confirmatory tests using Cefotaxime (30g), Ceftazidime (30g), and Ceftriaxon (30g)alone and in combination with clavulanic acid on inoculated Mueller-Hinton agar plates and incubated at 37°C for 24 hours. the inhibition zones of the drugs for bacteria were ≤27mm for Cefotaxime(30µg), ≤22mm for Ceftazidime(30µg), ≤25mm for Ceftriaxon(30µg) respectively.

The double disc synergy test was used to screen multi drug resistant*Pseudomonas aeruginosa*and *Proteus mirabilis.* a disc of amoxiclav (20 g amoxycillin and 10 g clavulanic acid) and a 30-g disc of each 3Gc (gas chromatography) test antibiotic were placed 15 mm apart on a lawn culture of the isolate to determine synergy. the zone of inhibitionwas increased towards the Amoxyclav disc.

Inoculums preparation for antibacterial assay:

The testedorganisms cultured at 37 °C for 18-24 hours in the Muller-Hinton Broth (MHB, HIMEDIA) and used as inoculums.

The standardization of the inoculums suspension turbidity adjusted to the

McFarland 0.5 turbidity standard (1.5×10^8 cfu/ml)(20).

Antibacterial assay procedure:

Antibacterial activity of the extracts were tested using the cup-plate agar diffusion method (25) was adopted to assess the antibacterial activity of the prepared extracts.

The test inoculum (0.5 McFarland's turbidity) was spread onto Muller- At first, a total of 0.1 ml of bacterial suspension was poured on each plate containing Muller-Hinton Agar (MHA, HIMEDIA). The lawn culture was prepared by sterile cotton swab and allowed to remain in contact for 1 min. Then the wells were punched by a sterile well puncture. Four concentrations of methanolic and water extracts (100,50, 25, and 12.5mg/ml) from seeds parts of P. harmala were prepared, and added to each well using micropipette, then incubated at 37°C for 24 hours. The presence of zones of inhibition was regarded as the presence of antimicrobial action. The diameter of zone of inhibition was measured in mm. antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured, and methanolic crude extract was dissolved in Dimethyl sulfoxide (DMSO) (1:3 w/v, final concentration 250mg/ml).

This experiment was carried out in triplicate. As positive controls, discs containing novobiocin 30 µg, colistin 10 µg, streptomycin 10 µg and methicillin 5 µg were used. All of these synthetic antibiotic discs were prepared from Difco. Discs impregnated with 80 % and methanol was also included to test if they had an inhibitory effect on the test bacteria.

Minimum inhibition concentration & minimum bactericidal concentration:

The broth microdilution method was used to determine Minimum inhibition concentration (MIC) and Minimum bactericidal (MBC) according to Clinical and Laboratory Standards Institute (CLSI). Sterile 96-well microplates were used for the assay. The stock extract (80 mg/mL) was dissolved in sterile distilled water. All tests were performed in Mueller-Hinton broth. Briefly, serial doubling dilutions of the extracts were prepared in a 96-well microtiter plate ranging from 40 mg/ml to 0.3125mg/ml.

Sterile 96-well microplates 0.1 mL (Mueller-Hinton broth, HIMEDIA). Test extract (0.1 mL) was added to the first well of each row and serial double-fold dilutions (40, 20, 10, 5, 0.25,

0.125, 0.625, and 0.3125 mg/ml) were made down to the desired minimum concentration. The wells (two rows for each microorganism) were inoculated with 0.1 mL suspension of each test bacteria (0.5 McFarland) and incubated at 35 ± 2 °C for 16-20 hours. The growth of each microorganism in the different dilutions of extracts was determined by measuring the optical density at 630 nm with ELIZA. The wells filled with Mueller-Hinton broth and the suspension of each test bacteria were included as a positive control in each assay. The wells filled with Mueller-Hinton broth and extract were used as a negative control. All assays were carried out in triplicate.

Results:

This research was conducted on the methanolic and water extracts of seeds parts ofmedically important plants *Peganumharmala.*

The researchwas done toevaluate the antimicrobial activity of *Peganumharmala* against reference standard microorganisms from American Type Culture Collection (ATCC), and multidrug resistance clinical isolates organisms using cup-plate agar diffusion method and broth microdilution method using 96-well microplates.

The reference standard microorganisms from American Type Culture Collection (ATCC) One Gram positive bacteria *Staphylococcus aureus*(ATCC 3889),three Gram negative bacteria *Eschericia coli*(ATCC 3887), *salmonella entrica* (ATCC 14028) and *Pseudomonas aeruginosa*(ATCC 9027), as well as one fungi *Candida albicans* (ATCC 10231).

The antimicrobial activity of seeds methanolic and water extracts of *pengum harmala*was screened for its antimicrobial activity against clinical isolated multi drug resistance microorganisms, one Gram positive bacteria Methicillin resistant *Staphylococcus aureus*(MRSA), and three Gram negative bacteria *Escherichia coli* , *Pseudomonas aeruginosa*, ,*klebsiella pneumoniae*and *proteus mirables*.

Extracts resulting in more 18mm growth inhibition zone are considered to possess high antimicrobial activity, those the resulting (14-18mm)are of intermediate activity and those resulting in zone below 14mm are inactive.

Yields percentage of methanolic and water extracts of *Peganumharmala* Plants:

Tables (1) showed the yield percentage of (99.8%) methanolic and water seeds extracts of *Peganumharmala*.

Scientific Name of Plant	Solvent	Weight of sample(g)	Yield (%)

Peganumharmala	Methanol	100 grams	3.7
	Water	100 grams	4.5

The antimicrobial activity of aqueous and Methanolic extracts of *Peganumharmala* against tested organisms :

According to the result obtained that are shown in Table 2, 3, and Figures1, and 2,The seed extracts of *Peganumharmala* have a broad antimicrobial activity against most of organisms under this study with moderately increasing in the methanolic extract activity compared with water extracts, and high antimicrobial effect against the reference standard microorganisms compared withthe clinical isolated multi drug resistance microorganisms.

The antimicrobial activity of the methanolic and water extracts of *Peganum harmala* directly proportional to their increasing in the concentration. The highest inhibition zone reached 32 mm to ward E. coli ATCC 8739at concentration 100 mg/ml and smallest inhibition zone zero mm toward *Pseudomonas aeruginosa* ATCC 9027 at concentration 12.5 mg/ml.

The obtained result indicate that the average inhibition zone of the methanolic extracts were 25.1.mm (100 mg/ml), 18.4 mm (50 mg/ml), 16.0 mm (25mg/ml) and 11.3 mm (12.5 mg/ml), while the average inhibition zone of the water extracts slightly decreased 21.8.mm (100 mg/ml), 17.8 mm (50 mg/ml), 12.4 mm (25mg/ml) and 7.4 mm (12.5 mg/ml).

The invitro antimicrobial activity shown that all tested microorganisms were sensitive to the methanolic extract at concentration of 100 mg/ml(Zone of inhibition >18 mm)with highly antimicrobial activity mainly against *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 8739,*klebsiella pneumoniae*, *Proteus mirabilis* and *Escherichia coli*, (Zone of inhibition >25mm), moderate antimicrobial activity against, *Salmonella enterica* ATCC 14028,*Candida albicans* ATCC 10231 and MRSA (Zone of inhibition >20 mm) and low antimicrobial activity mainly against *Pseudomonas aeruginosa* ATCC 9027 and MDR *Pseudomonas aeruginosa* (Zone of inhibition 18 mm) .While most of the organisms under this study indicate the sensitivity against the methanolic extract at the concentration of 50 mg/ml(Zone of inhibition >18 mm) except *Pseudomonas aeruginosa* ATCC 9027 and MDR *Pseudomonas aeruginosa* which indicate their resistance(Zone of inhibition 12 mm).

The results indicate that the methanolic extract possess high antimicrobial activity(Zone of inhibition >18 mm) against *Staphylococcus aureus* ATCC 6538, *E. coli* ATCC 8739 and *Proteus mirabilis* under all concentrations in this study (100, 50, 25, and 12.5 mg/ml), intermediate antimicrobial activity effect (Zone of inhibition >14 mm) against *Candida albicans* ATCC 10231,*Salmonellaenterica* ATCC 14028 and *E. coli*, at concentration of 50

and 25 mg/ml and no antimicrobial activity(Zone of inhibition < 14 mm) against MRSA and *Klebsiella pneumoniae*, at concentration of 25 and 12.5 mg/ml .

The water extract possess no antimicrobial activity(Zone of inhibition <14 mm) against all of the microorganisms under this study at concentration of 25 and 12.5 mg/ml except *Klebsiella pneumonia* and *Proteus mirabilis* with moderately intermediate antimicrobial activity against *Staphylococcus aureus* ATCC 6538 at concentration of 25 mg/ml.

The water extract possess high antimicrobial activity effect (Zone of inhibition >18 mm) against all of the microorganisms under this study at concentration of 100 mg/ml except *Pseudomonasaeruginosa* ATCC9027 and MDR *Pseudomonas aeruginosa* which had an intermediate antimicrobial activity(Zone of inhibition 18-14 mm).

The water extract possess high antimicrobial activity(Zone of inhibition >18 mm) against *Staphylococcus aureus*ATCC 6538 , E.coli ATCC 8739 , *S.enterica* ATCC 14028 ,*Klebsiella pneumoniae* and *Proteus mirabilis*at concentration of 50 mg/ml ,and an intermediate antimicrobial activity(Zone of inhibition 18-14 mm) against *Candida albicans* ATCC 10231 , MRSA and *Escherichiacoli* .

Pseudomonasaeruginosa ATCC 9027 and *Pseudomonas aeruginosa* were appear highly resistant to *Peganum harmala* water extract at 50 mg/ml, 25 mg/ml and 12.5 mg/ml.

Tables (2) showed Antimicrobial activity of Methanolic extracts of *Peganumharmala* against Reference and MDR Clinical Isolated microorganisms:

Organism Name	Methanolic Extracts of *Peganumharmala* Inhibition zone (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
S.aureus ATCC 6538	30 ± 0.31	22 ± 0.45	20 ± 0.4	18 ± 0.2
E.coli ATCC 8739	32 ± 0.40	28 ± 0.21	25 ± 0.2	20 ± 0.46
P.aeruginosa ATCC 90	18 ± 0.35	12 ± 0.42	12 ± 0.31	Zero
S.enterica ATCC 1402	24 ± 0.25	18 ± 0.15	15 ± 0.40	10 ± 0.45
C.albicans ATCC 1023	22 ± 0.30	16 ± 0.45	16 ± 0.44	12 ± 0.06
MRSA	20 ± 0.35	18 ± 0.06	12 ± 0.52	08 ± 0.20
Escherichia.coli,	26 ± 0.26	20 ± 0.45	14 ± 0.55	06 ± 00
Pseudomonas aerugino	18 ± 0.12	12 ± 0.55	10 ± 0.70	06 ± 00
Klebsiella pneumoniae	31 ± 0.10	26 ± 0.32	12 ± 0.60	09 ± 0.06
Proteus mirabilis.	30 ± 0.40	22 ± 0.25	24 ± 0.25	24 ± 0.30

Each sample was analyzed individually in triplicate. The diameter of cup/disc = 6 mm.

Key: MDIZ (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm); >18 mm: Sensitive, 14 to 18 mm: Intermediate, <14 mm: Resistant.

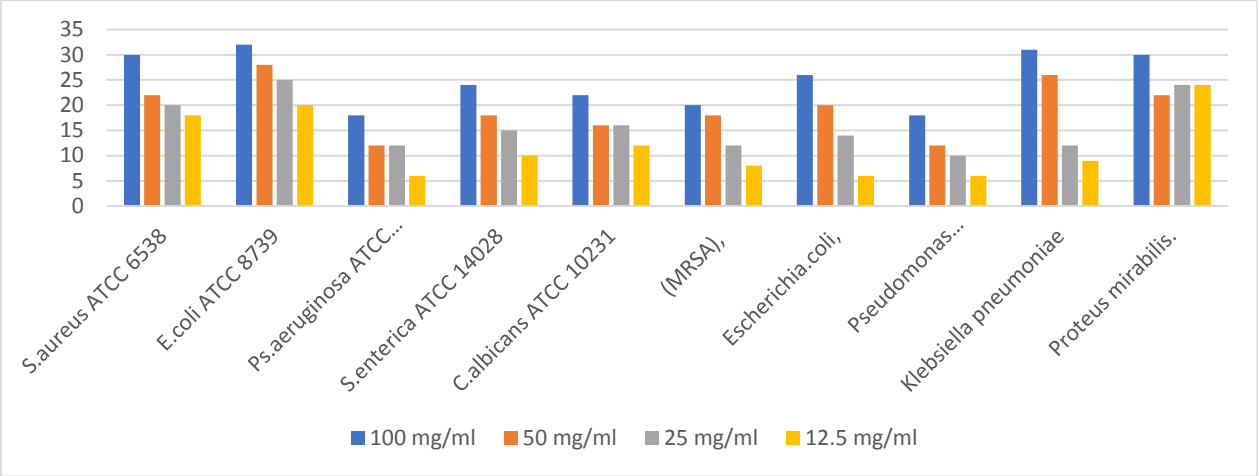


Figure **1**

Tables (3) showed Antimicrobial activity of Water extracts of *Peganumharmala* against

Reference and clinical MDR Isolated organisms:

Organism Name	Water extracts of *Peganumharmala* Inhibition zone (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
S.aureus ATCC 6538	28± 0.36	22 ± 0.40	16 ± 0.23	10± 0.2
E.coli ATCC 8739	25 ± 0.25	19 ± 0.5	12 ±0.13	6 ± 0
P.aeruginosa ATCC 9027	14 ± 0.49	10 ± 0.45	6 ± 0	6 ± 0
S.enterica ATCC 14028	20 ± 0.75	18 ± 0.15	12 ± 0.31	10 ± 0.45
C.albicans ATCC 10231	19 ± 0.57	16 ± 0.45	12 ± 0.07	9 ± 0.55
MRSA	18 ± 0.44	14 ± 0.06	10 ± 0.6	6 ± 0
Escherichiacoli,	20 ± 0.45	16 ± 0.29	10 ± 0.35	6 ± 0
Pseudomonas aeruginosa	15± 0.29	12 ± 0.47	6 ± 0	6 ± 0
Klebsiella pneumoniae	29± 0.75	22 ± 0.35	20 ± 0.25	6 ± 0
Proteus mirabilis.	30 ± 0.45	29 ± 0.21	20 ± 0.4	6 ± 0

Each sample was analyzed individually in triplicate. The diameter of cup/disc = 6 mm.

Key: MDIZ (mm) = Mean diameter of growth inhibition zone in mm. Interpretation of results: MDIZ (mm); >18 mm: Sensitive, 14 to 18 mm: Intermediate, <14 mm: Resistant.

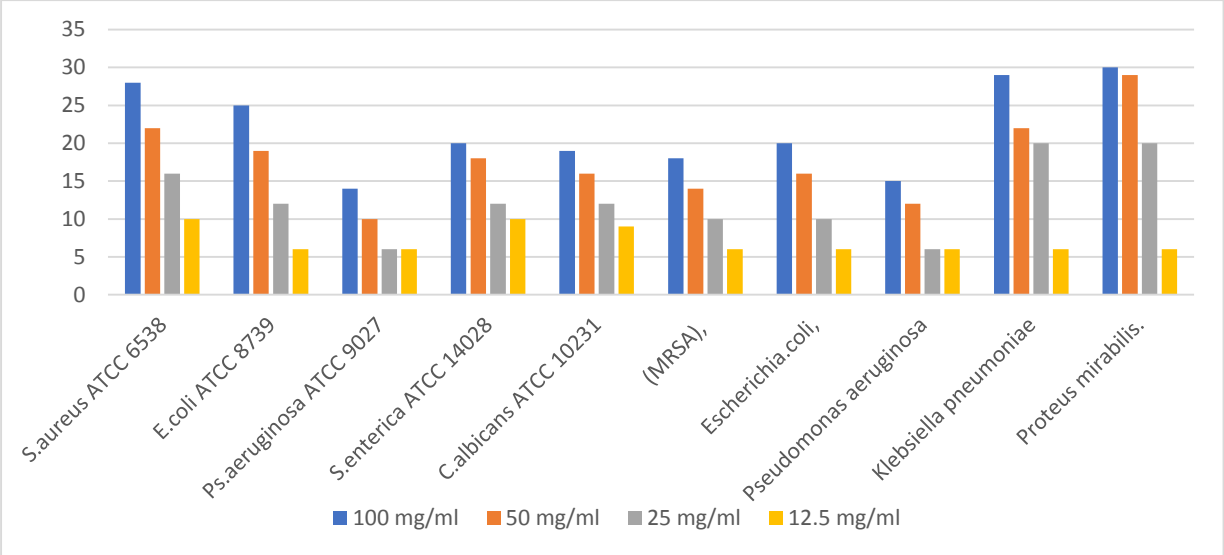


Figure**2**

Determination of theMICs and MBC for both Methanolic and Water extracts:

According to the result obtained that are shown in Table **5**, and Figure **3**, the methanolic extract has high effect at the concentration of**1.25**mg/ml against (*Ps.aeruginosa*ATCC 9027, *C.albicans*ATCC 10231,*Proteus mirabilis)* and moderate effect at the concentration of **2.5**mg/ml against (*E.coli* ATCC 8739, *S.enterica*ATCC 14028, MRSA and *Escherichia coli*, *Klebsiella pneumoniae*) and low effect against *Pseudomonas aeruginosa* at the concentration **10**mg/ml. while table **6** and figure **4** showed the high effect of water extract at the concentration of **2.5**mg/ml against (*C.albicans*ATCC 10231,*Proteus mirabilis*), and moderate effect at the concentration of **10**mg/ml (*S.aureus*ATCC 6538**,** *Ps.aeruginosa*ATCC 9027, and *S.enterica*ATCC 14028, MRSA and *Klebsiella pneumoniae*), and no effect at 40mg/ml concentration against *Pseudomonas aeruginosa*.

Table 5: The MIC and MBC values of Methanolic extracts of P. harmala against Reference and MDR clinical Isolated organisms:

Bacterial species	**MIC (mg/ml)**	**MBC (mg/ml)**
***S.aureus*ATCC 6538**	5mg/ml	10mg/ml
***E.coli* ATCC 8739**	2.5mg/ml	5mg/ml
***Ps.aeruginosa*ATCC 9027**	1.25mg/ml	5mg/ml
***S.enterica*ATCC 14028**	2.5mg/ml	5mg/ml
***C.albicans*ATCC 10231**	1.25mg/ml	2.5mg/ml
MRSA	2.5mg/ml	5mg/ml

Pseudomonas aeruginosa	10mg/ml	20mg/ml
Escherichiacoli	2.5mg/ml	5mg/ml
Klebsiella pneumoniae	2.5mg/ml	5mg/ml
Proteus mirabilis	1.25mg/ml	2.5mg/ml

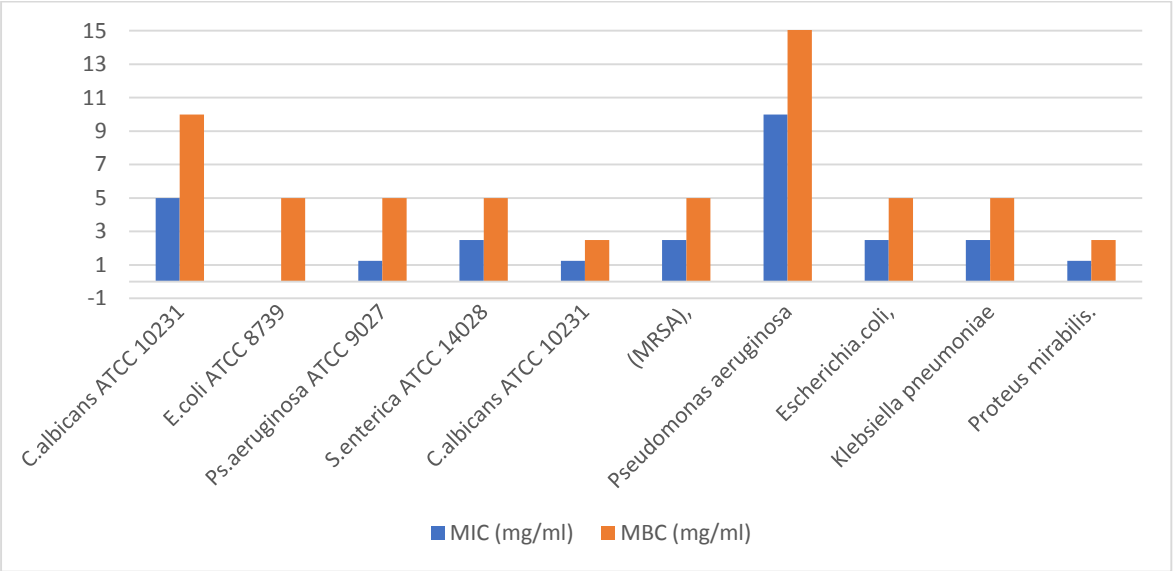


Figure **3**

Table 6: The MIC and MBC values of Water extracts of *P. harmala* against Reference and MDR Clinical Isolated organisms:

Bacterial species	**MIC (mg/ml)**	**MBC (mg/ml)**
***S.aureus*ATCC 6538**	10mg/ml	20mg/ml
***E.coli* ATCC 8739**	20mg/ml	40mg/ml
***Ps.aeruginosa*ATCC 9027**	10mg/ml	20mg/ml
***S.enterica* ATCC 14028**	10mg/ml	20mg/ml
***C.albicans*ATCC 10231**	2.5mg/ml	5mg/ml
(MRSA)	10mg/ml	20mg/ml
Pseudomonas aeruginosa	No Reaction at 40mg/ml	No Reaction at 40mg/ml
Escherichiacoli	5mg/ml	10 mg/ml
Klebsiella pneumoniae	10mg/ml	20mg/ml
Proteus mirabilis.	2.5mg/ml	5mg/ml

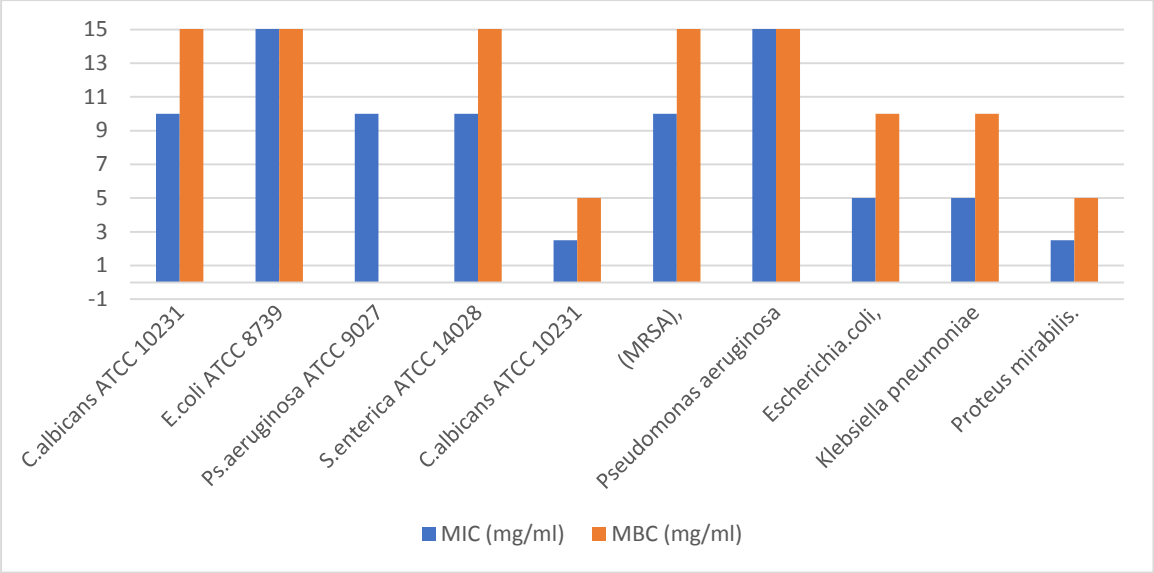


Figure **4**

Discussion:

Aromatic and medicinal plants, which are the mainstay of complementary and alternative medicine, have recently raised significant hopes for symptom relief and disease-related cures**(21).**Potential sources of natural materials that are useful in avoiding a variety of human ailments include medicinal plants. In the past, the Middle East, North Africa, and West China have utilized *P. harmala* as an emmenagogue and an abortifacient. It is a blooming plant that grows wild and is a member of the Zygophyllaceous family(22).

This experimental study was done in order to estimate antimicrobial activity of *P. harmala* and we found that The seed extracts of *P. harmala* have a broad antimicrobial activity against most of organisms under this study with moderately increasing in the methanolic extract activity compared with water extracts, and high antimicrobial effect against the reference standard microorganisms compared with the clinical isolated multi drug resistance microorganisms.

The antimicrobial activity of the methanolic and water extracts of *Peganumharmala* directly proportional to their increasing in the concentration. The obtained result indicate that the average inhibition zone of the methanolic extracts were 25.1.mm (100 mg/ml), 18.4 mm (50 mg/ml), 16.0 mm (25mg/ml) and 11.3 mm (12.5 mg/ml), while the average inhibition zone of the water extracts slightly decreased 21.8.mm (100 mg/ml), 17.8 mm (50 mg/ml), 12.4 mm (25mg/ml) and 7.4 mm (12.5 mg/ml).which is almost similar to study done in Iran 2016 to asses antimicrobial activity of *Peganum harmala*against*S.mutans* which indicate that the

average inhibition zone of the methanolic extracts were 25.1.mm (100 mg/ml), 18.4 mm (50 mg/ml), 16.0 mm (25mg/ml) and 11.3 mm (12.5 mg/ml), while the average inhibition zone of the water extracts slightly decreased 21.8.mm (100 mg/ml), 17.8 mm (50 mg/ml), 12.4 mm (25mg/ml) and 7.4 mm (12.5 mg/ml)(22). Furthermore, the results of this study showed that the *P.harmala* extracts inhibited the growth of all the microbial isolates tested. And the methanolic extract antimicrobial activity was higher Than the water extract which is similar to all litruters about *P.harmala* .furthermore extracts are equally broad spectrum in activity as their activities were independent of Gram reaction, this result is similar to (ahmed et al) (23) study in Libya 2021 which concluded that the actions of the extracts are as wide-ranging as their independence from the Gram reaction. Higher antimicrobial activity of the extracts was observed against Higher *S.enterica* flowed by pseudomonas as control strain group and the higher antimicrobial activity among multidrug resistant strain was against *protues spp*. Iranian study in 2011 showed that Among Gram positive bacteria, *S. epidermidis* and *Str. pyogenes* were the most sensitive strains to the seed and root extracts while among Gram negative bacteria, *K.pneumoniae* and *Br.melitensis* had the most sensitivity to the seed extract and *K.pneumoniae* and *E.coli* to the root extract too. *P. mirabilis* and *P. aeruginosa* were the most resistant strains to these two extracts. Antibacterial activity of the root extract against tested *Bacillus species* and *S. aureus* was better than the seed extract(24). The main differences in the susceptibility maybe due to assay methods that they used disc diffusion method.

Conclusion:

In conclusion, the extracts of*P. harmala's* have broad antibacterial activity against resistance organism.with high methanolic extract activitycompared with water extracts, the use of this plant as an antibacterial agent has promising future. its use may be more beneficial for resistant bacteria than the commonly used antibiotics. So additional antibacterial and cytotoxicity tests on animals or human beings are required.

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