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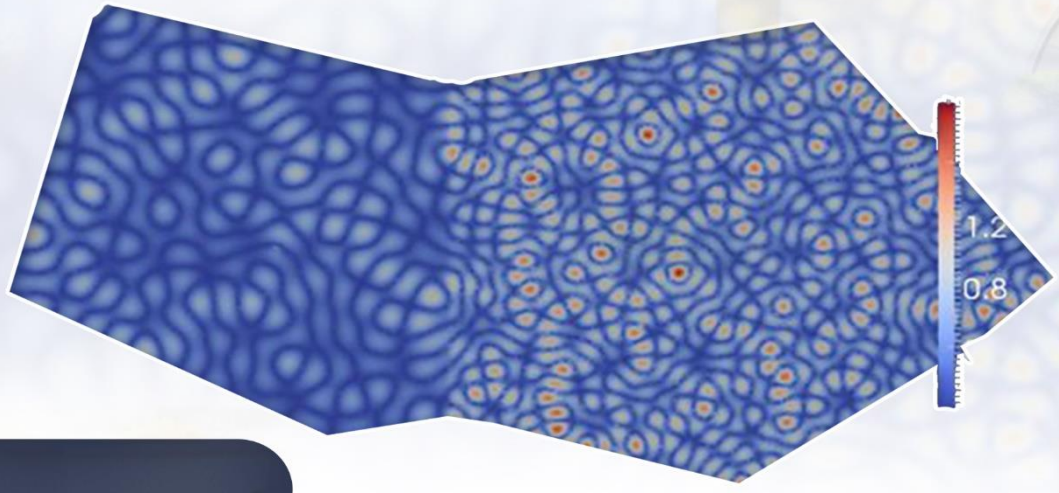

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Biological Studies of Harmala peganum Exracts as Antibacterial Agent

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A B S T R A C T

Harmala peganum is one of the most well-known medicinal plants in traditional Chinese medicine. The goal of this study was to look at the antibacterial effects of a methanol extract of several components of *H. peganum*, such as the stem and leaf, against certain common human pathogenic bacteria. The antibacterial capabilities of methanol extracts of the specified sections were tested using the disc diffusion method, as well as their synergism activity when combined with synthetic antibiotics. The leaf and stem extracts were the only portions of *H. peganum* that showed antibacterial action against all of the microorganisms tested, even at the lowest concentrations. Leaf extract out performed stem extract in terms of antibacterial activity against the majority of gram-positive bacteria tested. The extracts were tested for their biological activity against microorganisms such as *Streptococcus pneumoniae, Pseudomonas aeruginosa, Salmonella typh,* and *Escherichia coli*. Except for the distilled water extract of *H. peganum*, the results demonstrated that the extracts are active against all bacterium strains. However, the antibacterial activity of *Harmala peganum* leaf and stem extracts was higher than that of antibiotics used against the pathogens tested.

1 Introduction

Indicates that man used plants and herbs to treat some diseases that afflicted him or his pets, and he used them either in their natural form, or extracted as essential oils for a period of time approximately 622 Year. (Bin Marash, 2012). Medicinal and aromatic plants are used in folk medicine, and these fields go beyond perfuming, cosmetics, seasoning, and food, where activity is due to the presence of antioxidants and antimicrobials in their tissues, they are considered the natural source of natural antioxidants. (Omar, 2012.) The science of medicinal herbs in its modern concept is advancing a lot in different parts of the world, increasing interest in the study of medicinal plants and their use in treating various diseases, as plants contain a very large number of medically effective components that reflect the great therapeutic potential of these plants, it is known that some plant drugs have a greater therapeutic capacity than those of manufactured medicines in treating some diseases, and plant drugs contain nutrients and vitamins as well as active ingredients (Bin Marash, 2012). The recommendations of the medical and pharmacological conferences held in recent years have called for the necessity to limit the intake of these manufactured drugs, whose use has been proven to cause harmful side effects, and recommended the return to medicinal plants with interest in them as a safe source for the manufacture of medicines, and to make them in the service of health in a scientific way by applying And based on established scientific principles, where phyttochimie plays a vital role in extracting active substances or elements from the plant, the actif principe. (Nazni et al., 2006). This is by using different chemical, analytical and physical methods, and then comes the biological and pharmacists' role to conduct biological experiments (Ibn Arabiya, 2013). For this purpose, we decided through this work to study a plant that has been common since ancient times in many Arab countries, namely the H. *peganum* plant (Newall et al.,1996). It was widely used in ancient folk medicine the *Harmala* plant is considered a herbaceous flowering plant that spreads in Central Asia, Africa, and

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the Middle East, and it has also spread America and Australia. (Elsayem et al., 2012). It is one of the herbs used in folk medicine since ancient times for pain relief and sterilization, and its use has spread in cases of back pain, asthma, colic, and wheezing, and as a stimulant among many, and scientific research has found that the different parts of this plant carry different therapeutic effects. (Englisch et al., 2000; Lopez-Molina et al; 2003). The *H. peganum* plant is used industrially to produce a red dye used in carpet dyeing. Scientific research has found that the camel plant and its extracts have great importance in drug extraction and manufacture. This is due to the therapeutic effects it carries, and include its therapeutic benefits Anti-bacterial, anti-virus and anti-fungal effect. (Alghazee et al., 2012).

2 Materials and Methods

Leaves and stems of every species of selected plant were separated and washed with distilled water several times, then dried in open air. Its height is 60 cm, with lobed leaves, a distinctive aroma, and its large white flowers. It gives white top fruits, with small black seeds. The plant grows wild in most areas of Libya. Fresh of *Harmala peganum* washed two times distilled water and subjected to shade drying at room temperature the dried plant material was powdered using a mechanical grinder (Akinpelu *et al*., 2 008; Alshammary and Ibrahim, 2014). The powdered materials of *Harmala peganum* were extracted with methanol 10 grams of each plant powders were added to 100ml of methanol (80% w/v). Crude extracts were evaporated at 45^0C with the rotary evaporator the extracts were collected and stored at 4^0C until further use (Akinpelu et al., 2008; Alshammary and Ibrahim, 2014). The antimicrobial activity of the plants extracts was determined using the agar disc diffusion method (Sathishkumar *et al*., 2008), where Mueller-Hinton (MH) agar plates were seeded with bacterial strain on each plate wells were made by sterile standard cork borer. Each well was filled with 30µl of the different concentrations (0.8, 0.4, 0.2, 0.1, 0.01, 0.001, 0.0001 and 0.00001 g/ml) of incubated for 24 - 48 h at 37°C for bacteria. The of inhibition zones were measured, the results are presented as mean of triplicate. The minimal inhibition concentration (MIC) values were evaluated according to published procedures (Koneman et al., 1997; Iscan et al., 2002 and Guven *et al*., 2005). The minimal inhibitory concentration (MIC) was determined only with micro-organisms that displayed studied plants extracts and the plates were then inhibitory zones. MIC was determined by dilution of the plants extracts and pipetting 30µl of each dilution into wells dilutions of the extracts within a concentrations range of (0.8 - 0.00001 g/ml). MIC was defined as the lowest concentration that inhibited the visible microbial growth (NCCLS, 2005).

Aim of the study

This study aims to find out the inhibitory effect of medicinal plant extract of the medicinal plant *H. Peganum* used in folk medicine on some types of antibiotic-resistant bacteria.

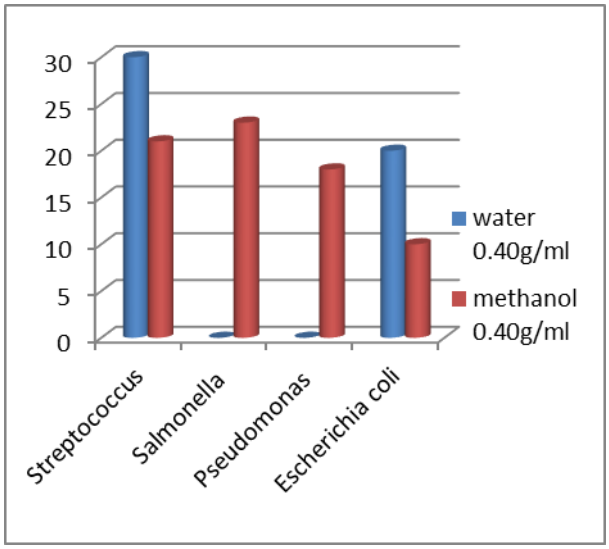

Figure (1). Antimicrobial activities of different concentrations of studied plant *Harmala* leaves extract against bacteria.

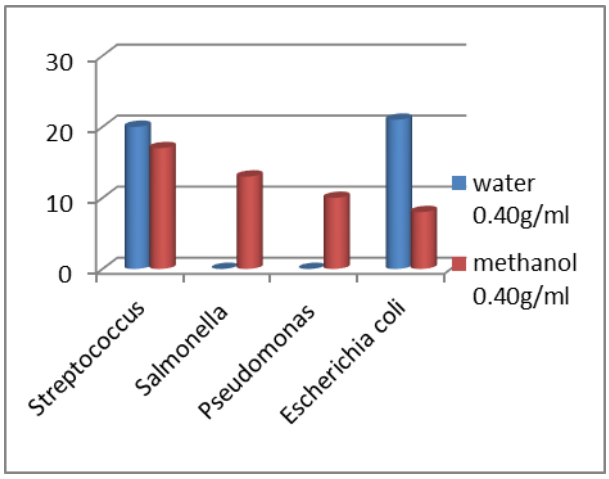

Figure (2). Antimicrobial activities of different concentrations of studied plant *Harmala* stems extract against bacteria.

2.1 Antibiotic Sensitivity Tests

In vitro antimicrobial susceptilit to four antibiotics in table. The inoculums was prepared by adding isolated colonies of the microorganism from an overnight nutrient agar plate into 2ml try tone soya broth (TSB). A sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked over the entire surface of the sterile Mueller Hinton Agar plate. This procedure was repeated by streaking two more times, rotating the plate approximately each time to ensure an even distribution of inoculums. plates were

allowed to dry for 5 minutes and then the antimicrobial disks were dispensed onto the surface of inoculated agar plates using an oxide antibiotic. Plates were then incubated at 37°C for 18-22 hours. The diameters of the zones of inhibition are measured to the nearest mm using a venier calipers (junior), zones diameters were interpreted as being susceptible sensitive (S) or resistant (R) according to (NCCLS, 2001).

Table (1). Antibiotic sensitivity testing**.**

Kanamycin	K	30mg/ml
Gentamicin	CN	10mg/ml
Tetracycline	TE	30mg/ml
Cefotaxime	CTX	30mg/ml

3 Results

Antibacterial activity showed different concentrations of studied plants extract against bacteria. The results showed that the inhibition zone and MIC in all extract It was resistant to bacteria. Except for distilled water of *H. peganum* no zones of inhibition did not show any effect on the S. typh and *P. aeruginosa*. Where it was found that the activity of the plant extract of the leaves and stems of the *H. peganum* plant was higher than the effect of antibiotics.

Discussion

If we notice that the *Harmala paganum* plant was a good resistance to different types of bacteria in all concentrations except for two types of bacteria that were resistant to the effect of this plant similar results observed by (Memon et al., 2003).

Table (2). Demonstrates the effect of *Harmala* plant extract (distilled water, methanol) against the tested bacteria**.**

Used bacteria				**concentrations**	**Solvent**	**Plant**
The diameter of the inhibition zone						
Escherichia coli	*P. aeruginosa*	*S. typh*	*Streptococcus pneumoniae*	0.40g/ml	Distilled water	*Harmala*
20	N.A	N.A	30			
10	18	23	21	0.40g/ml	Methanol	

Where inhibition zone diameters in (mm) **N.A: no activity**

Figure (3). Shows the effect of *Harmala* plant extract against *Streptococcus pneumoniae*

Figure (4). Shows the effect of *Harmala* extract agains *S. typh*

Figure (5). Shows the effect of *Harmala* plant extract against *P. aeruginos*

Figure (6). Shows the effect of *Harmala* plant extract against *Escherichia c*oli

Antibiotic Sensitivity

Table (3) show the rates of sensitivity of gram negative and gram positive bacteria results showed that the sensitivity pattern of *S. pneumoniae* was sensitive to K, CN, TE and resistant CTX figures (7). However, *S.typh* was resistant to K, CN, TE and sensitive CTX figures(8). Whereas *P.aeruginosa* was sensitive to CN, CTX and resistant to K, TE figures (9). Whereas *Escherichia coli*sensitive to CN, and resistant K, TE, CTX figures (10). Antimicrobial resistance developed by microbes against antibiotics open serious debates in this issue and recognized as a serious problem byglobal medicinal and research community (Finch,2004).

Table (3). Antibiotic sensitivity against bacteria

Antibiotic	Symbol	Concentration	Organism			
			S. pneumoniae	*S.typh*	*P.aeruginosa*	*E. coli*
Kanamycin	K	30mg/ml	S	R	R	R
Gentamicin	CN	10mg/ml	S	R	S	S
Tetracycline	TE	30mg/ml	S	R	R	R
Cefotaxime	CTX	30mg/ml	R	S	S	R

S-Sensitive

R-Resist

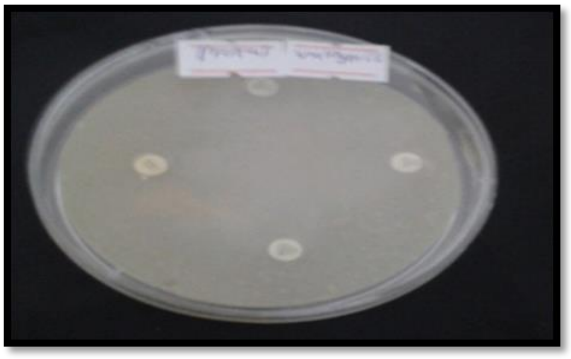

Figure (7). Antibiotic sensitivity testing of *S. pneumonia*

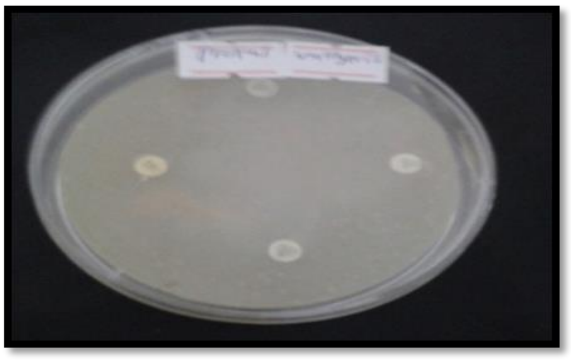

Figure (8). Antibiotic sensitivity testing of *S.typh*

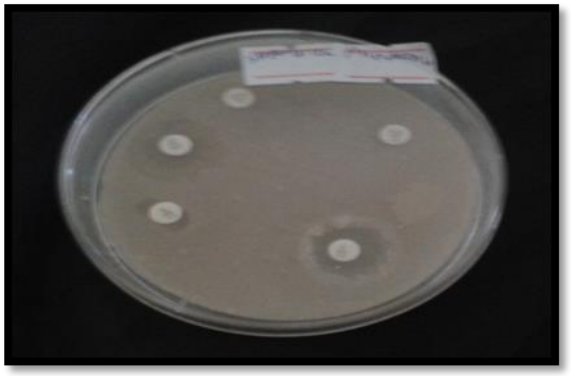

Figure (9). Antibiotic sensitivity testing of *E. col*

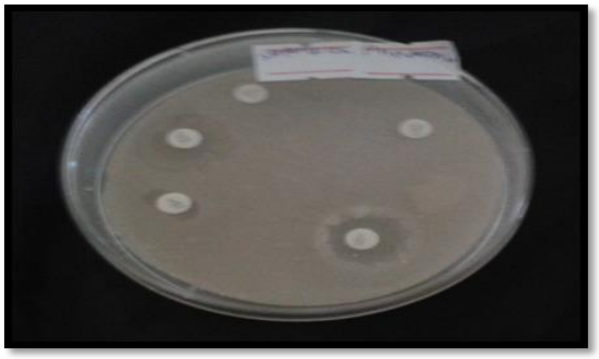

Figure (10). Antibiotic sensitivity testing of *P.aeruginosa*

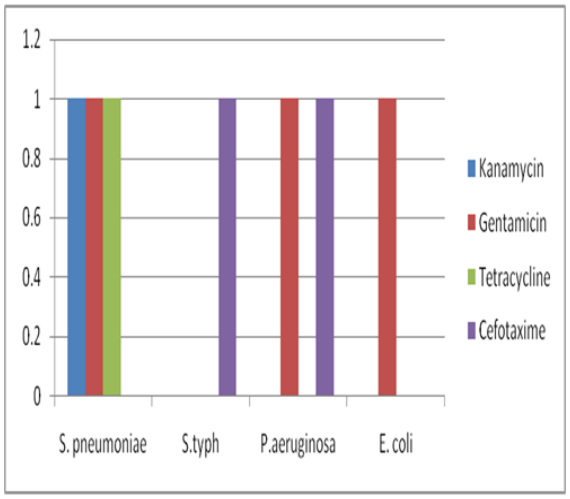

Figure (11). Antibiotics of against types different bacteria.

4 Conclusions

The present investigation proves that antimicrobial activity of leaves and stems *H. peganum* extracts was higher than that of antibiotic used against the tested microorganisms. The obtained results might be considered adequate to demonstrate that *H. peganum* extracts can be considered a good antibacterial agent, it can be used to an antibacterial overcoat against the strain that a major problem of resistance in hospitals.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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