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Assessment of the antibacterial activity of crude alkaloids extracted from seeds and roots of the plant *Peganum harmala* L.

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ABSTRACT

We evaluated the antimicrobial activity of crude extracts of alkaloids in seeds and roots of the plant Peganum harmala L. against some gram positive bacterial strains such as Staphylococcus aureus and Staphylococcus saprophyticus and gram negative such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis and Serratia spp, using the diffusion method in agar (antibiogram). Crude extracts of roots and seeds alkaloids have shown considerable activity against all organisms tested, seeds alkaloid showed a high activity at a concentration of 100mg/ml compared to roots alkaloidal at the same concentration, especially against Gram+ strains. The diameters of inhibition zones ranged from 11 to 22 mm for all treatments. The values of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. It was observed that the MIC and MBC of crude alkaloid extracted from the seeds Peganum harmala reached 0.78mg/ml, with the bacterial strain S.aureus (ATCC25923).

Keywords: *Peganum harmala*, Alkaloids, Antibiogram, MIC,MBC.

INTRODUCTION

Recently, searching for drugs and dietary supplements derived from plants have been accelerated. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids which have been found *in vitro* to have antimicrobial properties [1]. Since ancient times, the use of medicinal plants is related to diseases affecting humans, including the plant *Peganum harmala*, which belongs to the family of *Zygophylaceae*, distributed mainly in the Mediterranean region, also found in Central Asia, North Africa and also cultivated in America and Australia [2]. It is rich in alkaloids of type β-carboline and contains up to 2 - 7% total alkaloids [3]. Several studies have shown various biological activities and pharmacological characteristics of its seeds such as hypothermia [4], hallucinogen factor [5], antidepressant [6], inhibitor of the enzyme monoamine oxydase (MAO) [3] , antibacterial, antifungal and anti-virus [7,8]. It is effective for the treatment of dermatosis disease [9]. Its leaves are used as an antinociceptive [10]. It causes abortion in rats [11].

The aim of this study is to assess the antibacterial activity of alkaloids extracts from the seeds and roots of *P. harmala*, which can be the basis for the synthesis of new antibiotics. This is because the increase in the emergence of bacterial strains resistant to multiple clinical disease

MATERIALS AND METHODS

Plant material collection and identification

Different parts (roots and seeds) of *P.harmala* were collected from the Harmalia region (South east of the town of Ain M'lila, Algeria) in 2011. Sample collection was done in Jully for seeds and in September for roots. The botanical identity of the plant was confirmed by Dr. Youcef Halis, and a voucher specimen (No. 0118/HBPA) was deposited at the Herbarium of Laboratory of Biomolecules and Plant Amelioration, Larbi Ben m'hidi University of Oum El Bouaghi, Algeria. The seeds and roots were air dried at room temperature and kept in a dark-amber-colored bottle until processed.

GPS coordinates

The coordinates of the region of Harmlia (as google earth) are:

- Latitude: 35 ° 55'30 .95 "N
- Longitude: 6 ° 37'20 .19 "E
- Elevation: 795m

Alkaloids extraction

100g of each powder of roots and seeds of the plant was macerated in ethanol (70%), alcoholic extract was evaporated to one fifth of the initial volume by rotavapor, after, 20ml of hydrochloric acid HCl (0.1N) was added to the concentrated extract obtained is then filtered and extracted twice with 20ml of chloroform, the latter was treated twice with 10ml of hydrochloric acid (0.1 N), then we add ammonia NH_3 (0.1 N) to obtain an extract of pH = 9, then adding 30ml of chloroform, according to the classical method [12]. The operation is repeated 3 times then evaporates. residues were dissolved in 20ml of hydrochloric acid (0.02 N) and titrated with potassium hydroxide KOH (0.02 N) using methyl red as an indicator, then calculating the percentage of alkaloids seeds and roots of the plant by the formula :

$$\%\text{Alkaloïds} = \frac{\text{Volume of acid}(0.02\ \text{N}) - \text{base volume consumed}\,(0.02\ \text{N})}{\text{Powder mass (plant)}} \times 0.0046 \times 100$$

Sources and maintenance of microorganisms

Bacterial strains of Gram$^+$: *Staphylococcus aureus* ATCC25923 and *Staphylococcus saprophyticus* ATCC49453. Bacterial strains of Gram$^-$: *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Proteus mirabilis* ATCC29245, *Pseudomonas aeruginosa* ATCC27853 and *Serratia sp* ATCC39006. Bacterial strains are lots of ATCC (American Type Culture Collection), obtained from the Pasteur Institute of Algiers, maintained by subculture on nutrient agar medium favorable to their growth and incubated at 37 ° C for 24 h . So we obtain a bacterial suspension density of 10^6 colony forming units per milliliter (cfu/ml**).**

Preparing Disks

The alkaloid extracts of seeds and roots were recovered by distilled water to obtain an initial solution concentration of C_0 = 100mg/ml, from C_0 is conducting a series of dilutions. The sterile filter paper discs (6 mm diameter) were impregnated with 100 µl of different concentrations of each extract, finally preparing disks impregnated with sterile distilled water as control.

Diffusion method on agar medium

The antibacterial activity of different plant extracts was evaluated using the method of agar diffusion [13]. From colonies of 18 to 24 h, a bacterial suspension was made in sterile distilled water for each strain. The turbidity of this suspension is adjusted to 0.5 McFarland and then diluted 1/100. This gives an estimated inoculum to 10^6 cfu/ml. This inoculum is inoculated by flooding on Petri dishes containing Mueller-Hinton agar. Disks impregnated with various alkaloidal extracts of roots and seeds at concentrations 100mg/ml and 50mg/ml were then delicately deposited on the surface of the agar. The Petri dishes left for 1 h at room temperature for pre-release substances, before being incubated at 37 ° C in an oven for 24 h. The antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc [14].

MIC and MBC determination

To determine the MIC and MBC respectively, must be prepared a series of dilutions with sterilized (12.5, 6.25, 3.12, 1.56, 0.78 ..... etc.) mg/ml. Also prepared for each bacterial strain, an inoculum whose turbidity was adjusted to 0.5 McFarland (either 10^8ufc/ml) and reduced to 10^6ufc/ml in the Mueller-Hinton broth double concentrated, then adding in hemolysis tubes, 1ml of each concentration and 1ml of bacterial inoculum. A growth control tube was prepared, containing 1ml of sterile distilled water and 1ml of inoculum, and a control tube containing sterile 1ml of

sterile distilled water and 1ml of sterile broth. And all treaties tube and two witnesses were incubated at 37°C for 24 hours. After incubation, examine bacterial growth in each tube, resulting in turbidity. The MIC of an extract vis-a-vis a given strain will be the smallest concentration showing no visible growth of germs. To determine the MBC, we realize 24 hours earlier, a witness of bactericide by seeding in a streak on agar in Petri dishes, dilution 10°, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} of the initial inoculum, corresponding respectively to 100%, 10%, 1%, 0.1% and 0.01% of survivors. After reading the MICs, subcultures are performed by streaking on fresh agar plates, tubes without visible growth. These subcultures were then incubated at 37°C and 24 h are compared with the control of bacterial streaks. The MBC is the lowest concentration which shows the growth of transplanted germ lower or equal to 0.01% of survivors.

RESULTS

(Figure 1) shows that the highest rate of crude alkaloids was registered with the seeds of 3.89% compared with the rate of 2.63% of the roots.

The results of the antimicrobial activity of extracts of seeds and roots of *P.harmala* are presented in (Table 1). We observe that the sensitivity tests show the effect of crude extracted alkaloids from seeds and roots of different bacterial strains, giving varying diameters depending on the tested strains. There has been very important values with the strain of $Gram^+$. The highest diameter of inhibitory zone was recorded by *S. aureus* of 22mm with the crude extract of alkaloids in seeds at a concentration of 100mg/ml. The smaller diameter of inhibition zone was recorded with *S. saprophyticus* 18mm, at a concentration of 50mg/ml of the crude extract of alkaloids in roots (Figure 2 and Figure 3). the bacterial strains of $Gram^-$ shows that the highest diameter of the inhibitory zone was registered with *E. coli* 19mm, for the crude extract of alkaloids in seeds at a concentration of 100mg/ml, and the smallest diameter of the inhibitory zone was recorded with *K. pneumoniae* 11mm at a concentration of 50mg/ml for the crude extract of alkaloids in roots (figure 4 and figure 5). The (Table 1) show that alkaloids extracted from the roots were active in 6 out of 7 bacterial strains. It is found that the increase of the diameters of inhibitory zones is directly related to the increase of the concentration of crude extracts.

(Table 2) show the magnitude of the differential impact of alkaloids extracted from seeds and roots of the plant on different bacterial strains. The strains of *S. aureus*, *E. coli* and *S.saprophyticus* show a high sensitivity to these alkaloids, as their growth stops from 0.78 mg/ml. *K. pneumoniae* and *P. aeruginosa* strains, begin to stop their growth at concentrations of 3.12 mg/ml and 6.25 mg/ml with the crude extract of alkaloids in seeds and roots respectively. Strain *P.mirabilis* stops their growth at 3.12 mg / ml with two alkaloid extracts . Strain *Serratia spp* begins to stop their growth from 6.25 mg/ml with the alkaloid extract of the seeds, by the MIC against the alkaloid extract of the roots is higher than the concentration of 12.5 mg/ml. The minimal bactericidal concentration MBC strains *S. aureus*, *E. coli* and *S. saprophyticus* is equal to 0.78 mg/ml with the alkaloid extract of the seeds, the MBC values for the other strains were varied between (3.12 and 6.25) mg/ml, with the exception of *Serratia spp*, it is greater than 12.5 mg/ml with the alkaloid extract of the roots.

DISCUSSION

Study of quantitative evaluation of alkaloids has shown that the highest percentage was with the seeds, which can be considered as storage areas of the plant alkaloids studied [15]. The results obtained showed that the plant *P. harmala* almost prevents the growth of all microorganisms tested, but to varying degrees of effectiveness. Concentration of 100mg/ml of crude extract of alkaloids seeds inhibits the growth of all bacterial strains studied, and in the same concentration of the crude extract of alkaloids of the roots inhibited the growth of six of seven bacterial strains tested . Recording the highest microbial activity with the crude extract of alkaloids of the seeds against the $Gram^+$ bacterial strains (*S.saprophyticus*, *S. aureus*), compared to $Gram^-$ strains except *E.coli* in exception, and this is due the strong resistance of $Gram^-$ bacterial strains to antibiotics [16] which showed that aqueous and alcoholic extract of the plant *P. harmala* is very effective with $Gram^+$ bacterial as *Staphylococcus mutans*, and also stated [17] that the strongest antimicrobial activity with extracts from seeds of the plant *P. harmala* against *Condida albicans* and *lactobacillus.*

Table 1. Results of the microbial activity of crude extracts of alkaloids (seeds and roots) of the plant *P.harmala*, concentrations of 50mg/ml and 100mg/ml

Extracts / Organism	Diameters of inhibition (mm)			
	Alkaloid extract of the roots		Alkaloid extract of the seeds	
	50mg/ml	100mg/ml	50mg/ml	100mg/ml
S. aureus	19	21	20	22
S. saprophyticus	18	20	19	21
E.coli	16	17,5	17	19
K. pneumoniae	11	12	13	15
P. aeruginosa	12	13	14	16
P. mirabilis	12,5	14	13	15
Serratia spp	-	-	11,5	12

Table 2. Determining the values of MIC and MBC of extracts alkaloids (seeds and roots) of the plant *P.harmala*.

Extracts / Organism	**Concentrations (mg/ml)**			
	Alkaloid extract of the roots		**Alkaloid extract of the seeds**	
	CMI	**CMB**	**CMI**	**CMB**
S. aureus	**0.78**	**0.78**	**0.78**	**0.78**
S. saprophyticus	**0.78**	**1.56**	**0.78**	**0.78**
E.coli	**0.78**	**156**	**0.78**	**0.78**
K. pneumonia	**6.25**	**6.25**	**3.12**	**6.25**
P. aeruginosa	**6.25**	**6.25**	**3.12**	**6.25**
Proteus mirabilis	**3.12**	**3.12**	**3.12**	**6.25**
Serratia sp	**>12.5**	**>12.5**	**6.25**	**12.5**

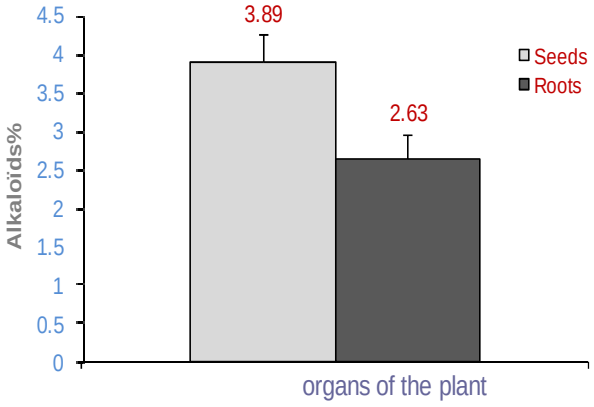

Figure 1. Rate of alkaloids in seeds and roots of *P.harmala*.

The zones of inhibition were found to increase when the concentrations of *P. harmala* extract increase. This may be attributed to the increased levels of the principle alkaloids, while increasing the extract concentrations, and these alkaloids (which are heterocyclic nitrogen compounds) have the ability to intercalate with DNA of the microorganisms [18]. The results of the MIC and MBC of extracts of alkaloids in seeds and roots of the plant *P.harmala* show that the value of the MIC and MBC equal to 0.78 mg/ml with *S. aureus* strain, known to his severe sensitivity to different antibiotics, as [19] showed that the values of MIC and MBC of

extracts of the plant *P. harmala* against MRSA (methicillin-resistant Staphylococcus aureus) are equal (0.625 mg/ml). also [20] shows that the values of compounds alkaloids (harmine, harmaline, harmane, harmalol) between(0.6-2)mg/ml with *S. aureus* strain.

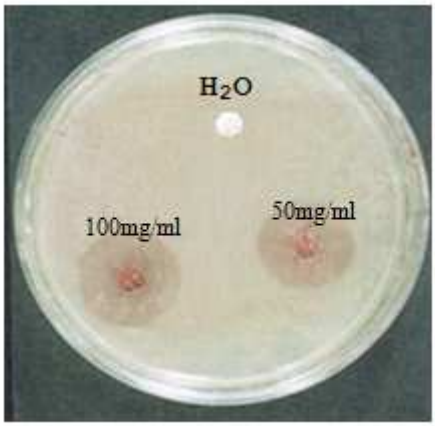

Figure 2. Effect of crude extract of alkaloid (seeds) on *S.aureus*.

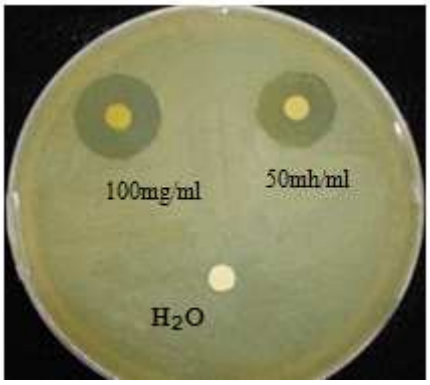

Figure 3. Effect of crude extract of alkaloids (roots) on *S.saprophyticus*

Figure 4. Effect of crude extract of alkaloids (seeds) on *E.coli*

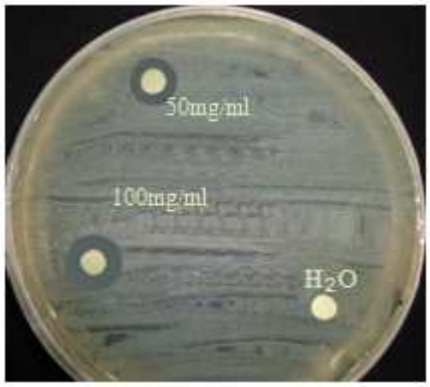

Figure 5. Effect of crude extract of alkaloids (Roots) on *K.pneumoniae*

CONCLUSION

Results from the present study have shown *P.harmala* as a potential source of antimicrobial drug against various pathogenic organisms. This is particularly important in combating the recent trend in the emergence of multiple drug resistant organisms. Further studies are however necessary for the development of new antimicrobials from this plant.

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