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Comparative Antibacterial Screening of some Medicinal Plants of Hunza Valley

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Abstract

In this research work, some plants were studied for their antibacterial activity typically by using aqueous or ethanol extractions. These plants were *Peganum harmala*, *Thuja occidentalis* and berry juice of *Hippophae rhamnoide*. Agar well diffusion technique was used to determine the susceptibility to bacterial strains of the above samples. The plant extracts were tested against four bacterial strains; out of these three were Gram-negative (*Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella Pneumonia*) and the fourth one was Gram-positive (*Staphylococcus aureus*). Smoke solution of *Peganum harmala* and *Thuja occidentalis* was also prepared with the help of Labbert vacuum pump apparatus to observe their antibacterial activity. All the samples under study have maximum antibacterial activity against *Pseudomonas aeruginosa* which is a Gram-negative bacterium except *Hippophae rhamnoide* which shows antibacterial activity against *Klebsiella pneumonia*.

Keywords: Antibacterial activity; *Peganum harmala*; *Thuja occidentalis*; *Hippophae rhamnoide*

Introduction

Hunza valley is a valley of Pakistan. It is a mountainous area. There are many medicinal plants in Hunza. In this research work, three common plants of Hunza valley that are *Peganum harmala*, *Thuja occidentalis* and *Hippophae rhamnoide* were studied. Their local names in brushaski language are Supandaro, Gal and Shass gonoo respectively. They are used as home remedies. All three plants under study have antibacterial activity and have much pharmaceutical importance.

Peganum harmala extracts are used for drug development. It is used for the relief of pain and as an antiseptic agent. It has antibacterial, antifungal, antiviral, antioxidant, anti-diabetic, anti-tumor, anti-insecticidal and cytotoxic activities. The seeds have hallucinogenic and hypothermic properties. Its fruit is used as analgesic and antiseptic in folk medicines. For long time, it has been used as a folklore medicine for treatment of various conditions such as lumbago, asthma, colic, and jaundice (Asgarpanah *et al.* 2012). This plant can also be used to assign a source of antibacterial compounds for treatment against multidrug resistant bacterial pathogens (Darabpour *et al.* 2011). The alkaloid content of *Peganum harmala* seed extracts is used to control hemosporidian infection in infected cattle, naturally or experimentally (Arshad *et al.* 2008). Its harmaline content is use as mild sleep inducer. It also has tremor generating effects. Its mechanism for tremor generating effects is under study (Brobst, *et al.* 2009). *Thuja occidentalis* is found valuable in nasal, vaginal, rectal, and urethral or bladder irritation. It is used as an antiseptic for the diphtheritic states of the mouth, nose and throat. It also cures syphilitic ulceration of the mouth, faces and nasal passage fluid. It is as the local remedy against goiter. Its fluid is applied at the effected site and the tumor size is greatly reduced. The drug dose not discolors the skin and has a relief effect. *Thuja occidentalis* essential oil and its active component α-thujone can be used for the treatment of PCOS (polycystic ovary syndrome) without inducing osteoporosis (Akkol *et al.* 2015). The oil of the *Thuja occidentalis* has been used for the insecticidal activity and characterized highly toxic. Its antifungal activity is also detected to control the bio contamination in libraries and documentations storage areas, showing that its oil had little inhibitory effect on the fungal combination (Moussa Kéïta *et al.* 2001). *Thuja occidentalis* oils reveal the highest antibacterial activity among the tested oils (Badawy *et al.* 2014). *Hippophae rhamnoide* berries are used to make juices (Arimboor et al., 2006). Traditionally, *Hippophae rhamnoide* has been known for its enormous medicinal, nutraceutical, and ecological value (Zeb, 2004). It has many important ingredients which are positively used in the cosmetic industry and in medicine (Zielińska *et al.* 2017). *Hippophae rhamnoide* has antimicrobial, anti-ulcerogenic, anti-oxidative, anti-inflammatory, anti-carcinogenic, immune-modulatory, hepato-protective and anti-hypertensive properties. These medicinal properties are result of the presence of important bioactive compounds present in *Hippophae rhamnoide* plant, primarily in seeds, berries and leaves (Bal *et al.* 2011). Research evidences suggested that *Hippophae rhamnoide* is a gifted plant that act as a natural remedy for the reduction of cardiovascular disease (CVD) risk and other health related problems such as inflammatory diseases, diabetes, cancer and thrombosis (Xu *et al.* 2011).

The main objective of this paper is to evaluate and compare the antibacterial activity of the samples under study. The antibacterial activity was studied with Agar well diffusion method. This paper represents the medicinal importance of the *Peganum harmala*, *Thuja occidentalis* and *Hippophae rhamnoide*.

Material and Methods

Sample Preparations

Peganum harmala, *Thuja occidentalis*, and berry juice of *Hippophae rhamnoide* were collected from Hunza Valley. *Peganum harmala* and *Thuja occidentalis* smoke solution was prepared with the help of Labbert vacuum pump. On other hand, weighed amounts of crushed dried samples (10 g) of *Peganum harmala* and *Thuja occidentalis* were soaked in 100 ml of ethanol for 2 weeks. The berry juice (10 ml) of *Hippophae rhamnoide* was added to 90 ml of ethanol to

obtain desired solution of 100 ml. To make samples in distilled water, 10 g of crushed dried *Peganum harmala* and *Thuja occidentalis* were soaked in 100 ml of distilled water for 2 weeks. The flasks were then labeled accordingly.

Bacterial Cultures

Bacterial cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Klebsiella pneumonia* were prepared to determine anti-bacterial activity of samples against them.

Preparation of 100ppm of standard antibiotic

By dissolving 1 mg of antibiotic ciprofloxacin into 10 ml distilled water, 100 ppm solution of antibiotic was prepared.

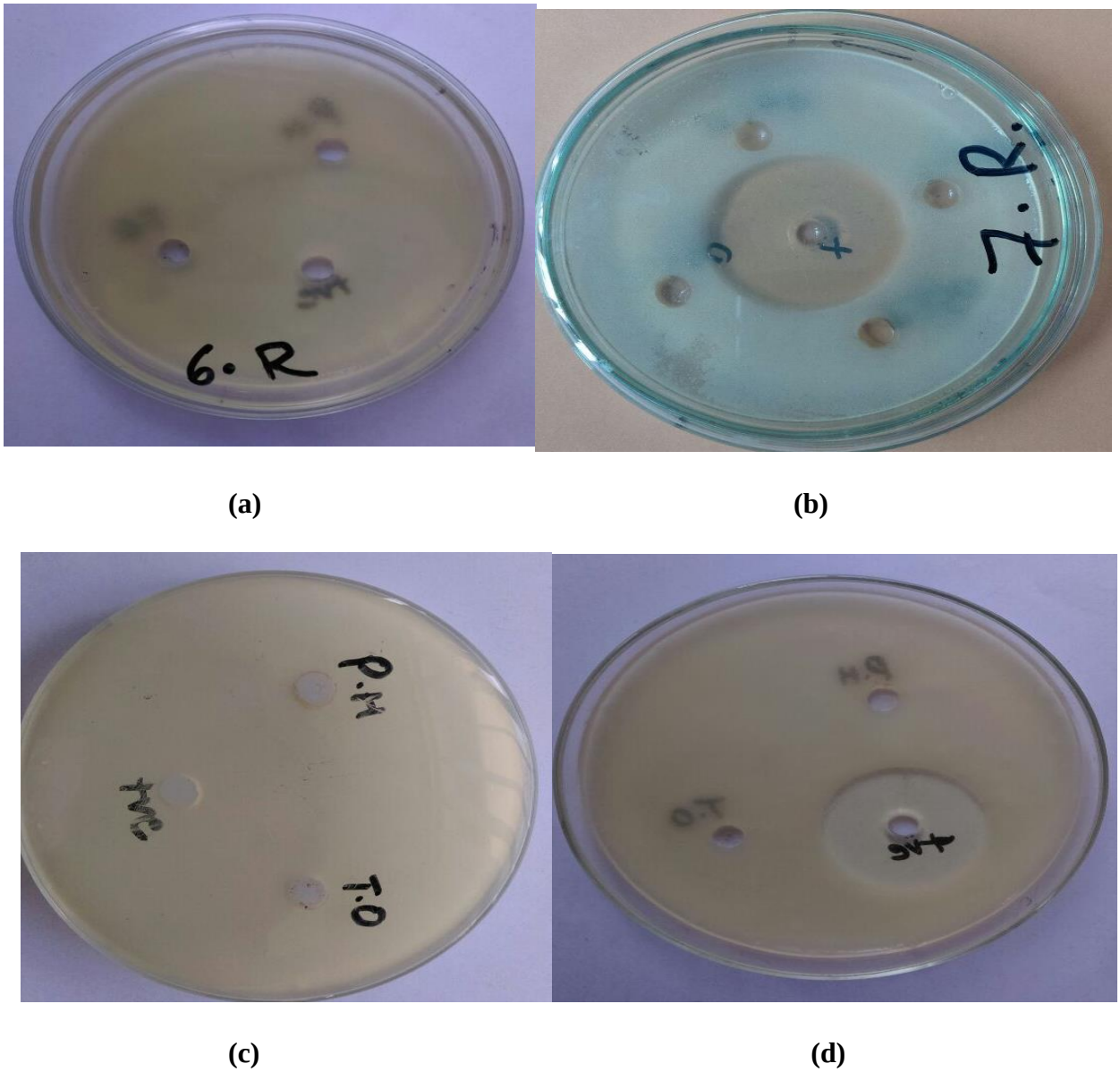
Antibacterial Activity

Agar well diffusion method was used to determine the antibacterial activity of samples. Zones of inhibition of different concentration of the samples of *Peganum harmala*, *Thuja occidentalis* and berry juice of *Hippophae rhamnoide* were observed. The zone of inhibition of standard antibiotic ciprofloxacin was also detected.

Results and Discussion

Agar well diffusion method represents to the movement of molecules through the gel agar matrix. The degree of the movement of molecules under controlled conditions depends on the concentration of the molecules. This phenomenon is used to determine the resistance of bacterial strain to an antibacterial agent. The zone of inhibition size depends on the effectiveness of the antibiotic in stopping the growth of bacterium. Larger will be the zone, if the antibacterial agent is stronger. At 50 µl concentration of the smoke solution of *Peganum harmala* and *Thuja occidentalis*, no zone of inhibition was observed against all the four bacterial strains. Antibiotic ciprofloxacin was used as positive control, showed zone of inhibition of 14 mm against *Proteus mirabilis* and *Pseudomonas pneumonia* in all petri plates of sample solutions. *Peganum harmala* extract in distilled water, showed zone of inhibition of 8 mm with bacterial strain of *Pseudomonas aeruginosa* which is Gram-negative bacteria. *Thuja occidentalis* extract in distilled water, showed zone of inhibition with bacterial strains of *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. It showed zone of inhibition of 6 mm and 8 mm with bacterial strain of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively. *Peganum harmala* and *Thuja occidentalis* extract in ethanol at 100 µl concentration showed zone of inhibition against bacterial strains of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa. Peganum harmala* showed zone of inhibition of 1 mm, 10 mm, and 3 mm against bacterial strains of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively. *Thuja occidentalis* showed zone of inhibition of 1mm, 14 mm and 6 mm against bacterial strain of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively. *Peganum harmala* and *Thuja occidentalis* extract in ethanol at 150 µl concentration showed zone of inhibition against bacterial strains of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. *Peganum harmala* showed zone of inhibition of 3 mm, 9 mm and 4 mm against bacterial strains of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* respectively. *Thuja occidentalis* showed zone of inhibition of 3 mm, 15 mm and 5 mm against bacterial strain of *Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa*

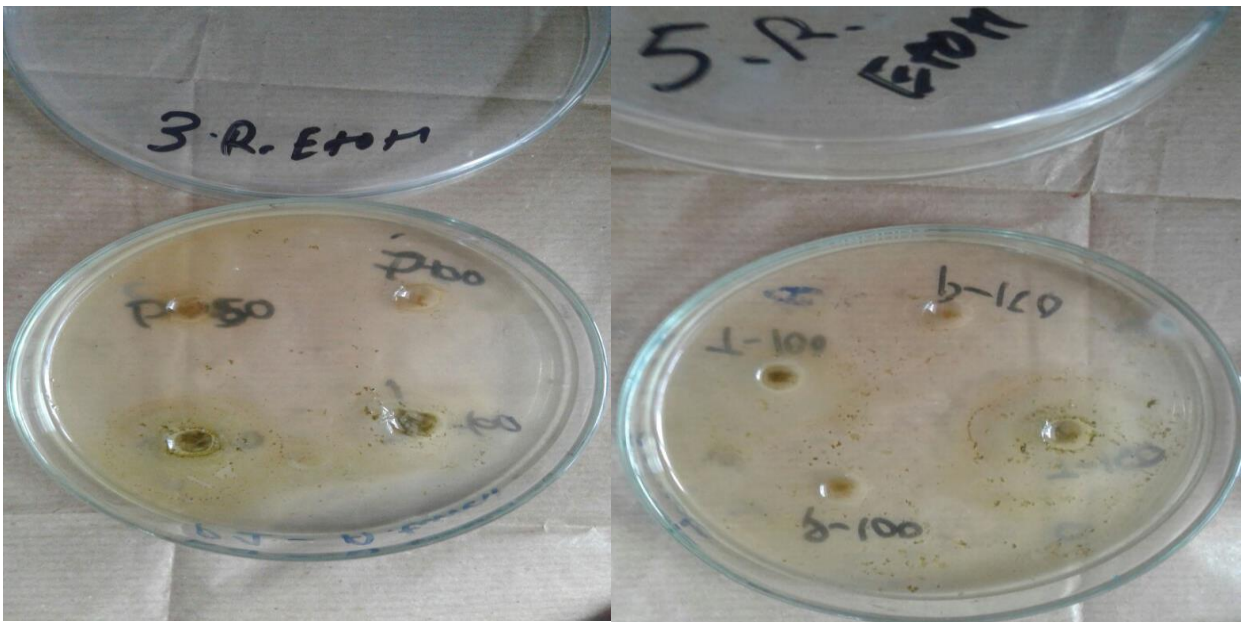
respectively. *Peganum harmala* and *Thuja occidentalis* extract in distilled water showed zone of inhibition of 8 mm with bacterial strain of *Pseudomonas aeruginosa* at 100 µl concentrations. *Hippophae rhamnoide* solution in ethanol showed zone of inhibition of 5 mm at 50 µl concentration and 6 mm zone of inhibition at 100 µl concentration.



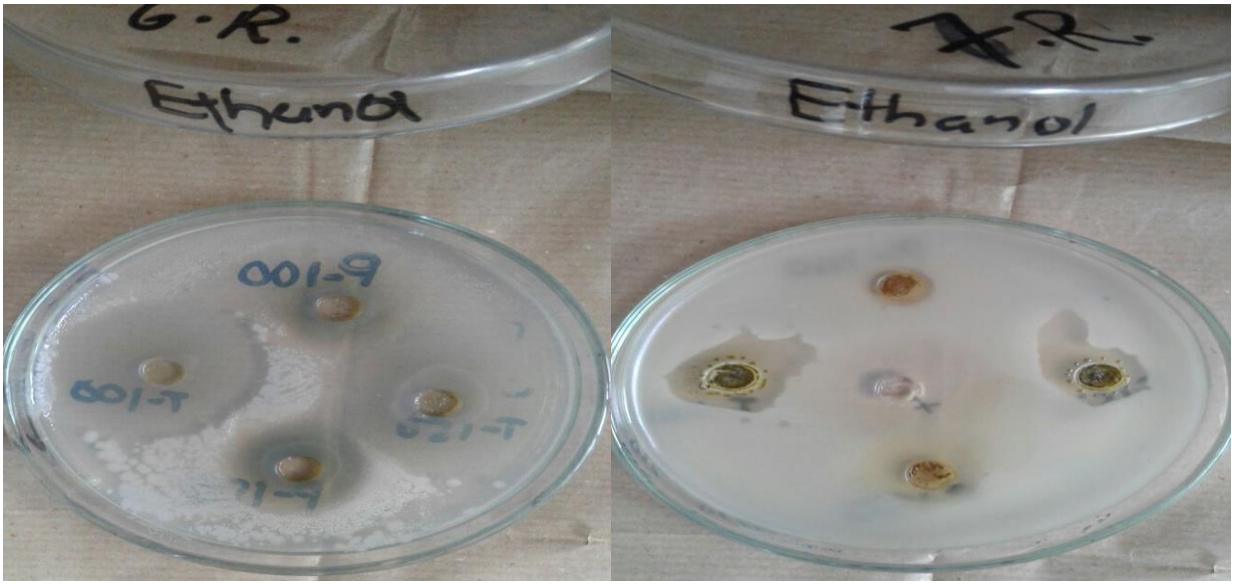
(a) **(b)**

(c) **(d)**

Fig. 1 Sample activity of Smoke solution of 50 µl concentration of *Peganum harmala* and *Thuja occidentalis* against a) *Staphylococcus aureus* b) *Proteus mirabilis* c) *Klebsiella pneumonia* d) *Pseudomonas aeruginosa*



(a) **(b)**



(c) **(d)**

Fig. 2 Sample activity of ethanol extract of *Peganum harmala* and *Thuja occidentalis* (100 µl and 150 µl concentration) against a) *Staphylococcus aureus* b) *Proteus mirabilis* c) *Klebsiella pneumonia* d) *Pseudomonas aeruginosa*

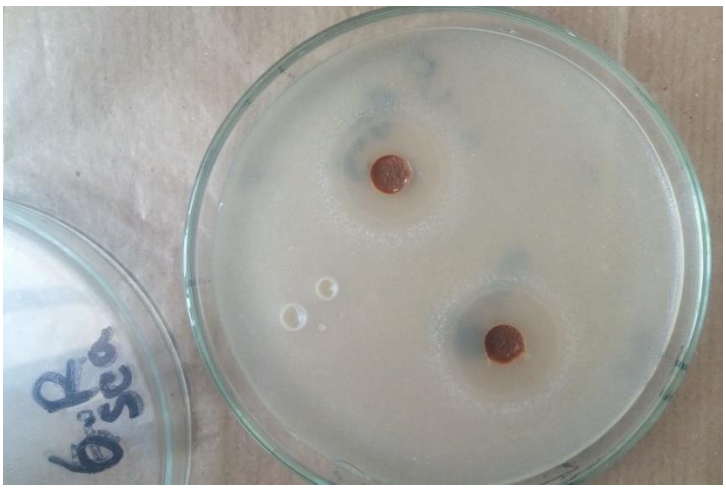
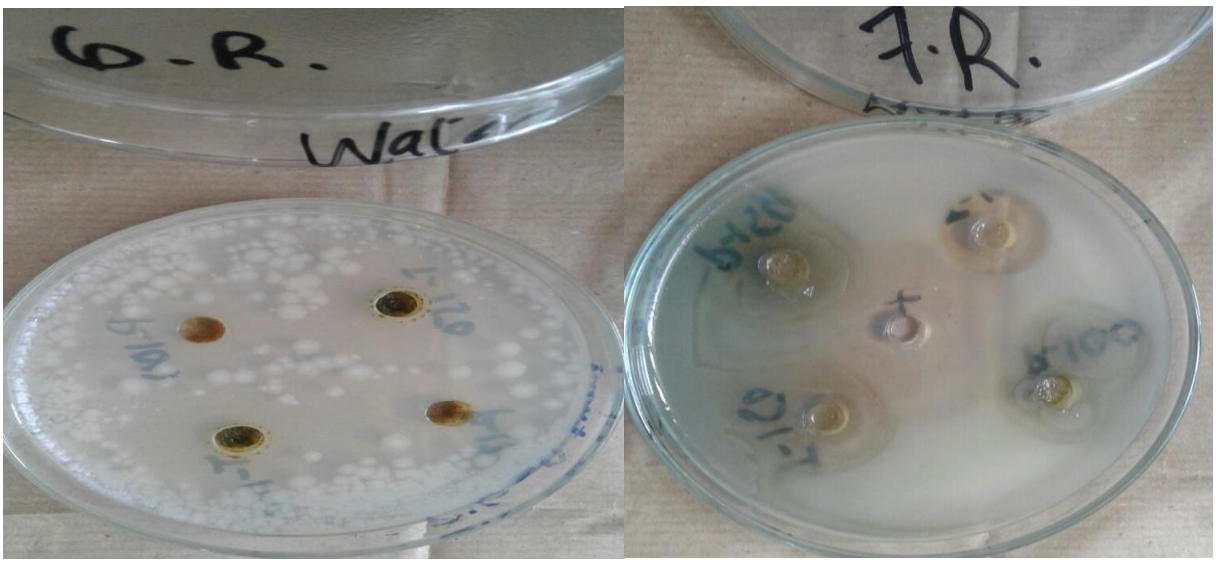


Fig. 3 Sample activity of ethanol extract of *Hippophae rhamnoide* (concentrations of 50 µl and 100 µl) against *Klebsiella pneumonia*

(a) **(b)**



(c) **(d)**

Fig. 4 Sample activity of distilled water extract of *Peganum harmala* and *Thuja occidentalis* (Concentrations of 100 µl and 150 µl) against a) *Staphylococcus aureus* b) *Proteus mirabilis* c) *Klebsiella pneumonia* d) *Pseudomonas aeruginosa*

Table 1: Antibacterial activities shown by samples against bacterial strains at 50 µl concentration of sample.

	Zone of inhibition (mm) at 50 µl concentration			
	In distilled water			**In ethanol**
Name of Strains	**Smoke sol. *P.harmala***	**Smoke sol. *T.occidentalis***	**Antibiotic Ciprofloxacin**	***Hippophae rhamnoide***
Staphylococcus aureus (G.P)	-	-	-	-
Proteus mirabilis (G.N)	-	-	**14mm**	-
Klebsiella pneumonia (G.N)	-	-	-	-
Pseudomonas aeruginosa (G.N)	-	-	**14mm**	**5mm**

Key words: G.P= Gram positive, (G.N) =Gram negative, (-) = no activity

Table 2: Antibacterial activities shown by samples against bacterial strains at 100 µl concentration of sample.

	Zone of inhibition (mm) at 100 µl concentration		
	In distilled water		
Name of Strains	***P.harmala***	***T.occidentalis***	**Antibiotic Ciprofloxacin**
Staphylococcus aureus (G.P)	_	_	_
Proteus mirabilis (G.N)	_	_	_
Klebsiella pneumonia (G.N)	_	**6mm**	_
Pseudomonas aeruginosa (G.N)	**8mm**	**8mm**	**14mm**

Key words: G.P= Gram positive, (G.N) =Gram negative, (-) = no activity

	Zone of inhibition (mm) at 100 µl concentration			
	In ethanol			
Name of Strains	***P.harmala***	***T.occidentalis***	**Antibiotic Ciprofloxacin**	***Hippophae rhamnoide***
Staphylococcus aureus (G.P)	-	-	-	-
Proteus mirabilis (G.N)	**1mm**	**1mm**	-	-
Klebsiella pneumonia (G.N)	**10mm**	**14mm**	-	-
Pseudomonas aeruginosa (G.N)	**3mm**	**6mm**	**14mm**	**6mm**

Key words: G.P= Gram positive, (G.N) =Gram negative, (-) = no activity

Table 3: Antibacterial activities shown by samples against bacterial strains at 150 µl concentration of sample.

	Zone of inhibition (mm) at 150 µl concentration		
	In distilled water		
sName of Strains	***P.harmala***	***T.occidentalis***	**Antibiotic Ciprofloxacin**
Staphylococcus aureus (G.P)	_	_	_
Proteus mirabilis (G.N)	_	_	_
Klebsiella pneumonia (G.N)	_	**8mm**	_
Pseudomonas aeruginosa (G.N)	**10mm**	**10mm**	**14mm**

Key words: G.P= Gram positive, (G.N) =Gram negative, (-) = no activity

	Zone of inhibition(mm) at 150 µl concentration		
	In ethanol		
Name of Strains	***P.harmala***	***T.occidentalis***	**Antibiotic Ciprofloxacin**
Staphylococcus aureus (G.P)	–	–	–
Proteus mirabilis (G.N)	**3mm**	**3mm**	–
Klebsiella pneumonia (G.N)	**9mm**	**15mm**	–
Pseudomonas aeruginosa (G.N)	**4mm**	**5mm**	**14mm**

Key words: G.P= Gram positive, (G.N) =Gram negative, (-) = no activity

Conclusion

The antibacterial activity of plant samples i.e. *Peganum harmala* and *Thuja occidentalis* were compared against the bacterial strains of *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and it was concluded that these plants have maximum antibacterial activity against bacterial strain of *Pseudomonas aeruginosa*, which was Gram-negative bacteria and showed no bacterial activity against *Staphylococcus aureus* bacteria which was Gram-positive bacteria. Thus, these results concluded that the samples under study showed antibacterial activity against Gram-negative bacteria only (*Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa*). In case of berry extract of *Hippophae rhamnoide*, it showed antibacterial activity against *Klebsiella pneumonia* only among the four bacterial strains.

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