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Research Article

TO STUDY ANTIMICROBIAL ACTIVITY OF *PEGANUM HARMALA.*

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ABSTRACT
Plants have been utilized as a natural source of medicinal compounds since thousands of years. Human is using numerous plants and plant derived products to cure from various ailments. These plants are used in traditional Chinese, Ayurveda, Siddha, Unani and Tibetan system of medicine. In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc. Maximum zone of inhibition was seen in methanolic extract of stem explant in comparison to ethanol or chloroform.

Key Words: *Peganum harmala, antimicrobial activity*

INTRODUCTION
Plants are the oldest source of pharmacologically active compounds and have provided mankind with many useful medicines for years. Biologically active plant extracts, pure compounds and their semi-synthetic derivatives serve as a promising therapeutic alternative to the currently available cost intensive options for the treatment of infection due to superbugs. Numerous plants have been screened for anti-infective properties as the probability of finding diverse chemistries have been implicated to serve as leads for the new anti-infective drugs. Thus, antimicrobial research is geared toward the discovery and development of novel antibacterial and antifungal agents (Prashanth and John, 1999).
The systematic screening of plant species with the purpose of discovery of new bioactive compounds is a routine activity in many laboratories. In particular, the search for components with antimicrobial activity is gaining increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms (Cowan *et al.,* 1999). Hence, there is a constant need for new and effective therapeutic agents. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs (Balick and Cox, 1997).
Peganum harmala, looking at the medicinal properties of the plant was selected for the proposed study to assess possible antimicrobial activities (Fig 1). (Family- Nitrariaceae) a bushy herb, 2.5ft. High, found in Ladakh and Kashmir, up to 1650 meter and in Punjab, upper Gangetic plain, western Bihar, Rajasthan, Gujarat, Deccan and Konkan. *Peganum harmala* has abortifacient, antioxidant, anticancer, insecticidal and antimutagenic properties (Almagboul *et al.,* 1985).

Review of Literature
A number of plants have been screened for their antimicrobial activity. The antimicrobial principles and their distribution have been extensively reviewed by Nickell (1959) who surveyed 174 plants belonging to 157 families of vascular plants. Antimicrobial activity of various plant parts has also been observed by several workers viz. *Drynaria quercifolia* (Ramesh *et al*., 2001); *Begonia malabarica* (Ramesh *et al*., 2002). Similarly antibacterial activity of different plant parts has also been observed by many workers (Parekh *et al*., 2006).Goswami and Reddi (2004) studied antimicrobial activity of isorhamnetin and kaempferol extracted from *Cassia angustifolia* against *Staphylococcus, Escherischia coli* and *Candida albicans*. Thus, it can be concluded that antimicrobial activity in plants is neither a generic character nor a family one but it is the feature of active principles present in the plant.

MATERIALS AND METHODS

Plant collection

Fresh plants or plant parts were collected from Ganganagar belt of Rajasthan state. Fresh plant material was washed under running tap water, air dried, homogenized to fine powder, and stored in tightened light protected containers**.**

Figure 1: Collected plant of *Peganum harmala*

Chemical and reagents

The entire chemicals used in this study were of analytical grade obtained from Sigma-Aldrich, Fluka, Loba, Merck, Renkem, and Sisco Research laboratory (SRL).

Glassware and plastic ware

The glassware used was of Borosil, Schott-Duran (Germany). Plastic were used of Tarsons, Taurus Scientific (USA). Petri plate was used of Hi-Media and Axygen. Vials (1ml) and micropipette tips were of Tarsons.

Equipments

The following equipment's were used in present study-

- YORCO VERTICAL Autoclave-YORK scientific industries PVT. LTD.(India)
- Laminar airflow-kirloskar Electrodyne
- Micropipette
- Water purification system-Millipore
- Hot plate- ACE-171, Size:10*12*6
- pH meter- Mettler Toledo, MPC-227, control dynamics
- Electron balance-WENSAR
- Oven-SEW India

Preparation of Extract

Plant parts (leaf, stem) were washed, air dried and grinded into powder form for preparation of extract. Aqueous plant extract was prepared by macerating the powdered plant sample with 50 ml sterile distilled water. The macerate was filtered and filtrate was centrifuged at 7000 rpm for 10 minutes. Supernatant obtained after centrifugation was heat sterilized at 1200 ºC for 30 minutes. Extract obtained (Fig 2) was preserved aseptically. Solvent extracts of plant parts were prepared in 70% methanol using Soxhlet extraction for 72 hours and extract was preserved at 40 ºC in air tight bottles. They were air dried and dissolved in Dimethyl sulfoxide (DMSO) in 1mg/1ml concentration and stored in refrigerator.

Figure 2: Extract of *Peganum harmala*

The reference antibiotic discs

Antibacterial activity of the test samples of plant extract was compared with the antibiotics known to be effective against the test bacteria in their established doses. Amoxycillin was used for bacteria.

Media preparation for nutrient agar media

The bacterial cultures of both gram positive and gram negative bacteria were maintained on nutrient agar medium (agar-agar 15 g, beef extracts 3 g, sodium chloride 5 g and peptone 5 g in one liter distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every antibacterial assay by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

Preparation of Inoculum

The antibacterial activity was tested by Whatman filter paper disc method. Stock cultures were maintained at 4°C on the slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth that were incubated with agitation for 24 hours at 37°C.

Test microorganisms

The bacterial strains studied are ***Pseudomonas aeruginosa*** and ***Escherichia coli.*** Microorganisms were maintained at 4 °C on nutrient agar slants. These test organisms were clinical isolates obtained from patients diagnosed for having bacterial infections and procured from the SMS Hospital Jaipur.

Antibacterial screening

The filter paper disc method was used for screening the extract for antibacterial activity. Standard size Whatman filter paper disc (6.0 mm diameter) were sterilized in an oven at 140°C for one hour, saturated with plant extracts such as stem leaf and air dried at room temperature to remove any residual solvent that might interfere with the determination of activity. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using saline solution) and air dried to remove the surface moisture. The thickness of agar medium was kept equal in all the pertriplates and the standard disc (streptomycin) was used as a control. Before incubation, the petriplates were placed for one hour in a cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured.

All the experiments were done in ten replicates and activity index was calculated for each of these using the formula .Leaves were the best source for assessing the antimicrobial activity.

$$\text{Activity index (A.I.)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

RESULTS

Table 1: Antimicrobial activity of *Peganum harmala* in methanolic, ethanolic, chloroform extract of stem and leaf

Bacterial species	Zone of inhibition (mm)								
	Standard Amoxycillin	Methanolic extract				Ethanolic extract			
		Leaves	AI	Stem	AI	Leaves	AI	Stem	AI
Escherichia coli	**7mm**	**7±0.57**	**1**	**8±1.00**	**1.1**	**5.66±0.33**	**0.7**	**-**	**-**
Pseudomonas aeruginosa	**14mm**	**6.33±0.33**	**0.4**	**-**	**-**	**-**	**-**	**-**	**-**
		Chloroform							
		Leaves	AI	Stem	AI				
		-	-	-	-				

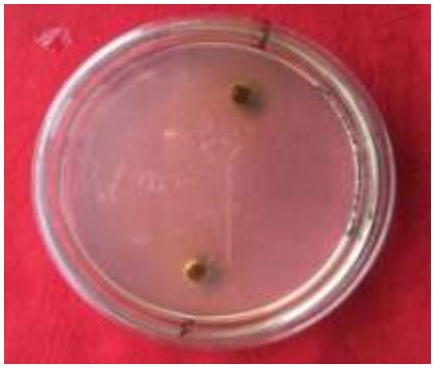

Figure 3: Inhibition zone formed in methanolic extract of *Peganum harmala*

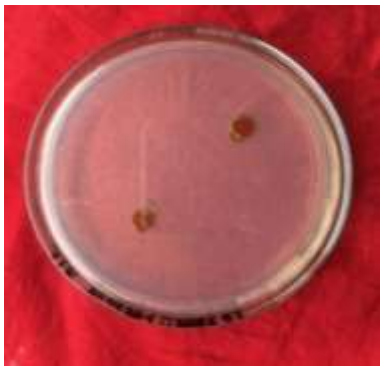

Figure 4: Inhibition zone formed in ethanolic extract of *Peganum harmala*

Figure 5: Inhibition zone formed in chloroform extract of *Peganum harmala*

The plant showed good results in antibacterial activity in Methanolic extract. Traditional herbal medicines must be granted the benefits of modern science technology on a global scale. The plant contains

phytomedicine, antioxidant and anti-depressive agents which in future will prove to be a boon in the field of medicine.

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REFERENCES

Almagboul AZ, Bashir AK, Farouk A, Salih AKM (**1985**). Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity. *Fitoterapia* **56**, 331-337.

Balick MJ and Cox PA (1997) Ethnobotanical Research and Traditional Health Care in Developing Countries. *Medicinal Plants for Forest Conservation and Health Care*, **92**, 12-24.

Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiological Review*. 1999 Oct **12**(4) 564–82.

Goswami A, Reddi A (2004). Antimicrobial activity of flavonoids of medicinally important plant *Cassia angustifolia* in vivo and in vitro. *Journal of Phytological Research* **17** 179-81. 14.

Jigna Parekh, Nehal Karathia and Sumitra Chanda (2006). Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegate* L. bark l *African Journal of Biomedical Research*, **9** *53 – 56*

Mahmoudian M, Jalipour H, Dardashti PS (2002). Toxicity of *Peganum harmala*: review and a case report. *Iranian Journal of Pharmacological Therapeutics.* **1**(1) 1-4.

Ramesh N, MB Viswanathan, A Saraswathy, K Balakrishna, P Brindha, P Lakshmanaperumalsamy (2001). Phytochemical and antimicrobial studies on *Drynaria quercifolia* Fitoterapia **72** 934_936**.**

Prashanth D, John S (1999). Antibacterial activity of *Peganum harmala*. *Fitoterapia.* **70** 438–9.

Ramesh N, MB Viswanathan, A Saraswathy, K Balakrishna, P Brindha, P Lakshmanaperumalsamy (2002). Phytochemical and antimicrobial studies of *Begonia malabarica**ous.*** *Journal of Ethnopharmacology* **79** 129–132