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In vitro evaluation of antibacterial activity of leaf and flower extracts of *Parthenium hysterophorus* L.

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ABSTRACT

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by bacteria has increased. Hence the present study was carried out with an objective to investigate the antibacterial potential of leaf and flower extracts of *Parthenium hysterophorus* L. The aim of the study is to assess the antibacterial activity and to determine the zone of inhibition of methanol and acetone leaf and flower extracts on some bacterial strains. The *in vitro* tests were conducted for their antibacterial activities against two Gram positive *i.e., Bacillus subtilis, Staphylococcus aureus* and two Gram negative *i.e., Escherichia coli*, *Pseudomonas aeruginosa* bacteria with agar disc diffusion method. Zone of inhibition of leaf and flower methanol and acetone extracts were recorded and compared with standard drug, streptomycin. The results showed that there was remarkable inhibition of the bacterial growth against the tested organisms. The bacterial growth inhibitory activity may be due to the presence of various secondary metabolites present in the tested plant. Hence, it is suggested that the plant can be further investigated to discover the bioactive compound that may serve as lead compound in the development of new pharmaceutical research activities.

Key words: *Parthenium hysterophorus,* Antibacterial activity, Agar disc diffusion method, Methanol and acetone solvents, leaf and flower extracts.

INTRODUCTION

Parthenium hysterophorus L. (Asteraceae) is a poisonous and problematic weed and posing a serious threat to crop cultivation and also to human and animal health. It is a noxious weed native to Tropical America. It has now naturalized in several tropical and subtropical parts of the world (Kohli *et al*., 2009). *Parthenium* weed has become widely distributed throughout India (Sushilkumar, 2012).

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Parthenium hysterophorus L. can grow anywhere and invade all types of territory lands and cause substantive losses in the yield of agricultural crops (Auld *et al*, 1983; Jayachandra, 1971; Krishnamurthy *et al*, 1975). The normal height of this erect plant is up to 1 m but under favorable conditions the height may reach up to 2 m having deeply penetrating taproot with many finely branched feeding roots and an angular, longitudinally grooved and profusely branched hairy stem (Parsons and Cuthbertson, 2001; Bhatt *et al*., 2012). It is light green with branching stems, finely lobed leaves, 3-20 cm long and 2-10 cm wide. Once stem elongation is initiated, smaller leaves are produced and the plant becomes multi-branched in its extremities. The plant flowers appears 4-8 weeks after germination and flowering continue until drought or frost kills the plant. In some areas, more than 340 million *Parthenium* seeds can be present per hectare in the surface soil, compared to 120,000 native grass seeds. *Parthenium* weed normally germinates in spring and early summer. It is

thought that most seed germinate within two years if conditions are suitable, although a portion of buried seed may remain viable for several years (Butler, 1984).

The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in the morbidity and mortality (Williams, 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs. Plants play an important role in modern medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious diseases. Hence, further exploration of plant antimicrobials need to be explored (Parekh et al 2007). Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants. Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity even though it play an important role in the modern medicine (Bhat *et al* 2009).The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan 2003). It is well known that even the most synthetic drugs have their origin from plant products (Sofowara 1982). On the basis of this background, in-vitro antibacterial activities of the leaf and flower extracts of *Parthenium hysterophorus L.* from methanol and acetone solvents were tested against clinically important pathogens.

Material and Methods

Collection and Processing of plant material:

Parthenium hysterophorus plants were collected from Hasanparthy village of Warangal district which is nearer to Kakatiya University and brought to the laboratory. The leaves and flowers were separated from the plant by plucking and shade dried and powdered using a blender. This powdered form was stored in an airtight container separately for further use.

Preparation of solutions for antimicrobial activity:

The methanol and acetone extracts of leaf and flower of the *Parthenium hysterophorus* were prepared as 10mg/10ml (0.1%), 5mg/10ml (0.05%) and 2.5mg/ml (0.025%) in DMSO (which did not influence the microbial growth).

Microbial samples:

Four microbial species were selected for analysis. The bacteria were taken from the Department of Microbiology, Kakatiya University, Warangal, Telangana, India. The bacteria selected were *Escherichia coli* ATCC 2343, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633.

Antibacterial activity experiment with Agar well disc diffusion method:

Antibacterial activity was done by Agar well disc diffusion method. The selected bacterial strains were inoculated into 10ml of sterile nutrient broth, and incubated at 37^0 C for 1 hour. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Five Agar wells were prepared with the help of sterilized cork borer with 6mm diameter. Using a micro pipette, different concentrations of leaf and flower extracts of *Parthenium hysteophorus* separately was added to the three wells in the plate, Standard antibiotic Streptomycin (1mg/ml) was used as standard drug in the middle well, and simultaneously controls were maintained employing 0.1ml of methanol and acetone separately in one of the five wells to observe the solvent effect. The plates were incubated at 37^0 C for 24 hours in Laminar flow ultra-clean air unit. The antibacterial activity was determined by measuring (in mm.) the diameter of inhibition zones after the incubation time. All the experiments were carried out in triplicate, and the results were recorded.

Results and Discussion

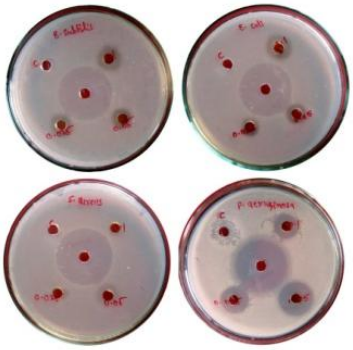
The usage of this plant for medicinal purpose has been reported by several researchers. Four bacterial strains were used as taxonomical representatives out of which two from Gram positive *Bacillus subtilis, Staphylococcus aureus* and two from Gram-negative

Table-1. Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in methanol against Human pathogenic bacteria.

S. No.	Pathogen	Zone of inhibition in mm				
		Std	Ctrl	0.025%	0.05%	0.1%
1	***Escherichia coli***	23	0	04	05	08
2	***Pseudomonas aeruginosa***	22	0	06	07	09
3	***Staphylococcus aureus***	19	0	07	08	10
4	***Bacillus subtilis***	21	0	05	07	09

Escherichia coli, *Pseudomonas aeruginosa* to evaluate the effect of candidate antimicrobial components against specific target microbes. The methanol and acetone extracts of *Parthenium hysterophorus* leaf and flower exhibited the antibacterial activity against four isolates of bacteria. The antibacterial activities of *Parthenium hysterophorus* could be compared favourably with that of standard antibiotic (streptomycin). Extracts of *Parthenium hysterophorus* exhibited significant antibacterial activity against different bacterial genre in agar well diffusion assay.

Figure-1..Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in metahnol against human pathogenic bacteria.



Antibacterial activity of *Parthenium hysterophorus* and standard antibiotics such as streptomycin, depicting zone of inhibition on agar-well diffusion method is shown as Fig. 1, 2, 3 & 4. The standard antibiotics, streptomycin was effective against most of the organisms tested with zone of inhibition higher that of *Parthenium hysterophorus*. However significant inhibition of the plant extracts on various bacterial strains were recorded.

The inhibition zone values of the plant leaf extracts in methanol ranges from 4 to 8 mm against *Escherichia coli*, 6 to 9 mm against *Pseudomonas aeruginosa,* 7 to 10 mm against *Staphylococcus aureus* and 5 to 9 mm against *Bacillus subtilis* **(Table-1, Fig-1**). The inhibition zones of the plant leaf extracts in acetone ranges from 7 to 11 mm against *Escherichia coli*, 7 to 15 mm against *Pseudomonas aeruginosa,* and 5 to 9mm against *Staphylococcus aureus* and 6 to 12 mm against *Bacillus subtilis* (**Table-2, Fig-2**).

The inhibition zone values of the plant flower extracts in methanol ranges from 4 to 17 mm against *Escherichia coli*, 8 to 13 mm against *Pseudomonas aeruginosa,* and 4 to 9 mm against *Staphylococcus aureus* and 5 to 12 mm against *Bacillus subtilis* (**Table-3, Fig-3**).

The inhibition zone values of the plant flower extracts in acetone ranges from 9 to 17 mm against *Escherichia coli*, 9 to 13 mm against *Pseudomonas aeruginosa,* and 5 to 9 mm against *Staphylococcus aureus* and 5 to 12 mm against *Bacillus subtilis* (Table-4, Fig-4).

The results from this study strongly suggest that *Parthenium hysterophorus* plant extracts possess bactericidal activity. The antibacterial activity of this plant extracts presumed to be due to destruction of

Table-2. Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in acetone against Human pathogenic bacteria.

S. No.	Pathogen	Zone of inhibition in mm				
		Std	Ctrl	0.025%	0.05%	0.1%
1	***Escherichia coli***	24	0	07	09	11
2	***Pseudomonas aeruginosa***	26	0	07	09	15
3	***Staphylococcus aureus***	27	0	05	07	09
4	***Bacillus subtilis***	24	0	06	08	12

Figure-2. Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in acetone against human pathogenic bacteria

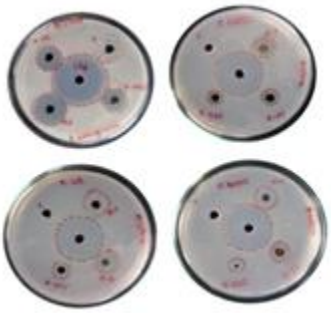


Table-3. Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in methanol against Human pathogenic bacteria.

S. No.	Pathogen	Zone of inhibition in mm				
		Std	Ctrl	0.025%	0.05%	0.1%
1	*Escherichia coli*	24	08	04	10	17
2	*Pseudomonas aeruginosa*	25	0	08	10	13
3	*Staphylococcus aureus*	25	0	04	05	09
4	*Bacillus subtilis*	24	06	05	12	12

Figure-3. Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in methanol against human pathogenic bacteria.

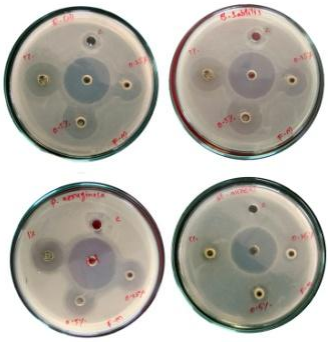
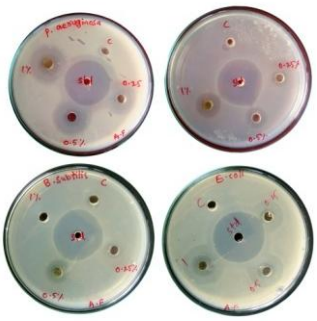


Table-4. Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in acetone against human pathogenic bacteria.

S. No.	Pathogen	Zone of inhibition in mm				
		Std	Ctrl	0.025%	0.05%	0.1%
1	*Escherichia coli*	22	0	09	12	17
2	*Pseudomonas aeruginosa*	24	0	09	11	13
3	*Staphylococcus aureus*	25	0	05	07	09
4	*Bacillus subtilis*	24	0	05	09	12

Figure-4. Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in acetone against human pathogenic bacteria.



bacterial cell wall and leakage of cytoplasmic constituents by the active principle present in them. Hence the active principle present in this plant can be exploited as a natural antibacterial agent for the production of new drugs against various infections.

Isolation of different extracts and many other chemical compounds from plants with efficient antimicrobial activities can be of enormous impact in the healthcare. Medicinal action of the plants is defined to some important chemical compounds that

pass on a definite physiological action on the human body (Satish et al 2008; Choudhury et al 2012). In concern to negative aspect of the conservative medicine, the utilization of natural products as an alternate way to the convectional action in healing of different ailments has been increased in the previous few decades (Saeed et al 2007). The interest in this specific biological activity of *Parthenium hysterophorus* is based on different evidences of its effectual use for the alleviation of many diseases.

Conclusion

The methanol and acetone extracts of *Parthenium hysterophorus* showed good antibacterial activity. In our study it is accomplished that extracts obtained from the leaf and flowers of *Parthenium hysterophorus* has shown maximum antibacterial activity. The chemical constituents of the extract which is accountable for this effect should be investigated further.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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