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Study on antibacterial activity of *Parthenium hysterophorus* L. leaf and flower extracts

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ABSTRACT

The aim of the study was to find the effect of the antibacterial activity. Leaf and flower extracts in methanal and acetone of Parthenium hysterophorus were tested in vitro for their antibacterial activities against, Gram positive Bacillus subtilis, Staphylococcus aureus; Gram-negative Escherichia coli, Pseudomonas aeruginosa with the agar diffusion method. Methanol and acetone was the best solution for extracting the effective antibacterial materials from the Parthenium hysterophorus used in this experimental and compared with standard drug, streptomycin. The highest antimicrobial potentials were observed for the methanol and acetone extracts of Parthenium hysterophorus. In the present study show the importance of in producing new bioactivity compounds having antibacterial activity.

Key words: *Parthenium hysterophorus leaf and flower extract, Antimicrobial activity, Agar well diffusion method.*

INTRODUCTION

Parthenium hysterophorus L. (Heliantheae: Asteraceae) is a poisonous and problematic weed, is now posing a serious threat to crop cultivation and also to human and animal health. It is commonly known as congress grass, feverfew, ragweed, parthenium or white top. 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 and no state is able to say that they of free of *Parthenium* weed (Sushilkumar, 2012).

Medicinal properties of leaf extracts have been reported by many workers (Kirtikar KR, 1991). *Parthenium hysterophorus L.* (Asteraceae) is labeled as serious weed in the agricultural crops. It can grow anywhere and invade all types of pasture lands and cause substantive losses in the yield of agriculture (Auld BA *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 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).

Recently scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore the search for new drugs from plants continues to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease hence, further exploration of plant antimicrobials need to occur (Parekh et al 2007). Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also 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). There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Cowan 1999). In the present study was undertaken to evaluate antibacterial activity of the *Parthenium hysterophorus.*

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MATERIALS 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.

Solvent extraction:

250 grams of plant material was soaked in 500 ml of methanol and acetone separately for 24 hours at room temperature of 25 to 30 degrees Celsius. The solvents were filtered with the help of a filter paper and the filtrate was evaporated at room temperature. This is used as a crude extract and is diluted to required concentrations as required according to the experiments conducted.

Preparation solution for antimicrobial activity:

The methanol and acetone extract 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 analyzed. The bacteria were taken from the Department of Microbiology, Kakatiya University,

Warangal, Telangana, India. The bacterias are *Escherichia coli* ATCC 2343, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633.

Antibacterial activity experiment with Agar well diffusion method:

Antibacterial activity was done by Agar well 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 effects. 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.) diameter of inhibition zones after the incubation time. All the experiments were carried out in triplicate, and the results were recorded.

RESULTS AND DISCUSSION

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 evidence of its effectual use for the alleviation of many diseases as referred in literature.

The usage of this plant for medicinal purpose has been reported by several researchers. Four bacterial strains were used as taxonomical representatives such as Gram positive *Bacillus subtilis, Staphylococcus aureus;* Gram-negative *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. Antibacterial activity of *Parthenium hysterophorus* and standard antibiotics such as streptomycin, depicting zone of inhibition on agar-well diffusion method is also 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*.

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 No.1, Fig. 1**). The inhibition zone 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 No. 2, Fig. 2**).

The inhibition zone 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 No. 3, Fig. 3**). The inhibition zone 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 No. 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 bacterial cell wall and leakage of cytoplasmic constituents by the active principle present in them. The bioactive compound that is isolated and identified is parthenin and the effect of this compound has to be compared with the crude extracts that were tried in this present work. 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.

Table No. 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

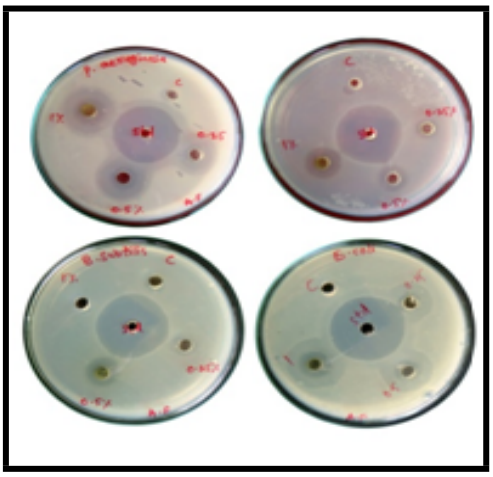

Fig. 1: Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in metahnol against human pathogenic bacteria.

Table No. 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

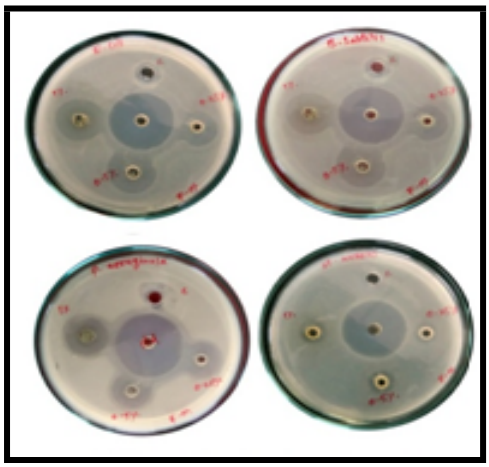

Fig. 2: Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* leaf extract in acetone against human pathogenic bacteria.

Table No. 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

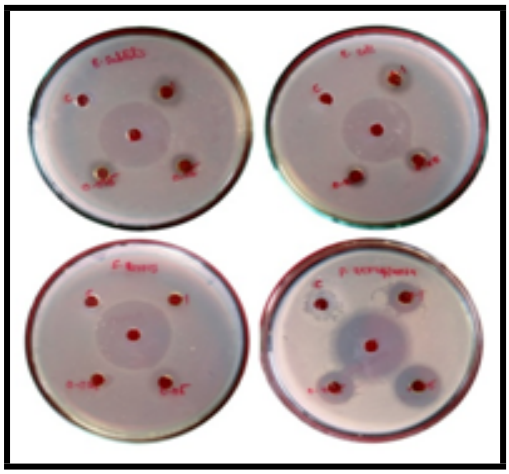

Fig. 3: Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in metahnol against human pathogenic bacteria.

Table No. 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

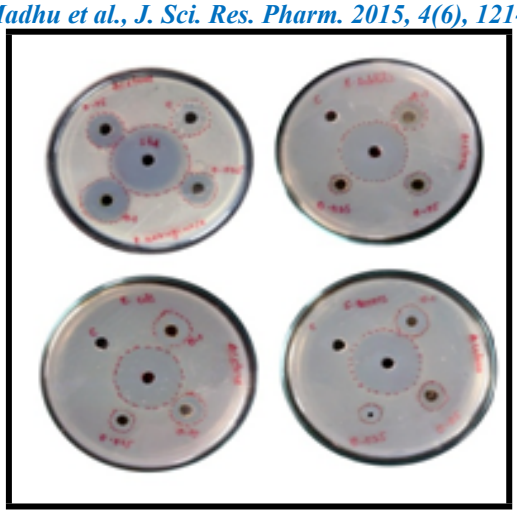

Fig. 4: Photograph showing Zone of inhibitory activity of *Parthenium hysterophorus* flower extract in acetone against human pathogenic bacteria.

CONCLUSION

The methanol and acetone extracts of *Parthenium hysterophorus* showed good antimicrobial activity. Our study it is accomplished that extracts obtained from of leaf and flowers of *Parthenium hysterophorus* has shown maximum antimicrobial activity. The chemical constituents of the extract which is accountable for this effect would be investigated for these pharmacological in our phytochemical studies however to be performed in upcoming.

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