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ANTIMICROBIAL POTENTIAL OF A WEED PLANT *PARTHENIUM HYSTEROPHORUS*: AN INVITRO STUDY

Dr. Sunita Sharma^{*1}, Nidhi Gupta¹,

¹Department of Biotechnology Madhav Institute of Technology and Science, Gwalior, 474005, Madhya Pradesh, India

ABSTRACT

Extracts in organic solvents viz. Methanol, Dichloromethane, Petroleum ether and Acetone and Aqueous extract of a herbal medicinal weed plant - *Parthenium hysterophorus* was evaluated for its antibacterial activities against *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Micrococcus luteus* and *Citrobacter freundii* and antifungal activities against *Microsporum gypseum*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Aspergillus niger*, *Mucor*. These were carried out by taking the organic and aqueous extracts of inflorescence of the plant at a concentration of 1000 μ g, 500 μ g, 250 μ g, 125 μ g/ml and their activities were recorded by estimating zones of inhibition as produced by agar solid-diffusion method on Mueller-Hinton agar media for bacteria and on Sabouraud Dextrose agar media for fungus. All of the extracts exhibited different antibacterial and antifungal activities and the activities varied from solvent to solvent and the concentrations of solvent. All types of organic extracts and aqueous extract exhibited greater antibacterial activity but surprisingly all the extracts except methanol were inactive against *Klebsiella pneumoniae*. The methanolic extract of inflorescence of tested plant showed highest antibacterial activity against all the bacterial strains. *P.aeruginosa* and *C.ferudii* were inhibited by all types of extracts whether organic or aqueous. None organic extracts showed antifungal activity, the aqueous extract of inflorescence exhibited antifungal activity at concentrations of 1000 μ g/ml and 500 μ g/ml. The antibacterial and antifungal activities were compared with positive control chloremphenicol and nystatin respectively.

Key words: *Parthenium hysterophorus*, Antibacterial, Antifungal, Chloremphenicol, Nystatin, DMSO, CFU.

Correspondence to Author

Dr. Sunita Sharma

Department of Biotechnology
Madhav Institute of Technology and
Science, Gwalior, 474005, Madhya
Pradesh, India

Email: Sunitasharma75@yahoo.co.in

INTRODUCTION

In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally [1]. An upward trend has been observed in the research on herbals. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases [2]. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines [3]. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species [2].

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses [1]. *Parthenium hysterophorus*, a dreaded weed plant is evaluated for their therapeutic efficacies in this study.

Parthenium hysterophorus L. an aggressive and exotic weed of family Asteraceae, at present has occupied almost all parts of India [4]. It is native to subtropics of North and South America [5] and was accidentally introduced in subcontinent in 1955 through imported food grains [6, 7, 8, 9]. It is also known as congress weed, carrot weed, star weed, white top, chatak chandani, bitter weed, ramphool and gajar grass[10]. *Parthenium hysterophorus* is an erect herb with alternate, deeply dissected leaves, growing upto 2m tall with much branched inflorescences. Bearing white flower heads and numerous obovoid, smooth and black achenes[11].

Adverse effects of *Parthenium* not only on human beings but also on animal health have been well documented. It is known to cause asthma, bronchitis, dermatitis and hay fever in man and livestock [12]. The chemical analysis has indicated that all the plant parts including trichomes and pollen contain toxins called sesquiterpene lactones. The major components of toxic being 'parthenin' and other phenolic acids [12, 13].

In ancient Indian literatures, it is written that every plant on this earth is useful for human beings, animals and also for other plants [14, 15, 16] although *Parthenium* is considered as toxic plant but many medicinal, allelopathic [17, 18, 19, 20] and industrial [21] uses have been well documented in literatures. In Homoeopathy, whole plant, gathered when it flower, is used for preparing drug. The mother tincture is obtained by expressing the juices of the whole plant, gathered fresh and mixing it with twenty parts of alcohol [22]. *Parthenium* is also reported as promising remedy against hepatic amoebiasis [23]. South American Indians uses the decoction of roots to cure amoebotic dysentery [24], whereas parthenin, a toxin of *Parthenium*, is found pharmacologically active against neuralgia and certain types of rheumatism [25]. In book titled 'Compendium of Indian Medicinal Plants' 1991 *Parthenium hysterophorus* is described as medicinal plant and reported that parthenin induced dosedependent damage to human leucocyte chromosomes *in vitro*[26]. It also induced micronuclei formation in polychromatic erythrocytes of mice. It's decoction has been used in traditional medicine to treat fever, diarrhoea, neurologic disorders, urinary infections, dysentery and malaria and as emmenagogue [27]. Sublethal doses of parthenin exhibited antitumour activity in mice and that the drug could either cure mice completely or increase their survival time after they had been injected with cancer cells [28].

The present investigation has been done to evaluate the antibacterial and antifungal potency of *Parthenium hysterophorus* against important human pathogenic bacteria and fungus.

MATERIALS AND METHODS

Plant Material

Fresh inflorescence of *Parthenium hysterophorus* were collected from the farmyard of Madhav Institute of Technology and Science. The plants were identified taxonomically by the Botany Department of Jiwaji University, Gwalio, Madhya Pradesh (M.P), India. Fresh Inflorescence was washed thoroughly with distilled water and then

shade dried. The dried inflorescence were powdered with the help of mixer grinder and further used for extract preparation.

Preparation of the extract

15 g of the powder is mixed with 100ml of solvents like Methanol, Acetone, Dichloromethane, Petroleum ether and distilled water in a 250ml conical flask and was kept on rotary shaker at 25°C for 48h. The suspension was filtered through a Whatman no.4 filter paper and filtrate was evaporated to dryness by vacuum dryer at 40°C. The extracted powder was resuspended in the Dimethyl sulfoxide (DMSO) at a concentration of 1mg/ml before it was tested for the antimicrobial activity.

Test Microorganisms

Bacterial strains : Six species of human pathogenic bacteria were used for testing, two gram positive (*Bacillus cereus*, *Micrococcus luteus*) and four gram negative (*Escherichia Coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Citrobacter freundii*) obtained from Microbiology Laboratory of Department of Biotechnology, Madhav Institute of Technology and Science, Gwalior (M.P, India). Each Organism was maintained in Mueller –Hinton Agar (MHA). Inocula were prepared by adding an overnight culture of the organism in nutrient broth. The cells were allowed to grow until they obtain the McFarland standard 0.5 (approximately 10^8 CFU/ml).

Fungal Strains: Five fungal strains were used in this study *Microsporum gypseum*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Aspergillus niger*, *Mucor* these potentially pathogenic strains were obtained from Botany Department of Jiwaji University where strains were maintained by regular subculturing. Loops full of all fungal cultures were inoculated in the Sabouraud dextrose broth at 37°C for 72h.

Antimicrobial Assay

Antimicrobial activity of the various solvents extracts from inflorescence of *Parthenium hysterophorus* obtained by infusion and maceration was determined by agar solid diffusion method. The extracts, in concentration 1000µg, 500µg,

250µg, 125µg/ml, were dissolved in DMSO for a final concentration.

Agar –Solid diffusion method

Antimicrobial activity assay of different Aqueous extract and solvent extracts; Methanol, Petroleum Ether, Acetone, Dichloromethane were determined by disc diffusion method on Mueller Hinton Agar and Sabouraud dextrose Agar (SDA) for bacterial and fungal strains respectively. The antimicrobial spectrum of the extracts was determined qualitatively for the bacterial and fungal species in terms of zone sizes around wells cut in plates of Sabouraud Dextrose and Mueller Hinton Agar. 100µl of various microbial species were spreaded on SDA and MHA containing 20µl of the tested material dissolved in DMSO in wells. The bacterial and fungal plates were incubated for 24h at 37°C and 28°C respectively and zone of inhibition if any around the wells were measured in millimeter (mm).

Positive and Negative Control

Chloremphenicol (30µg/ml) was used as a positive control for bacteria. Nystatin was used as a positive control for fungus. DMSO was used as negative control.

Data Analysis

The experiment was performed in triplicates. Results were expressed as mean ± SE.

RESULTS AND DISCUSSION

Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. They are rich source of bioactive compounds and thus serve as important raw materials for drug production. Now-a-days multiple drug resistance has developed due to the indiscriminate use of

Commercial antimicrobial drugs commonly used in the treatment of infectious diseases [29]. This situation forced scientists to search for new antimicrobial substances [30]. Therefore, this work is carried out to fulfill a need of developing alternative antimicrobial drugs for the treatment of infectious diseases from a herbal medicinal weed plant.

Comparative analysis of antibacterial activity

To search for traditionally used medicinal plants with potent antibacterial properties against gram negative and gram positive bacteria and antifungal properties, different organic extracts of inflorescence of *Parthenium hysterophorus* was screened at concentrations of 1000µg,500 µg,250 µg,125 µg/ml . . The extract was very effective against some bacteria and showed the highest activity than standard antibiotics that were used in the study as shown in Table 1.

Highest antibacterial activity was observed against *P. aeruginosa* and *C. freundii* by all types of organic extracts of inflorescence of *P. hysterophorus*. Methanolic extract of inflorescence of *P. hysterophorus* was found to be antibacterial against all types of Bacterial strains used in this study and considered as broad spectrum antibacterial extract. All types of organic extracts were not active against *K. pneumoniae* except Methanolic extract which showed antibacterial activity against *K. pneumoniae* at 1000µg/ml.

Aqueous extract was inactive against *E. coli*, *B. cereus*, *K. Pneumoniae*, *M. luteus* but showed

Table 1. Antibacterial activity of *P.hysterophorus* extracts against pathogenic bacterial strains.

S. No	Test Organisms	Zone of inhibition(mm of diameter)																				
		Aqueous (µg/ml)				Methanol (µg/ml)				Dichloromethane (µg/ml)				Petroleum ether(µg/ml)				Acetone (µg/ml)				
		1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125	
1	<i>E.coli</i>	—	—	—	—	10.33±0.33	5.00±0.00	3.66±0.33	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	<i>B.cereus</i>	—	—	—	—	13.33±1.2	7.00±0.577	4.00±0.577	—	11.33±0.33	5.33±0.166	3.33±0.33	—	—	—	—	—	11.00±0.577	5.66±0.33	3.33±0.33	—	
3	<i>K.pneumoniae</i>	—	—	—	—	5.00±0.57	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
4	<i>P.aeruginosa</i>	9.00±0.577	6.33±0.33	2.66±0.166	—	15.66±0.88	8.16±0.40	5.00±0.00	2.53±0.17	13.66±0.88	11.66±0.88	5.66±0.33	2.66±0.33	4.33±0.33	4.00±0.00	—	—	15.66±0.33	7.66±0.166	4.33±0.66	2.1±0.57	
5	<i>Micrococcus.luteus</i>	—	—	—	—	18.33±0.88	14±1.154	9±1.0	5.33±0.88	14.33±0.33	7.33±0.33	5±0.33	—	—	—	—	—	12.33±	6.33±0.33	2.66±0.33	—	
6	<i>Citrobacter freundii</i>	10.00±0.00	4.83±0.166	—	—	16.33±0.33	8.33±1.2	5.66±1.2	5.66±1.2	11±0.577	6.66±0.33	4.33±0.33	3.00±0.577	4.66±0.33	—	—	—	12.00±0.577	5.33±0.33	4.33±0.33	—	

Comparative analysis of antifungal activity

Different extracts of Inflorescence of *Parthenium hysterophorus* were also tested against different human pathogenic fungi such as *Microsporium gypseum*, *Penicillium chrysogenum*, *Rhizopus stolonifer*, *Aspergillus niger*, *Mucor* at Available online on www.ijprd.com

good activity against *P. aeruginosa* and *Citrobacter freundii* both are gram negative bacteria. In methanolic extract, Inflorescence shows highest antibacterial activity of 5.66 mm even in low concentration of 125µg/ml and 18.66 mm in 1000µg/ml concentration against *C. freundii* and *M. luteus* respectively. The antibacterial activity of methanolic extract of inflorescence decreases in the order of *M. luteus*≥*C. freundii*> *P. aeruginosa*>*B. cereus*>*E. coli*>*K. pneumoniae*.

Dichloromethane extract of inflorescence showed antibacterial activity in the order *M. luteus*>*P. aeruginosa*>*B.cereus*>*C. ferudii*. DCM showed no activity against *K. pneumoniae* and *E. coli*. Petroleum ether extract showed little activity of 4.33mm and 4.66mm against *P. aeruginosa* and *C.freundii* respectively in 1000 µg/ml and nil against other strains.

The acetone extract showed antibacterial activity in the order of *P. aeruginosa*>*M. luteus*> *C. ferudii* >*B. cereus* and inactive against *E. coli* and *K. pneumoniae*.The inhibitory activities of all the extracts reported in table 1 are comparable with standard antibiotic Chloremphenicol.

concentration of 1000µg,500 µg,250 µg,125 µg/ml . The extract was found to be less effective against fungal strains as shown in Table 2. Organic extracts of inflorescence of *Parthenium hysterophorus* displayed no activity against any type of fungi indicating that fungus are resistant to the tested

plant. On the other hand aqueous extract of inflorescence of tested plant was found effective at higher concentrations of 1000 µg/ml and 500 µg/ml. The antifungal activity of aqueous extract was in the order *Penicillium chrysogenum* > *Microsporium gypseum* > *Rhizopus stolonifer* and

inactive against Aspergillus niger and Mucor. In case of fungus, aqueous extract was found effective this may be due to the better solubility of the antifungal components in water. The inhibitory activities of all the extracts reported in table 2 are comparable with standard antibiotic nystatin.

Table 2. Antifungal activity of *P.hysterophorus* extracts against pathogenic fungal strains.

S.No	Test Organisms	Zone of inhibition(mm of diameter)																			
		Aqueous (µg/ml)				Methanol (µg/ml)				Dichloromethane (µg/ml)				Petroleum ether(µg/ml)				Acetone (µg/ml)			
		1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125	1000	500	250	125
1	<i>Microsporium gypseum</i>	11.66±0.33	6.33±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	<i>Penicillium chrysogenum</i>	33.31±0.88	16.33±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	<i>Rhizopus stolonifer</i>	10.66±0.66	5.66±0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5	<i>Mucor</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

CONCLUSION

The literature survey revealed that antibacterial activity of *Parthenium hysterophorus* has already been seen in different parts of plant except the inflorescence .So in this study different organic extracts of inflorescence of *Parthenium hysterophorus* including aqueous extract were considered to determine the antibacterial and antifungal activity of plant.

The present study showed that there is higher antibacterial and lesser antifungal activity in the inflorescence of *Parthenium hysterophorus* .So this study supports the use of this plant as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the active compound and carry out further pharmacological evaluation. It is quite sure that such components could be useful in developing drugs.

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