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Research article

Antimicrobial activity of the water-soluble ash extract from the invasive weed *Parthenium hysterophorus* L.

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ABSTRACT

Parthenium hysterophorus L., also known as congress grass or carrot grass, is a widely distributed invasive weed which is a big challenge for sustainable agriculture. For the past several years, a number of attempts have been made to evaluate its medicinal properties. The present study aimed to evaluate the antimicrobial activity of the water-soluble ash extract of *P. hysterophorus* against different bacterial and fungal pathogens. The disc diffusion method was used to measure zone of inhibition (ZOI) whereas broth microdilution method was used to evaluate minimum inhibitory concentration (MIC) of the extract. The extract was found most effective against *Staphylococcus aureus* with a ZOI value of 33.5 mm at 33 mg/mL concentration when compared with ofloxacin (ZOI = 39.5 mm at 5 μg/disc). The extract was also found active against *Salmonella enterica, Staphylococcus epidermidis* and *Escherichia coli* with ZOI values of 25.5, 32.5 and 25.5 mm, respectively. The present study revealed that the ash extract of *P. hysterophorus* has potential to inhibit various bacterial species. However, the tested fungal pathogens like *Candida albicans* were found unaffected after treating with the ash extract.

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INTRODUCTION

The genus *Parthenium* comprises of 15 species belongs to the family Compositae, one of the largest families having 1911 genus and 32913 species (The Plant List, 2013). *Parthenium hysterophorus* L., also called as carrot grass in English or gajar ghas in Hindi, is a toxic invasive weed. It is a native of America and the West Indies and now grown in more than 40 countries of the five continents, which is still spreading at an alarming rate (Dhileepan and Strathie, 2009). It was reported to be one of the seven most dangerous weeds of the world (Bhateria et al., 2015). It is believed that the seeds of this plant have entered in India with contaminated cereal grains in the early nineteenth century; however, it was first reported in India in 1956. The carrot grass can grow in a wide range of landscapes and habitats due to its adaptive nature. Today, this weed is growing everywhere in agricultural fields, wastelands, and forests. Carrot grass is a fast-growing, much-branched erect annual herb of up to 150-200 cm height. The plant has deep-penetrating taproots and erect shot. Its stems are cylindrical, straight, covered with hairs and many branches whereas the leaves are pale-green, alternate, deeply-dissected with fine hairs on lowers surface. The flowers are creamy-white of 4 mm size, arise from the leaf forks and the time of flowering is March-April and August-September. The seeds are 4-5 black, wedge-shaped produced by each flower (Kaur et al., 2014).

The trichomes and pollen of *P. hysterophorus* contain sesquiterpene lactones like phytotoxins. Parthenin, a bitter sesquiterpene lactone, is found as a major constituent of the plant. Several other constituents are also identified e.g. hysterin, ambrosin, coronopilin, conchasin A, charminarone, dihydroisoparthenin and tetraneurin A (Fig. 1). The

phytochemicals like parthenin, hymenin and ambrosin are responsible for its toxic nature (Patel, 2011). This weed has been reported to have a rich mineral composition of N, P, K, Fe, Mn, Cu and Zn (Apurva et al., 2010). Till the date, numerous methods including mechanical, chemical, cultural and biological have been applied to control this weed but the success is still out of reach (Bhateria et al., 2015).

On the one hand, its pollens and dust of other parts cause contact dermatitis in human mainly due to parthenin content whereas its long-term exposure causes skin inflammation, asthma, bronchitis, eczema, blisters, hay fever, allergic rhinitis, diarrhoea, papular urticarial and breathlessness (Narasimhan et al., 1984; Maishi et al., 1998). On the other hand, it showed various pharmacological properties such as antidiabetic (Patel et al., 2008), antioxidant (Kumar et al., 2013) antitumor (Mukherjee and Chatterjee, 1993), antimicrobial (Talakal et al., 1995), antimalarial (Kumar et al., 2011) and neuromuscular relaxant (Vijayalakshmi et al., 1999). In view of the global challenge for the management of *Parthenium hysterophorus*, the present study is designed to evaluate the antimicrobial activity of the water-soluble ash of its aerial parts for a possible solution of this weed by utilizing it as a medicine.

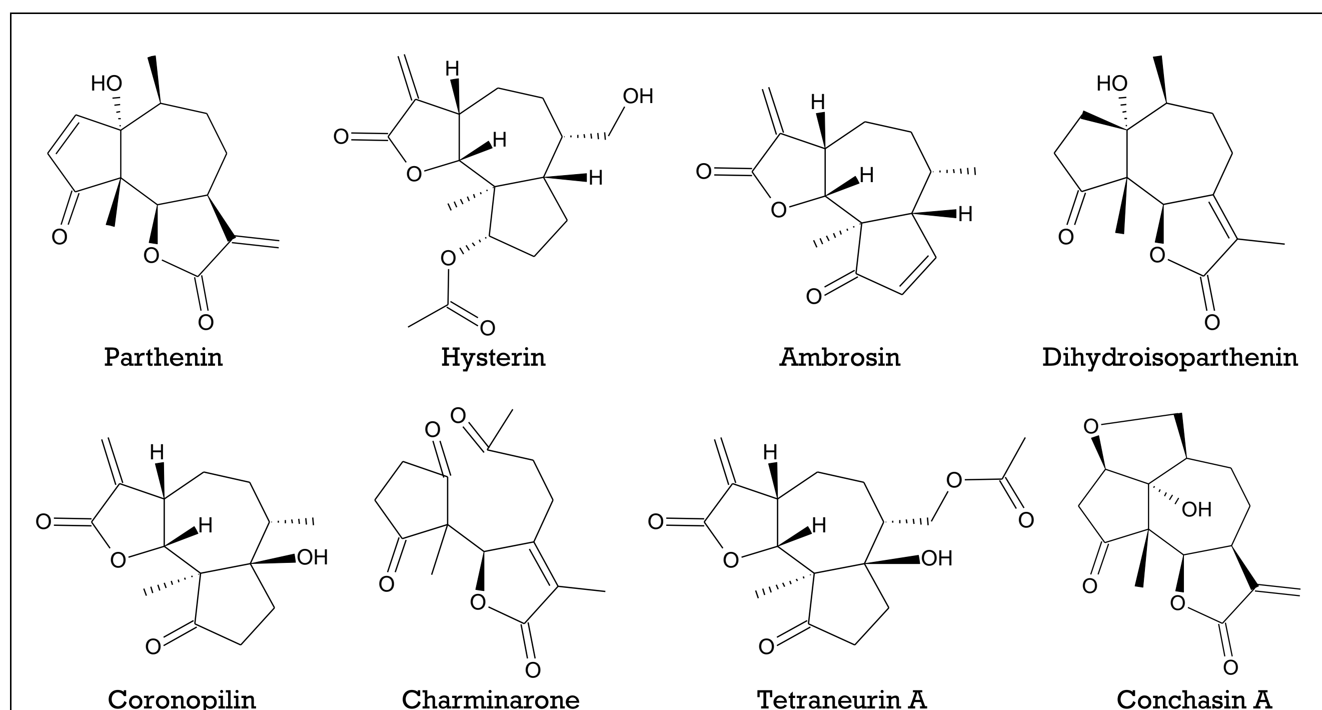

Fig. 1. Major sesquiterpene lactones found in *Parthenium hysterophorus*

MATERIAL AND METHODS

Collection of plant material

Fresh aerial parts of *P. hysterophorus* were collected during December 2019 from Harrawala, Dehradun. The plant was authenticated from the Research and Development Center, Uttarakhand Ayurved University, Dehradun where a specimen is preserved for future record.

Preparation of water-soluble ash

The collected plant material was dried in a hot air oven at 60 °C and burnt in an iron container to make crude ash. This ash was further put in a muffle furnace at 600-800 °C for 6 h to burn the remaining organic matter exhaustively. The ash thus obtained was dissolved in water and left at room temperature for 24 h with occasional shaking. The mixture was filtered and the water-soluble ash was concentrated using vacuum rotary evaporator and used for the antimicrobial studies.

Antimicrobial analysis

Microbial strains

The antimicrobial activity of the water soluble ash of *P. hysterophorus* was evaluated against 18 bacterial {*Salmonella enterica* (MTCC1165), *Escherichia coli* (MTCC724, MTCC729 and MCC3099), *Pseudomonas fluorescens* (MTCC2421), *Staphylococcus epidermidis* (MTCC435), *Klebsiella pneumoniae* (MCC3094), *Pseudomonas aeruginosa* (MCC3097), *Staphylococcus aureus* (MCC2408), *Staphylococous spp.* (MCC3042), *Shigella flexineri* (MCC3095), *Yersinia intermedia* (MCC3579), *Enterobacter cloacae* (MCC3111), *Salmonella spp.* (NCL5284), *Bacillus cereus* (MCC2086), *Rhodococcous spp.* (MCC2645), *Shigella boydii* (MTCC11947) and *Acinetobacter spp.* (MCC2024)} and 8 fungal {*Aspergillus spp.* (MCC1074), *Candida*

albicans (MCC1155), *Geotrichum capitatum* (NCCPF480010), *Sporothrix globosa* (NCCPF-220119), *Cryptococcus neoformans* (NCCPF25012), *Rhodotorula mucilaginosa* (NCCPF50005), *Fusarium chlamydosporum* (NCCPF580016) and *Acremonium kiliense* (NCCPF530013)} strains.

Preparation of sample

The ash extract of carrot grass at 33 mg was dissolved in 1 mL of 1X PBS to make a stock solution for the antimicrobial analysis.

Sterility of test sample

The ash extract (100 µL each) of the tested concentrations was streaked onto a sterile nutrient agar plate. The media plates were incubated at 37 °C for 48 h and afterwards, the plates were examined for any microbial contamination.

Activation of microbial culture

Each of the bacterial strains was revived before susceptibility testing by transferring them from stock culture to freshly prepared nutrient broth and incubated overnight at 37 °C.

Antibacterial susceptibility testing

Antibacterial activity of the sample was checked by disc diffusion method. Culture plates were prepared by pouring 20 mL sterilized Muller Hinton Agar (MHA) into pre-sterilized Petri dishes. Thereafter, 0.1 mL of inoculums suspension of each bacterial strain was spread uniformly over the agar medium using sterile glass spreader in plates. The inoculums size was adjusted by using 0.5 Mcfarland standard with approximately 1.0 x 10^8 CFU/mL. Sterile standard wells (6 mm) were made on the agar surface; afterwards, 100 µL of the test sample was added in the wells. The plates were incubated at 37 °C for 24 h. The mean diameters of zone of inhibition (mm) were measured and recorded. Standard antibiotic ofloxacin (Hi-Media, Mumbai) at 5 µg/disc was used as positive control while blank MHA media plates and PBS alone were used as control. Experiments were carried out in triplicate and averages diameter of zone of inhibition (ZOI) was recorded (Semwal and Rawat, 2009).

Antifungal susceptibility testing

Similar to the antibacterial activity, the antifungal activity of the sample was checked by disc diffusion method. Culture plates were prepared by pouring 20 mL sterilized Potato Dextrose Agar (PDA) into pre-sterilized Petri dishes. Inoculums suspension (100 µL) of each fungal strain was spread uniformly over the agar medium using sterile glass rod in plates. Sterile standard wells (6 mm) were made on agar surface and the sample (100 µL) was added in the wells. The plates were incubated at 28 °C for 5 days. The mean diameters of zone of inhibition (mm) were measured and recorded. Standard antibiotic itraconazole (Hi-Media, Mumbai) at 30 µ/disc was used as positive control while blank PDA media plates and PBS alone were used as control. Experiments were carried out in triplicate and averages diameter of ZOI was recorded.

Evaluation of minimum inhibitory concentration

The antibacterial potency of the test article against sensitive bacterial strains was also evaluated by measuring the minimum inhibitory concentration (MIC) values as per the Clinical and Laboratory Standards Institute (CLSI) guidelines using Broth Microdilution Method. The 96-well microplates were used to determine the MIC of test articles against sensitive bacterial strains. A stock solution of the ash extract was prepared by dissolving 33 mg of test article into 1 mL of 1X PBS. The stock solutions were diluted to obtain serial log by tenfold dilutions; 125 µL of these dilutions was added to each well containing Muller-Hinton broth medium (MHB–HiMedia), resulting in test concentrations ranging from 33 mg to 0.515 mg and 100 µL of the bacterial suspension of each sensitive strain prepared in MHB (final concentration, 10^5 CFU/mL) was added to each well, with a final volume of 250 µL per well. All the plates were covered with a sterile plate sealer and incubated for 24 h at 37.2 °C and 180 rpm agitation. After the incubation, MIC values were quantified by taking absorbance at 600 nm by using a microplate reader (Envision, Perkin Elmer USA). The MIC qualitative analysis of the samples was detected after incubation following addition (50 µL) of 0.4 mg/mL *p*-iodonitrotetrazolium chloride (INT) and kept at room temperature for 30 min. in above plates. Viable bacteria produced a pink colour by the reduction of yellow dye. MIC was determined with the lowest test sample concentration that prohibited the colour change in the medium which results as the inhibition of bacterial growth, which was observed by the turbidity in the wells. Test articles with MIC values <10% were considered significantly active.

RESULTS AND DISCUSSION

The present study revealed that out of 18 bacterial strains only *S. enterica* (MTCC1165), *S. epidermidis* (MTCC435), *E. coli* (MTCC724 and MTCC729), *K. penumoniae* (MCC3094) and *S. aureus* (MCC2408) were found sensitive to *P. hysterophorus* ash extract at 33 mg/mL with ZOI values of 25.5±0.7, 32.5±0.7, 25.5±0.7, 28±1.4, 11.5±0.7 and 33.5±0.7, respectively (Table 1). The results from MIC study found that 16 out of total 18 bacterial strains showed 50% and above inhibition

at a concentration of 16.5 mg/mL and only *S. aureus* (MCC2408) and *S. epidermidis* (MTCC435) showed 50% and above inhibition at a concentration of 8.25 mg/mL (Table 2). However, all the fungal strains did not show any activity as compare to the itraconazole taken as a positive control (Table 1).

Table 1. In vitro antibacterial activity of *P. hysterophorus* ash extract and ofloxacin.

S.No.	Strains	Zone of Inhibition (mm diameter)		
		***P. hysterophorus* (33 mg)**	**Ofloxacin (5 μg)**	**Itraconazole (30 μg)**
Bacterial strains				
1	*S. enterica* MTCC1165	25.5±0.7	29.5±0.7	NT
2	*S. aureus* MCC2408	33.5±0.7	39.5±0.7	NT
3	*S. epidermidis* MTCC435	32.5±0.7	21.5±0.7	NT
4	*E. coli* MTCC724	25.5±0.7	32.5±0.7	NT
5	*E. coli* MTCC729	28.0±1.4	32.0±0.7	NT
6	*K. penumoniae* MCC3094	11.5±0.7	18.5±0.7	NT
7	*P. fluorescens* MTCC 2421	NA	17.5±0.7	NT
8	*S. flexineri* MCC3095	NA	28.5±0.7	NT
9	*Y. intermedia* MCC3579	NA	33.5±0.7	NT
10	*E. cloacae* MCC3111	NA	21.2±0.7	NT
11	*Salmonella spp.* NCL5284	NA	13.4±0.7	NT
12	*B. cereus* MCC2086	NA	20.5±0.7	NT
13	*Rhodococcous spp.* MCC2645	NA	29.0±0.7	NT
14	*E. coli* MCC3099	NA	12.0±0.7	NT
15	*S. boydii* MTCC11947	NA	30.5±0.7	NT
16	*Acinetobacter spp.* MCC2024	NA	23.0±0.7	NT
17	*P. aeruginosa* MCC3097	NA	29.5±0.7	NT
18	*Staphylococcous spp.*	NA	12.0±0.7	NT
Fungal strains				
1	*Aspergillus spp.* MCC1074	NA	NT	23.0±1.0
2	*C. albicans* MCC1155	NA	NT	24.0±1.8
3	*G. capitatum* NCCPF480010	NA	NT	31.4±1.5
4	*S. globosa* CCPF220119	NA	NT	30.2±1.3
5	*C. neoformans* NCCPF25012	NA	NT	31.0±1.5
6	*R. mucilaginosa* NCCPF50005	NA	NT	25.7±0.9
7	*F. chlamydosporum* NCCPF580016	NA	NT	16.6±1.0
8	*A. kiliense* NCCPF530013	NA	NT	11.1±1.2

ZOIs are given as mean of three values ± standard deviation. NA = Not active; NT = Not tested.

Table 2. Percentage inhibition at different concentrations to measure MIC of *P. hysterophorus* (% inhibition)

S.No.	Strains	Percentage inhibition at different concentration						
		33 μg	**16.5 μg**	**8.25 μg**	**4.125 μg**	**2.06 μg**	**1.03 μg**	**0.515 μg**
1.	*S. enterica* MTCC1165	80	81	28	21	21	14	07
2.	*E. coli* MTCC724	80	75	19	09	02	00	00
3.	*P. fluorescens* MTCC 2421	75	63	28	17	14	04	02
4.	*S. epidermidis* MTCC435	79	67	52	32	24	14	10
5.	*E. coli* MTCC729	79	75	16	13	01	00	00
6.	*K. penumoniae* MCC3094	78	54	17	16	04	02	00
7.	*S. flexineri* MCC3095	75	61	27	18	16	05	02
8.	*Y. intermedia* MCC3579	81	73	45	27	20	11	01
9.	*E. cloacae* MCC3111	80	52	20	09	05	03	00
10.	*Salmonella spp.* NCL5284	82	74	30	14	10	03	00
11.	*B. cereus* MCC2086	76	61	47	23	23	18	07
12.	*Rhodococcous spp.* MCC2645	65	53	31	13	04	01	00
13.	*E. coli* MCC3099	83	64	24	27	08	09	01
14.	*S. boydii* MTCC11947	76	59	49	20	14	16	08
15.	*Acinetobacter spp.* MCC2024	79	66	15	15	13	10	01
16.	*P. aeruginosa* MCC3097	73	58	23	10	04	00	00
17.	*S. aureus* MCC2408	80	74	56	38	16	12	06
18.	*Staphylococcous spp.*	76	60	19	21	12	11	08

DISCUSSION

The infections of *Salmonella enterica* represent a chief concern to public health, food industry and animals, it is a foodborne pathogen. Till the early twentieth century, around 2600 serovars of *S. enterica* has been identified in which most of the serovars are pathogenic to both humans and animals (Bangtrakulnonth et al., 2004). However, few of them have been found beneficial for public health. Although the most type of *E. coli*, commonly found in the intestine and gut of humans and warm-blooded animals, are harmless and play a significant role in the digestive system, however, sometimes they can cause diarrhoea, pneumonia and urinary tract infections (UTI). *E. coli* transmits to humans mainly through consumption of contaminated foods including raw meat, vegetables and milk (WHO, 2018). Similarly, *K. pneumoniae* is commonly found in the intestine and harmless when remains in intestine. However, after spreading to other parts like lungs, bladder, brain, liver, eyes, and blood, it can cause severe infections such as pneumonia, thrombophlebitis, UTI, cholecystitis and upper respiratory tract problems (Denkovskienė et al., 2019). *S. aureus* is one of the most common pathogens which causes bacteremia and infective endocarditis together with various skin and soft-tissue infections and spread through contact with pus from an infected wound and contact of skin to skin or skin to objects of an infected person like towel, sheet or other cloth (Tong et al., (2015). The results from the present study revealed that the ash extract of *P. hysterophorus* has potential to inhibit *S. enterica, E. coli, K. pneumoniae* and *S. aureus*. Hence, it can be developed as an antimicrobial agent against these pathogens.

It is also well-established that carrot grass causes dermatitis and respiratory problems in humans and its major component parthenin is found responsible for its toxicity (Ramos et al., 2002). Its pollens cause induction of allergic rhinitis which is also known as hay fever (Rao et al., 1985). As the toxicity of this plant is well-known, therefore, a water-soluble ash was used in this study to diminish its toxicity. The plant was found rich in potassium (0.32%), phosphorus (0.10%), zinc (178 ppm), manganese (125 ppm), iron (84 ppm) and copper (76 ppm) (Yadav, 2015). Since, various metallic nanoparticles can adhere to and diffuse into biofilms which resulting to disruption of the membrane potential, enhanced lipid peroxidation and DNA binding. Moreover, disturbing the normal functioning of these processes can decrease the ability of bacterial pathogens to form biofilms (Wang et al., 2017). This may be the mechanism behind the antimicrobial activity of *P. hysterophorus* ash extract because of the presence of metallic contents. Although the antimicrobial activity of this plant has been reported earlier (Kumar et al., 2014; Kaur et al., 2016), a novel approach to use water-soluble ash extract has applied for the first time against a wide range of microbial pathogens.

CONCLUSION

The present study concludes that the water-soluble ash extract of *P. hysterophorus* has antibacterial activity against *S. enterica, E. coli, K. pneumoniae* and *S. aureus*. The extract showed more than 50% inhibition against *S. aureus* and *S. epidermidis* at a concentration of 8.25 mg/mL. As the extract was found inactive against fungal pathogens including *Candida albicans*, it can be considered as an antibacterial against selected pathogens. However, further studies are warranted to develop it as a medicine against bacterial infections.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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