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Full Length Article

In-vitro Antibacterial Activity of *Parthenium hysterophorus* against Isolated Bacterial Species from Discolored Rice Grains

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Abstract

The frequency of bacterial population was estimated in discolored rice grains of seven different local and exotic varieties. Twelve bacterial species were isolated on Nutrient agar (N.A.) medium and maximum frequency among bacteria (19.32%) observed in KSK-133 and minimum (11.96%) in 140-4-1-2-5 among all rice varieties. *Burkholderia pseudomallai* and *Burkholderia glumae* had higher frequency almost in all tested rice varieties, while *Acidovorax facilis* had higher frequency only in CB- 38. Different extracts/solvents (benzene, ether and chloroform etc) were used to check the antibacterial activity on each rice variety. The aqueous extract showed evidence of low antibacterial activity as compared to other solvents. The maximum inhibition *in vitro*, was observed in benzene and ether solvent (up to 3.0-3.7 cm) against *B. glumae*, *B. pseudomallei, K. oxytoca, K. sibirica and M. rhodesianum* followed by chloroform extract with inhibition zone of 3.0 cm and 3.7 cm against *B. glumae* and *K. oxytoca*, respectively. Among different treatments, minimum inhibitory concentration (MIC) varied among 0.25-4.0 mg/mL when tested with all four solvent extracts of *P. hysterophorus* against rice pathogens. The main objective of the study was to minimize the bacterial diseases by using different extracts/solvents and to increase the high yield potential. The present investigation strongly indicated antibacterial potential of leaf extracts of *P. hysterophorus* against bacterial pathogens of rice in sustainable disease management and also helpful for increasing the yield of rice crop. © 2013 Friends Science Publishers

Keywords: *P. hysterophorus*; Solvents; Leaf extracts; Rice grains; Pathogenic bacteria antimicrobial assay

Introduction

Rice (*Oryza sativa* L.) is most important staple food for more than half of total population in the world. Its productivity depends on different ecological factors i.e., weather, soil type, moisture and nutrients (Luo *et al*., 1998). The growing area (grain yield/unit area) of rice is low down in Pakistan as compare to other developed countries of the world and reason behind of this less production is ascribed to several biotic and abiotic factors. There is 40% per acre yield gap between Pakistan and the neighboring countries including India and other developing countries while this breach widens to 100% if compared with the developed world. Low yield per acre is due to poor disease management, lack of proper inputs utilization and use of low quality seeds. To enhance the productivity and to fill up the yield gap, farmers should be trained with all new advanced techniques for increasing the yield potential of the crop to compete with other countries (Khush, 1995).

Crop diseases are the most important biotic factor which results in reduced yield. The extensive damage caused due to different diseases in rice and infection severity differs among varieties and species (Asghar *et al*., 2007). Rice is attacked by 76 diseases caused by fungi, bacteria, viruses, mycoplasma like organisms and nematodes. Discoloration, blights, rots, blotch are a complex disease symptoms of rice due to infection by certain microorganisms on the glumes, kernels, or both (Arshad *et al.,* 2009). Rice diseases can be managed by the use of pesticides, resistant cultivars, agronomic practices and biotechnological methods (Ribot *et al.,* 2008). However, the use of resistant cultivars is the most economical and environment friendly method for the management of disease but the resistance is subject to break down due to appearance of new/more virulent races of the pathogen (Ghazanfar *et al.,* 2009). Similarly plant diseases may be controlled by using different natural products as compare to synthetic pesticide due to their lower negative impacts on the environment. These practices are very successful in plant disease control due to harmless and non-phototoxic

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effect on ecosystem (Mushatq *et al.,* 2012).

The antimicrobial property resides in weed that would be an added advantage. This concept of weed has been used by man since prehistoric times and many of them have been used in the past for food, drug and fiber. Many of weeds would still be useful, but they have been superseded by plants of greater productivity and superior flavor (Tahir *et al.,* 2008). There are many antimicrobial compounds in weeds, which are affective against harmful microorganisms in plant and human. The extracts of weeds, medicinal plants, and various other species have shown to possess antimicrobial functions naturally and could serve as a source for antimicrobial agents against food spoilage and pathogens (Khan *et al.,* 2011). *P. hysterophorus* is one of the common weeds in Pakistan that have antimicrobial property and serve as an important bio-control agent against plant pathogens.

P. hysterophorus belonging to family Asteraceae, is a common invasive species, found in all disturbed land, including farms, pastures and roadsides. In some areas, its outbreak has been of almost affecting crop production, livestock and human health. It can trigger allergies and is a common cause of pollen allergy. Its decoction has been used in traditional medicine to treat fever, diarrhea, neurologic disorders, urinary infections, dysentery and malaria (Khan *et al.,* 2011). Consequently it is an important and critical issue to work on innovative antimicrobial compounds with diverse chemical structures and novel mechanisms of action against reemerging and new infectious microbes. As a result, scientists are focusing their attentions on folk medicine, looking for new leads to develop better drugs against microbial diseases or infections (Fazal *et al.,* 2011). The objectives of this study were to (1) check the antibacterial potential of leaf extracts of *P. hysterophorus* against bacterial species of different rice lines in sustainable disease management. (2) Association study of bacterial species with respect to their different morphological traits (3). Increase the yield of rice crop by controlling of rice diseases by using different solvent extracts/bio-control agents.

Materials and Methods

Source of Rice Varieties

The sample of discolored rice grain of different rice varieties/lines i.e., KSK-133(M_4), PK-8480-8-1-1-1(M_1), Super Basmati, K8C-634-10, CB-38, CB-42 and 140-4-1-2-5 were collected from Rice Research Institute Kala Shah Kaku, Lahore, Pakistan, District Narowal and USDA, Arkansas, USA in the year 2010-2011. The experiments were conducted in the Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. The diversity of bacterial population was estimated in diseased rice grains. The sources of different rice varieties/lines of diseased rice grains and their collection site are given in Table 1.

Collection of Weed

Leaves of *P. hysterophorus* were collected from of various places at University of the Punjab, Lahore, Pakistan. All the collected leaves were washed and shade dried for three weeks. The dried leaves were then homogenized by using a Mixer Grinder to make fine powder.

Preparation of Aqueous Extract

Shade dried plant material (30 g) was macerated in 60 mL of sterile distilled water by using pestle and mortar. The macerated material was filtered through four layer of muslin cloth and then centrifuged at 8,000 rpm for 15 min at room temperature. Remaining supernatant was cleaned by filtration with Whatman No. 1 filter paper and autoclaved at 121ºC, 15 psi. Finally obtained extract was preserved in a brown bottle aseptically; at 4ºC for future use (Mushatq *et al.,* 2012).

Organic Solvents Extraction

Powder of shade dried plant materials (30 g) was put in the thimble separately and extracted successively with 300 mL each of solvents (Benzene, Chloroform and Ether) for 48 h by using a soxhlet extractor. These extracts were concentrated using rotary flash evaporator, weighed and preserved in airtight bottles at 4ºC until further use (Mushatq *et al.,* 2012).

Final Working Concentration of Extracts

Each organic solvent (1 g) was dissolved in 10 mL of sterile 100% dimethylsulfoxide (DMSO). These dissolved solvents were used as the working concentration for antibacterial activity assay. Sterile 100% DMSO served as negative control (Mushatq *et al.,* 2012).

Culture Media

Nutrient agar (NA) medium (Difco) was used for bacterial isolation and antibacterial activities. NA medium (High media) was prepared by dissolving Peptone: 5.0 g; NaCl: 5.0 g; Beef extract: 1.50 g; Yeast extract: 1.50 g and Agar: 15.0 g in distilled water. Before adding agar pH was adjusted to 7.4. Media was sterilized by autoclaving at 15 lb. pressure for 15 min and plates were prepared.

Surface Sterilization of Diseased Rice Grains

Diseased rice grains were washed by using different sterilizing agent to study the damage affects on rice grains. Surface sterilization was done by stepwise washing in 70% ethanol for 5 min, 1% sodium hypochlorite solution for 5 min, and 70% ethanol for 30 s, followed by three rinses in sterile distilled water (Araujo *et al.,* 2002).

Isolation and Identification of Bacteria

The surface sterilized diseased rice grains were cut into pieces 2 to 3 mm long, which were placed on prepared NA

Table 1: List of different rice varieties with their source

Name of Rice Varieties	Accession No.	Source	Collection site
KSK-133 (M_4)	--	Pakistan	RRI-KSK
PK-8480-8-1-1-1 (M_1)	--	Pakistan	RRI-KSK
Super Basmati	--	Pakistan	Narowal
K8C-634-10	GSOR-311723	Suriname	USDA, Arkansas
CB- 38	--	Pakistan	RRI-KSK
CB- 42	--	Pakistan	RRI-KSK
140-4-1-2-5	GSOR-310197	Suriname	USDA, Arkansas

medium plates. Incubation was carried out at 37°C for 24 h to allow growth of endophytic bacteria from the cut pieces. In a further experiment, fragments of diseased rice grains were homogenized in 5 mL of sterile autoclave saline solution with a blender and serial dilutions (1 mL of 105) were spread with sterilized spreader onto NA medium (Araujo *et al.,* 2002). The plates were placed in an incubator at 37°C for 24 h and distinct individual colonies were purified by streaking on a new agar plate. Identification of bacterial species was done by recording phenotypic characteristics e.g., colony morphology, colony color, cell shape, motility and growth rate. The purified colonies were subjected to gram staining and characterized using biochemical tests and consulting the pertinent literature (Benson, 1996; Koneman *et al.,* 1997; Holt *et al.,* 2000). The relative abundance/frequency (%) of each bacterial isolated by dilution plating was also calculated as: (Number of colonies of a bacterial species/total number of bacterial colonies) × 100 (Mukhtar *et al.,* 2010).

Antibacterial Activity Assay

Antibacterial activity of aqueous and solvent leaf extracts (benzene, chloroform and ether) of *P. hysterophorus* was done on N.A. medium by using well-diffusion technique (Mushatq *et al.,* 2012). Well were made in nutrient agar plate using sterile cork borer (0.8 cm) and inoculum containing 10^6 CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 60 µL each of all solvent extracts were poured in the wells of the inoculated plates. In control treatments, Penicillin (5 µg/disc) was used as positive and DMSO as a negative control. These plates were incubated at 37ºC for 24 h and antibacterial activity was evaluated (measured in cm) by qualifying inhibition zones (IZ) of bacterial growth if any around the wells. Entire antibacterial assay was carried out under strict aseptic conditions. Three replicates of each treatment were repeated at least twice against each of the test bacterium. On the basis of readings, minimum inhibitory concentration (MIC) was exhibited as the least concentration of solvent extracts, which inhibited the growth of pathogenic bacterial species.

Data Analysis

Data were presented in tables and graph was prepared using the excel spread sheet. The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates. The means were compared with each other (Table 5).

Results

Frequency Distribution of Bacterial Species from Rice Grains

Bacterial species isolated on the NA medium from different varieties of rice collected from Lahore, Narowal and Arkansas, were used in this study. All isolates were purified by serial dilution with sterile distilled water and plating on NA medium. The bacterial colonies on these plates were examined for their morphological characteristics. Single colonies with different cultural characteristics, from each culture plate were touched with a flamed bacteriological loop and streaked on NA medium, incubated at 37°C for 24 h and then stored in micro-centrifuge tubes at -80°C in 30% glycerol. Each isolate was given an Accession number from First Fungal Culture Bank of Pakistan (FCBP). Bacterial strains were removed from the freezer as needed for identification and plated on NA medium. All bacterial species were identified by recording microscopic and biochemical characters and consulting the pertinent literature of Bergey's Manual of Determinative Bacteriology (9^{th} Edition) (Holt *et al.,* 2000). The identified bacterial community that was isolated from rice grains included: *Acinetobacter haemolyticus, Acinetobacter junii, Klebsiella sibirica, Methylobacterium rhodesianum, Xanthobacter agilis, Burkholderia pseudomallai, Burkholderia glumae Citrobacter diversus, Kurthia oxytoca, Enterobacter asburiae, Acidovorax facilis* and *Acidovorax temperans.* Frequency distribution of bacterial species was different within varieties and location on NA medium (Table 2). The frequency of bacteria from KSK-133 (M_4), PK-8480-8-1-1-1 (M_1), and Basmati super was higher than that of K8C-634-10, CB- 38, CB-42, 140-4-1-2-5. *B. pseudomallai* and *B. glumae* had high frequency almost in all tested varieties, while *A. facilis* has high only in CB- 38. Moreover, other all species were present in all varieties up to 2-5%. However, Out of twelve bacterial species two were gram positive and ten gram negative, whereas cell shapes of four bacterial species were bacilli and rest were cooci (Table 3).

Table 2: Frequency distribution of bacterial species from different varieties of rice grains

Diseased Rice grain sample collection site	Rice varieties	Bacterial species	No. of colonies	Frequency (%)	Culture media (g/L)
Rice Research Institute Kala Shah Kaku, Pakistan	KSK-133 (M_4)	*Burkholderia glumae*	03	7.00	Nutrient Agar(NA)
		Acinetobacter junii	01	2.32	=
		Acidovorax temperans	02	5.00	=
		Burkholderia pseudomallei	02	5.00	=
Rice Research Institute Kala Shah Kaku, Pakistan	PK-8480-8-1-1-1 (M_1)	*Burkholderia glumae*	03	7.00	=
		Acidovorax temperans	02	5.00	=
		Enterobacter asburiae	01	2.32	=
		Methylobacterium rhodesianum	01	2.32	=
District Narowal, Punjab, Pakistan	Super Basmati	*Burkholderia pseudomallei*	03	7.00	=
		Acidovorax facilis	01	2.32	=
		Citrobacter diversus	01	2.32	=
		Xanthobacter agilis	02	5.00	=
United States Department of Agriculture, Arkansas, USA	K8C-634-10	*Acinetobacter junii*	02	5.00	=
		Burkholderia glumae	02	5.00	=
Rice Research Institute Kala Shah Kaku, Pakistan	CB-38	*Kurthia sibirica*	01	2.32	=
		Acidovorax facilis	03	7.00	=
		Xanthobacter agilis	02	5.00	=
Rice Research Institute Kala Shah Kaku, Pakistan	CB-42	*Acinetobacter haemolyticus*	01	2.32	=
		Burkholderia pseudomallei	03	7.00	=
		Citrobacter diversus	02	5.00	=
United States Department of Agriculture, Arkansas, USA	140-4-1-2-5	*Acidovorax facilis*	01	2.32	=
		Acinetobacter haemolyticus	01	2.32	=
		Burkholderia glumae	02	5.00	=
		Klebsiella oxytoca	01	2.32	=
	Total		43	103	

Antibacterial Activity of *P. hysterophorus*

The present study with the aqueous and solvent extracts (benzene, chloroform and ether) of *P. hysterophorus* gave variable results. The results of antibacterial activity and inhibition zone of leaf extracts against different bacterial strains isolated from diseased rice grains were shown in Table 4, 5 and Fig. 1.

Antibacterial Activity against Aqueous Extract

The aqueous extract showed low antibacterial activity as compared to solvents. *K. oxytoca, K. sibirica* and *X. agilis* showed high resistance ranging from 1.6-1.7 cm. The growth of *A. temperans, A. haemolyticus, E. asburiae, M. rhodesianum* and *C. diversus* were moderately inhibited ranging from 1.4-1.5 cm. On the other hand *A. facilis, A. junii, B. glumae* and *B. pseudomallei* were showed maximum growth and slightly inhibited by aqueous extract with range of 1.00-1.30 cm (Fig. 1).

Antibacterial Activity against Solvent Extract

The antibacterial efficacy of three solvent extracts of *P. hysterophorus* demonstrated varied level of inhibition zone against bacterial rice pathogens. In case of benzene extract, among treatments: *K. oxytoca*, *K. sibirica* and *M. rhodesianum* was scored maximum inhibition zone of 3.7, 3.0 and 3.2 cm, respectively. Further, chloroform extract was effective against *B. glumae* and *K. oxytoca*, which recorded significant inhibition zone of 3.0 and 3.5 cm, respectively. A significant inhibition zone of bacteria *B. pseudomallei* and *M. rhodesianum* were found in ether solvent extract showing 3.0 and 3.1 cm inhibition, which was followed by 3.2 cm of *B. glumae* and *K. oxytoca* inhibition from same extract of *P. hysterophorus.* Whereas, chloroform and ether extracts were found to be ineffective or showed poor inhibition against *A. facilis, X. agilis* and *E. asburiae.* However, other four bacterial species (*A. temperans, A. haemolyticus, A. junii* and *C. diversus*) were less significant against three solvent extracts (Fig. 1).

Table 3: An Outline of the morphological and biochemical characteristic of bacterial species isolated from diseased rice grains (+): positive reaction; (-): negative reaction

Morphological and Biochemical Characteristic	Name of Bacterial species											
	A. haemolyticus	*M. rhodesianum*	*X. agilis*	*A. junii*	*B. pseudomallei*	*K oxytoca*	*C. diversus*	*K. sibirica*	*B. glumae*	*E. asburiae*	*A. temperans*	*A. facilis*
Cell shape	cocci	rod	cocci	rod	cocci	rod	cocci	rod	cocci	cocci	cocci	cocci
Gram Stain	+	-	-	+	-	-	-	-	-	-	-	-
Spore Stain	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	-	-	+	-	-	+	-	-	-	+	-
Pigment	-	-	+	-	-	-	-	-	-	-	-	-
Nitrate Reductase Test	+	-	-	-	-	-	-	-	+	-	-	+
Oxidase Test	-	-	-	+	-	-	-	-	-	-	+	-
Urease Test	-	+	-	-	-	-	+	-	-	-	-	-
Methyl Red Test	-	-	-	-	-	-	-	-	-	-	-	+
Citrate Utilization Test	-	-	-	-	-	-	-	-	-	-	-	-
Hydrogen Sulfide Production Test	+	-	-	+	-	-	+	-	-	-	-	+
Catalase Test	-	-	-	-	-	-	-	-	-	-	+	-
Indole Test	+	-	+	-	+	-	-	-	-	-	-	-
Glucose Test	-	-	-	-	+	-	+	+	-	+	-	+
lysine Test	+	-	+	-	-	+	-	+	-	-	+	-
Rhamnose Test	-	+	+	+	+	+	-	-	-	-	-	+
Mannitol Test	+	-	-	-	-	+	+	-	-	+	+	-
Xylose Test	-	+	+	+	-	-	-	-	-	-	-	-
Sorbitol Test	+	-	-	-	+	-	-	-	-	+	-	+
Arabinose Test	-	-	-	+	-	+	-	+	+	-	+	-
Raffinose Test	-	+	+	-	-	-	-	-	+	-	-	+

Table 4: Minimum inhibitory concentration (MIC) of solvent extracts of *P. hysterophorus* for antibacterial activity

Extracts	MIC mg/ml				
	B. glumae	*B. pseudomallei*	*K. sibirica*	*M. rhodesianum*	*K. oxytoca*
Benzene	4.00	4.00	4.00	4.00	4.00
Chloroform	2.00	2.00	1.00	1.00	2.00
Ether	4.00	2.00	2.00	1.00	4.00
Penicillin	8.00	8.00	8.00	10.00	9.00

Minimum Inhibitory Concentration (MIC)

P. hysterophorus extract in chloroform showed MIC of 2.0 mg/mL against pathogenic bacteria *B. glumae B. pseudomallei and K. oxytoca*, whereas 1.0 mg/mL for *K. sibirica* and *M. rhodesianum*. Benzene leaf extract showed MIC of 4.0 mg/mL against all tested bacteria. Furthermore ether extract showed MIC of 1.0 mg/mL against *M. rhodesianum* bacteria, whereas MIC of 2.0 mg/mL was found against *K. sibirica* and *B. pseudomallei* in both treatments. Consequently, the MIC of 4.0 mg/mL was found against *B. glumae* and *K. oxytoca* bacteria when ether extract was used. Besides, the range of MIC from 8.0 to 10.0 mg/mL was exhibited in control condition.

Discussion

The antibacterial activity of *P. hysterophorus* has been studied previously. Detailed studies of their leaf extracts are lacking, however, some have been individually sporadically tested among other afflictions. Despite the wide use of bio-control potential of this weed, detailed knowledge and studies are scarce except for some preliminary reports. Nature based compounds in plants have enormous therapeutical potential as they can be used as antimicrobial agent without any side effects that are often associated with synthetic antimicrobials. The preliminary screening of organic solvents of benzene, ether, chloroform and aqueous extracts of *P. hysterophorus* leaves were investigated for antibacterial activity against pathogenic bacterial species isolated from rice grains. Besides it was observed from the present study, that leaves extracts of *P. hysterophorus* (aqueous and solvents) revealed pronounced activity against all the tested bacterial pathogens. Leaves extracts of *P. hysterophorus* using benzene, chloroform and ether as extracting solvents presented a better inhibitory effect on the test organisms. This could be attributed to the concentration of the active substance causing the inhibitory effect which could have been higher in the leaves. The use of benzene, chloroform and ether as extracting solvents proved to be more efficient

Table 5: Inhibition zone of aqueous and solvent extracts *P. hysterophorus* against bacterial strains isolated from rice grains

Bacterial species	Mean Diameter of Inhibition Zone (cm)				
	Control	Aqueous extract	Benzene extract	Chloroform extract	Ether extract
A. facilis	0.9±0.0	1.20±0.02	2.50±0.06	1.50±0.02	2.00±0.06
A. temperans	0.9±0.0	1.50±0.12	2.00±0.03	2.90±0.06	2.60±0.13
A. haemolyticus	0.9±0.0	1.50±0.09	2.80±0.13	2.60±0.03	2.90±0.11
A. junii	0.9±0.0	1.00±0.10	2.30±0.06	2.00±0.17	2.00±0.16
B. glumae	0.9±0.0	1.20±0.05	2.90±0.09	3.00±0.14	3.20±0.02
B. pseudomallei	0.9±0.0	1.30±0.07	2.90±0.02	2.50±0.04	3.00±0.08
C. diversus	0.9±0.0	1.50±0.09	2.30±0.01	2.00±0.06	2.40±0.04
E. asburiae	0.9±0.0	1.40±0.20	2.00±0.04	2.00±0.08	1.80±0.09
K. oxytoca	0.9±0.0	1.70±0.16	3.70±0.16	3.50±0.06	3.20±0.16
K. sibirica	0.9±0.0	1.60±0.07	3.00±0.18	3.00±0.04	2.80±0.02
M. rhodesianum	0.9±0.0	1.50±0.09	3.20±0.07	2.80±0.02	3.10±0.15
X. agilis	0.9±0.0	1.70±0.05	2.00±0.04	1.80±0.01	2.00±0.17

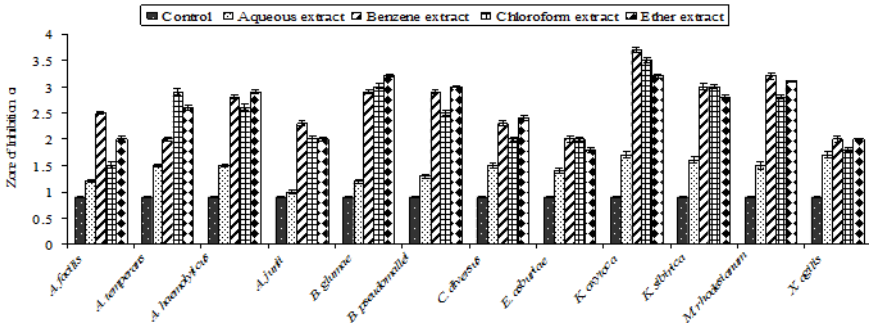

Fig. 1: Antibacterial activity of aqueous and solvent leaf extracts *P. hysterophorus* against bacterial strains isolated rice grains

in extracting the active compounds. This could be ascribed to the alcoholic aqueous environment which promotes easy extraction as reported by Nostro *et al*. (2000). The insolubility of the active components in aqueous extract indicates the absence of antibacterial activity of *P. hysterophorus*. The antibacterial activities of aqueous and solvent extracts compared favorably with Penicillin as standard antibiotics and have appeared to be broad spectrum as its activities were independent of gram reaction. Among different treatments standard antibiotics have exhibited low antibacterial activity as compared to tested bacterial cultures used. Sukanya *et al.* (2009) studied different extracts of *P. hysterophorus* leaves i.e., aqueous, methanol, ethanol, ethyl acetate and chloroform extracts against *Escherichia coli, Staphylococcus aureus, Xanthomonas vesicatoria* and *Ralstonia solanacearum*, therefore this report also support our results. Furthermore, minimum inhibitory concentration (MIC) of all three solvents was checked against pathogenic bacterial species. The MIC ranged between 0.35 to 4.0 mg/mL and 0.25 to 4.0 mg/mL used when tested with all three solvent extracts. Madan *et al*. (2011) reported the antibacterial activity of *P. hysterophorus* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Conversely, the antibacterial activity of *P. hysterophorus* against bacterial species such as *A. facilis, B. glumae, B. pseudomallei, K. oxytoca, K. sibirica* and *X. agilis* in our present report has been confirmed for the first time. Additionally insignificant results of *A. haemolyticus* and *A. junii* were found low activity against aqueous and all tested solvent extracts. Fazal *et al.* (2011) evaluated different solvent extracts of *P. hysterophorus* against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis, Enterococcus* sp, and *Staphylococcus aureus* by using agar well diffusion method. In addition, *P. hysterophorus* has well known potential for its antimalarial, antiamoebic and allelopathic properties (Hopper *et al.*, 1990; Sharma and Bhutani, 1998; Sukanya *et al.*, 2009), it successful to inhibit the tested bacterial species where a good to excellent inhibition was obtained. The inhibition zone of solvent extracts of *P. hysterophorus* leaves was found highest for *K. oxytoca*. The benzene extract showed the highest activity against *K. oxytoca* (3.70±0.04), *K. sibirica* (3.00±0.02) and *M. rhodesianum* (3.20±0.05). The current study is in close proximity with that of Sukanya *et al*. (2009). They evaluated the antibacterial activity of methanolic extract of *P. hysterophorus* against *E. coli*, which was 9 mm but no activity against *S. aureus* but the authors evaluated the activity against *E. coli* and *S. aureaus* viz., 13.00±0.12 and 26.00±0.12, respectively. Leaf extracts of *P. hysterophorus* illustrates a wide range of antibacterial activity against plant pathogenic bacterial species. In advance studies new synthetic natural antibacterial compounds would be achievable by primary screening of these extracts. Sharma

and Bhutani (1998) also confirmed *P. hysterophorus* as well known plant used for various medical purposes and providing active extracts against pathogenic organisms. It is need of the day to investigate plant-derived antimicrobials on advance basis. Future research is needed to study the antibacterial compounds within this plant and also to determine their full efficacy. Hence the recent study demonstrates that the leaf extracts of *P. hysterophorus* exhibit antibacterial effect, which offers a scientific basis for using this weed as a good source of biocontrol agent against plant diseases. Further work on its formulation by phytochemical and pharmacological studies is yet essential to synthesize less harmful and biodegradable natural bactericides.

In conclusion, *P. hysterophorus* showed appreciable activity against bacterial species isolated from different diseased rice grains of different varieties than the standard antibiotics used. Results indicate that this weed is a very good source of antibiotics for the treatment of certain bacterial diseases of rice and other cereal crops for getting maximum yield production and it is equally beneficial both for farmers and scientific community. However, further experimental and research efforts on this weed and their extracts are needed to be able to specify the pharmacological implication.

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