Antimicrobial activity of *Parthenium hysterophorus* against five bacterial strains

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Abstract
Microbial resistance to classical antibiotics and have raised serious concern in the treatment of infectious diseases. Recently, many studies have been directed towards finding promising solutions to overcome these problems. Phytochemicals have exerted potential antibacterial activities against sensitive and resistant pathogens via different mechanisms of action. *Parthenium hysterophorus* is a yearly and local herb used for antibacterial, antifungal, antiviral, anti-parasitic and anthelmintic properties. The present study was conducted to find out the antibacterial potential of *Parthenium hysterophorus*. Fresh and dry samples of leaves, stem and flowers in ethanol, methanol and acetone extracts were tested against bacterial strains through agar well diffusion assay. Variable results of antimicrobial activity against *Klebsiella pneumoniae*, *Salmonella paratyphi A*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli* were obtained. Fresh flower, fresh leaves and fresh stem extracts with ethanol, methanol and acetone of *P. hysterophorus* showed no specific effect on *P. mirabilis*. Maximum effect was observed both in case of fresh as well as dry plant extracts on *E. coli* and *P. aeruginosa*. The findings provide support for the use of *Parthenium hysterophorus* in producing new bioactivity compounds having antimicrobial activity.

Keywords: Antimicrobial activity; *Escherichia coli*; Gram negative bacteria; Plant extracts; *Parthenium hysterophorus*; *Pseudomonas aeruginosa*; *Salmonella paratyphi A*; Well diffusion assay.

Introduction
Infectious diseases caused by bacterial pathogens is a major health problem these days. These bacterial infections are treated with antibiotics. Due to the increased and regular usage of antibiotics multi drug resistant strains have emerged that are difficult to treat with the drugs available in the local market [1]. The use of plant based products is a safe and alternate approach to treat such multi drug resistant bacterial pathogens as plants contains a huge range of chemicals including alkaloids, tannins, flavonoids, chlorophyll pigments, linalool, eugenol, caffeine etc. that have the potential to negatively affect bacterial pathogens [2-4]. *Parthenium hysterophorus* is a flowering plant that belongs to the Asteraceae family. It
is a local plant of the American tropics. The plant have other Common names including Santa-Maria, feverfew, white top weed, carrot grass, Gajar Ghans or Congress grass. It was seen for the first time in Pakistan in the 1980s in District Gujarat [5-7]. It is a yearly weed that grows in a distressed environment such as river and sea banks, on tracks of railway, around buildings, and on wasteland. It is also present in crop fields, plant nurseries, villages, and gardens [8-10]. P. hysterophorus is a branched, fragrant, and rigid herbaceous plant with vital growth habit. The plant grows about 30-90 cm in height, but up to 1.5 m, or 2.5 m, in exotic situations and reproduces through seed [11, 12]. It is locally utilized as a home-grown solution for different intestinal and skin issues, treatment of wounds, ulcerated injuries, fever, heart issues, toothaches, bubbles and pimples treatment, fevers, against a sleeping disorder and to treat diabetes [13, 14]. During this study fresh and dry plants was used to make extracts and investigate its antibacterial potential against common bacterial isolates.

Materials and Methods
Sample collection
Plant sample
Parthenium Hysterophorus was collected from different areas of Takht Bhai, Mardan and taxonomically identified by the Department of Botany, Islamia College Peshawar.

Bacterial strain
Pathogenic bacterial isolates i.e. Klebsiella pneumoniae, Salmonella paratyphi A, Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli were used in this study. Almost 10 samples of each bacterial isolate were collected from tertiary care hospitals of Mardan and Peshawar.

Preparation of plant extracts
Three different solvents namely Methanol, Ethanol and Acetone were used for extraction. 30 grams leaves sample (Fresh and dry) were suspended in conical flasks containing 50mL of methanol, ethanol and acetone. The mixture was kept at 36° C for 48 hours with constant shaking. Each preparation was filtered through sterilized whatman filter paper. The filtrates were dried in water bath for at 64.7° C for methanol, 78.37° C for ethanol and 56° C for acetone. The obtained residues were weighed and used for phytochemical screening.

Antibacterial assay
Inoculum suspensions of the bacterial isolates (Klebsiella pneumoniae, Salmonella paratyphi A, Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli) were swabbed on Mueller-Hinton Agar (MHA) plates and allowed to dry for five minutes. After five minutes, 6mm wells were made with the help of blue tip and 20μl of each extract (Fresh and Dry), negative control (Distilled) was added in its respective well. Chloramphenicol discs was used as positive control and the plates were incubated at 37° C for 18-20 hrs.

Results and Discussion
Dry plants extracts
As evident from (Fig. 1). Dry flower extract with methanol and ethanol showed slight activity against Klebsiella pneumoniae, Proteus mirabilis and Salmonella paratyphi A and no effect against Escherichia coli and Pseudomonas aeruginosa. For acetone extract of dry flower slight activity was observed against Escherichia coli, Klebsiella pneumoniae, and Salmonella paratyphi A. Strong inhibitory effect against E. coli and Pseudomonas aeruginosa and slight activity against Klebsiella pneumoniae, Salmonella paratyphi A, and Proteus mirabilis was observed in case of dry Stem with Methanol extract. Similarly, ethanol extract of dry stem was highly effective against Pseudomonas aeruginosa followed by Salmonella paratyphi A and E. coli and minimum effect against Klebsiella pneumoniae and Proteus mirabilis. Acetone
extract of dry stem was only effective in inhibiting the growth of Salmonella paratyphi A while other were not much effected. Methanol extract of dry leaves was able to inhibit the growth of E. coli and Pseudomonas aeruginosa to some extent while others were not much effected. Ethanol and acetone extract of dry leaves showed maximum inhibitory effect against Proteus mirabilis and Salmonella paratyphi A respectively.

**Fresh plants extracts**

Flower with methanol showed minimum antimicrobial activity against Salmonella paratyphi A, and Klebsiella pneumoniae while no effect in case of Pseudomonas aeruginosa, Proteus mirabilis and Escherichia coli. The flower with ethanol shows the greater effect on E. coli, slight inhibitory response against Salmonella paratyphi A and Klebsiella pneumoniae and no effect on Pseudomonas aeruginosa and Proteus mirabilis. The flower with acetone the greater effect on Pseudomonas aeruginosa slight activity against Salmonella paratyphi A and Klebsiella Klebsiella pneumoniae and no effect on E. coli and Proteus mirabilis. Stem with ethanol extract showed maximum activity against Pseudomonas aeruginosa while negligible effects were observed for the rest of the pathogenic isolates. Variable results were obtained in case of leaves extracts where methanol leaves extract showed greater activity against E. coli, Pseudomonas aeruginosa and Salmonella paratyphi A while ethanol and acetone extract were not much effective in inhibiting the growth of pathogens Such phytochemical screening of various plants is reported by Siddiqui [15]; Chitravadiy [16]; Ashok [17]; Savithramma [18]. The overall results have been summarized in (Fig. 2).
Figure 1. Summary of dry plant extracts activity against gram negative bacteria in comparison with positive and negative controls
Figure 2. Summary of fresh plant extracts activity against gram negative bacteria in comparison with positive and negative controls
Conclusion
Fresh and dry Plant extracts in three different solvents i.e. Methanol, ethanol and acetone were tested for antibacterial activity against common gram-negative bacterial pathogens. Varying degree of antibacterial activities were observed against the tested bacteria. Fresh flower, fresh leaves and fresh stem extracts with ethanol, methanol and acetone of *P. hysterophorus* showed no specific effect on *P. mirabilis* and maximum effect on *E. coli* and *P. aeruginosa* was observed both in case of fresh as well as dry plant extracts. It can be concluded that the plant possesses potent pharmacologically active compounds which can be utilized to manufacture new and improved antibacterial agents. The difference in the zone of inhibition against different bacteria is due to the different diffusing rate of the solvents.

Authors’ contributions
Conceived and designed the experiments: N Sharif, H Zahir & H Shumail, Performed the experiments: N Sharif, H Zahir, H Shumail & SA Taskeen, Analyzed the data: S Khalid & SIU Haq, Contributed materials/ analysis/tools: S Khalid, N Shareef, H Nazeer & H Shumail, Wrote the paper: SIU Haq & NS Jilani.

References