Original Article

Antimicrobial Activity of Selected Medicinal Plants against Clinical Bacterial Species

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Abstract: The current study was conducted to determine the antimicrobial potential of herbal plants against gram negative including *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*, and gram positive bacteria including *Staphylococcus aureus* and Enterococcus species. Aqueous extracts of these herbs were used to check the antimicrobial susceptibility. The antimicrobial activity of these herbal extracts was determined by using agar well diffusion technique. The aqueous extract of *Zanthoxylum alatum* exhibited maximum antibacterial activity against *Enterococcus fecalis* and *E. fecium* with the inhibitory zone in the range 35 mm and 30 mm at 100 uL respectively. While aqueous extract of *Gerwia asiatica* showed maximum antimicrobial activity against *E. fecalis* with the inhibitory zone 28 mm. whereas, aqueous extract of *Juglans regia* showed maximum antibacterial activity against *P. aeruginosa* and *E. fecalis* with the inhibitory zones 28 mm and 20 mm respectively. Some of the microbes showed no inhibitory activity such as, *S. aureus* exhibited resistance against both G. asiatica and *J. regia* antimicrobial contents. The results confirmed the therapeutic potency of these herbs for use in folk medicine. The results also encourage the usage of plants exhibiting antimicrobial activity against most of the food-borne and food-spoilage microorganisms.

Keywords: Antimicrobial activity, *G. asiatica*, *J. regia*, *Z. alatum*, therapeutic potential, medicinal plants

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1. Introduction

The extant of infectious diseases have increased in recent years [1]. On globe these are the second, while in developing countries these are the third major cause of death [2]. Different factors including: improper use of broad-spectrum antibiotics, organ transplantation, immunosuppressive drugs, intravenous catheters and infections like immunodeficiency virus syndrome (HIV) contributed to enhanced antibiotic resistance in bacterial strains [3]. According to estimates, 2.22 million hospitalized patients experienced adverse pharmacological effects in a given year, with 106,000 patients dying as a result of those effects [4]. Scientists are now looking for possible antimicrobial compounds from a variety of sources in order to address the problems caused by multidrug-resistant bacteria strains as well as the side effects associated with manufactured antimicrobial medications [5].

Globally and particularly in developing countries having insufficient public health infrastructure, antibiotic have prominent impact including the reduction of morbidity and mortality rate in human that are being caused by bacterial infections [6]. In general population, there is an increased rate of antibiotic resistance in several microbial strains due to over and misused of antibiotics [6-7]. Various chemical compounds derived from plants with antibacterial capabilities are being investigated in scientific studies to see if they have therapeutic effect against antibiotic resistant infections [8-10].

A range of medicinal plants have been identified as significant sources of natural antibacterial substances for the treatment of many troublesome bacterial diseases [11]. According to World Health Organization (WHO), many medicinal plants could be used for the production of various drugs due to the antimicrobial properties of phytochemicals metabolically produced by these plants [12-14]. Examples of various secondary metabolites produced by these plants found to exhibit in vitro antimicrobial traits include: flavonoids, alkaloids, tannins and phenolic compounds [15-16].

2. Materials and Methods

2.1 Plant Collection and Extraction

Plant material was obtained from two locations i.e. (i) Sagyam Suplah, Kotli (Azad Kashmir) and (ii) Ghazi Elahi Baksh Girls Degree College Mirpur (Azad Kashmir) for the determination of antimicrobial activity. The samples collected were taken to Post-graduated Laboratory Department of Zoology, Mirpur University of Science and Technology (MUST), Mirpur (Azad Kashmir).

Fresh fruit obtained from mature plants of *G .asiatica, Z. alatum* and *J .regia* were firstly washed with tap water and rewashed twice with distilled water. With the help of pestle and mortar, the fruit of *G. asiatica, J. regia* and *Z. alatum* were crushed after cutting into pieces by sterilized knife. Then their extracts were obtained with cotton bandage in separate and sterilized test tubes. These test tubes containing extracts were labeled and stored in refrigerator before further use.

2.2 Culture and Maintenance of Microbes

Pure cultures of six bacterial strains i.e. (i) *E. coli*, (ii) *P .aeruginosa*, (iii) *E. faecium*, (iv) *S. aureus*, (v) *E .faecalis* and (vi) *K. pneumonae* were collected from Microbiology section of Pathology Lab located in Combined Military Hospital (CMH), Rawalpindi. These clinical strains were cultured and maintained on nutrient agar medium by frequent subculturing. The prepared subcultures were stored at 4 °C before further use.

2.3 Preparation of Bacterial Culture Plates

In the current study, antibacterial activity of the selected plants was determined by using agar well diffusion method on nutrient agar against bacterial strains. After autoclaving, the nutrient agar medium was poured into sterilized petri plates and let them solidify. Then the plates were placed in incubator for 24 hours at 37 °C. After 24 hours, the streaking was done by the help of wire loops after sterilizing it by making it red hot with flame. A colony was picked with the help of loop wire from each bacterial culture plates and streaked on agar plates. Then the plates were incubated at 37 °C for 24 hours.

2.4 Preparation of Bacterial Inoculums

Saline solution was used for the preparation of bacterial inoculums. For the preparation of 100 mL saline solution, 0.9 mg sodium chloride was dissolved in 100 mL distilled water. Six clean test tubes were taken and 9 mL saline solution was transferred in each test tube. Then these tubes were autoclaved and incubated at 37 °C for 24 hours. And after 24 hours, some bacterial cells from the colonies in fresh culture plates were transferred into saline solution by the help of sterilized wire loop.

2.5 Determination of Antimicrobial Activity

Antimicrobial activity of selected plants extracts was determined by using agar well diffusion method. Sterilized cotton buds were used for inoculating the agar plate with bacterial species by dipping the cotton buds into the inoculated saline solution.

With the help of cork borer having 6 mm diameter, four wells were prepared on each inoculated agar plate. Different volumes of each plant extract i.e. 20, 40, 60, 80 and 100 μL were used to test their antimicrobial activity. Then micropipette was used for transferring the plant extract in one of four wells by using autoclaved tips. After that the plates were left at room temperature for an hour to allow the liquids completely diffuse into agar. Then the plates were given overnight incubation at 37 °C. After incubation, inhibition

zone appeared as clear zone around the well containing plant extract and the diameter (in millimeter) of inhibition zone was measured..

3. Results and Discussion

Antibiotic resistance describes bacteria's inability to be healed or prevented by antibiotics. It is one of the most serious bacteria-caused risks since it not only causes fatal infections but also causes prolonged sickness, expensive costs, and increased morbidity. The factors which contribute to the development of antibiotic-resistant bacterial strains include: poor management, inexperienced experts, an unsanitary environment, overuse and misuse of antibiotics [17]

The discovery of medicinal plants having antimicrobial potential have given a hope for the treatment of infections caused by antibiotic-resistant bacterial strains. The use of plant extracts with recognized antibacterial properties can be quite beneficial in the treatment of bacterial infections [18].

In this study, antimicrobial potential of plant extracts were determined against various pathogenic bacterial strains including: (i) *E. coli*, a common cause of different infections including: gastroenteritis, neonatal meningitis, urinary tract infections *etc*, (ii) *K .pneumonia* which causes pneumonia, (iii) *S. aureus*, a causative agent of toxic shock syndrome, septicemia and endocarditis, (iv) *P .aeruginosa*, which causes infections of burns, pulmonary tract, wounds and urinary tract, (v) *E. faecalis* and (vi) *E. faecium*, both are causative agents of various infections such as endocarditis, prostatitis, intra-abdominal infection, wound infection, urinary tract infections, cellulitis and concurrent bacteremia.

Agar well diffusion method was used to determine the antimicrobial activity of three selected plants against the bacterial strains by observing the inhibition zone created by plant extract. Different concentrations of aqueous plant extracts (20, 40, 60, 80 and 100 µL) were subjected to antimicrobial activity test. The results showed that among three plant extracts, *Z. alatum*, having local name "timber", showed remarkable resistance towards the selected bacterial pathogenic species used in this study. Maximum antimicrobial activity was achieved by 100 µL *Z. alatum* extract against two bacterial strains *i.e. E. fecalis* and *E. fecium* having zone of inhibitions with diameters 30 mm and 35 mm respectively. While lower activity exhibited against S. *aurues* and *K. pneumonia* having zone of inhibition with diameter 11 mm as depicted in Fig. 01. The extract of *Z. alatum* showed moderate level of activity against *E. coli* and *P. aeruginosa* with a zone of inhibition 15mm and 11mm at 100 µL concentration. Plant activity was remarkable against *E. fecalis* and *E. fecium* with a maximum zone of inhibition while other pathogens showed more resistance. Type of phytochemical constituents present in *Z.alatum* named as linalool, *β*-fenchol and alkaloids might be responsible for antibacterial activity against pathogenic bacteria. In a study conducted by Guleria *et al*., in 2013 [19] found out the antimicrobial activity of *Z. alatum* against three pathogenic bacteria *i.e. E. coli*, *S. aureus* and *B. subtilus*.

The results also showed that *G asiatica* exhibited antimicrobial activity towards the bacterial strains used in this study. Maximum antimicrobial activity was achieved by 100 µL *G. asiatica* extract against *E. fecalis* having zone of inhibition with 28 mm diameter.

While in case of *E. coli* and *E. fecium*, the zones of inhibition achieved by *G. asiatica* had 15 and 24 mm diameter respectively as shown in Fig. 02. Two bacterial strains *i.e. S. aureus* (gram-positive) and *K. pneumonia* (gram-negative) exhibited resistance to antimicrobial components present in plant extract and no zone of inhibition was observed. *E. coli* exhibited less sensitivity against extract of *G. asiatica*, zones of inhibition increased as concentration of the extract increased. Other studies reported the presence of flavonoids, tannins, phenolic compound protein, amino acid and vitamin C in aqueous and methanolic extracts of *G. asiatica*. Quercetin, kaempherol and their glucoside were detected and isolated from pure leaf extracts of *G. asiatica*. Shukla *et al*., in 2016 conducted a study in which they reported the antimicrobial potential of *G. asiatica* against bacterial pathogens.

Maximum antimicrobial activity was achieved by *J. regia* aqueous extract against *E. fecalis* having zone of inhibition with 28 mm diameter at 100 ul concentration and minimum antimicrobial activity was achieved against *E. fecium* having zone of inhibition with 18 mm at 100 ul concentration. The zones of inhibition of *K. pneumoniae* and *P. aeruginosa* were 20 mm and 19 mm respectively at 100 µL concentration. Whereas *S. aureus* and *E. coli* exhibited resistance to *J. regia* aqueous extract and no zone of inhibition was observed as depicted in Fig. 03. Jafer *et al*., in 2020 [20] found that *J. regia* had antimicrobial potential against some bacteria including gram positive and gram negative strains. The results obtained may contribute to the traditional use of plants in medicines.

In the current study, the antibacterial activity exhibited by the aqueous extracts of three plant samples might be due to the presence of therapeutic components. The presence of biologically active secondary metabolites such as flavonoids, saponins, tannins, glycosides, coumarins and steroids present in these plants may contribute to their antimicrobial potential observed in this study. These biologically active compounds follow various mechanisms to perform antimicrobial activity. Tannins are involved in interfering protein synthesis by binding to proline rich proteins. Flavonoids are produced by plants to combat microbial infection and they are found to exhibit in vitro antimicrobial activity against a wide range of microbes.

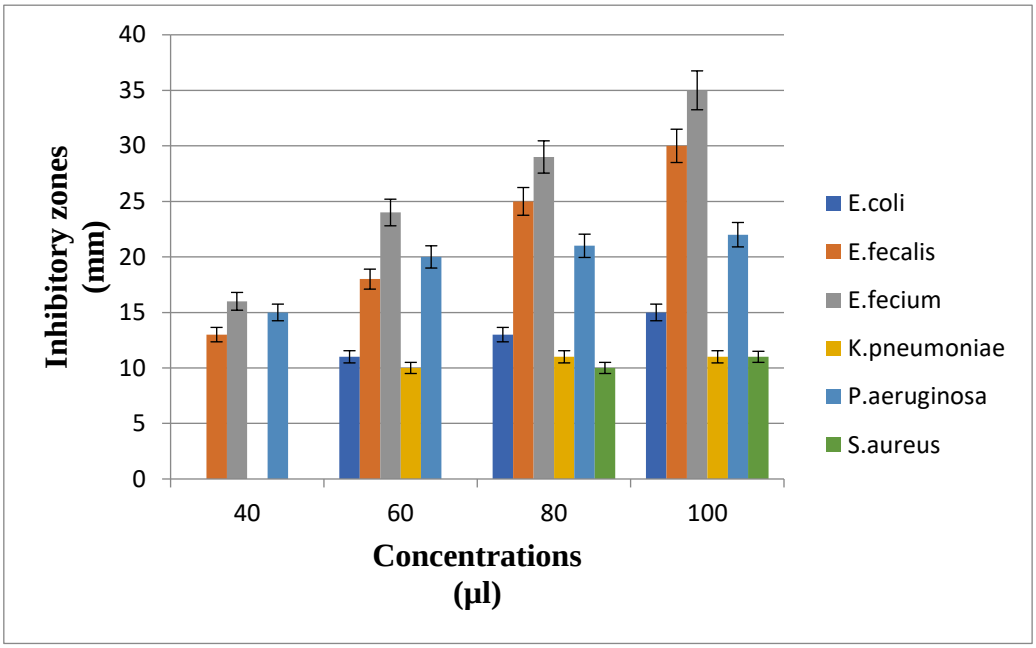

Fig. 01: Zone of inhibition of aqueous extract of *Z. alatum* against bacterial strains

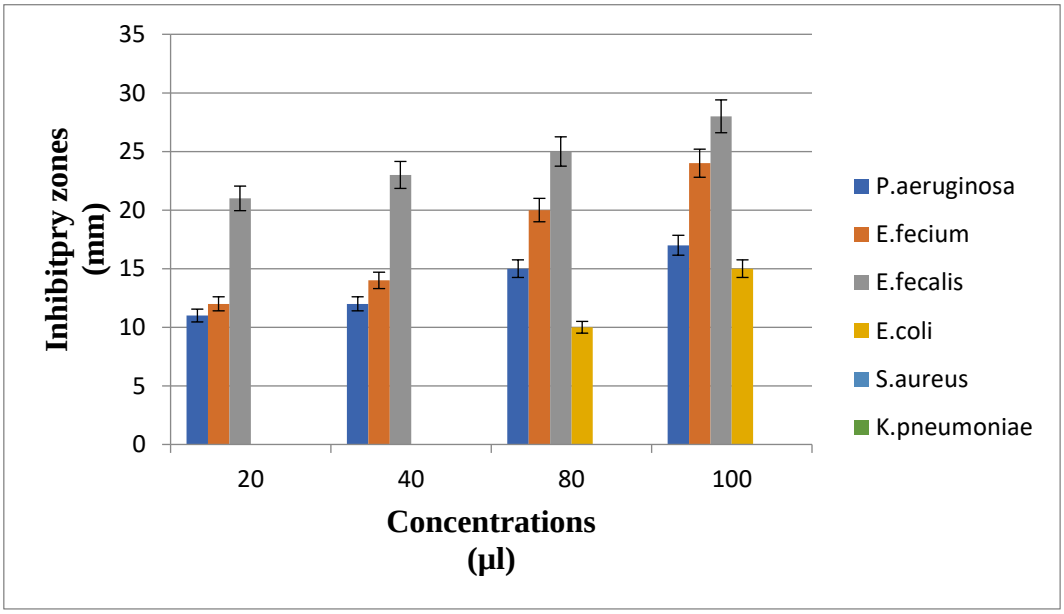

Fig.02: Zone of inhibition of aqueous extract of *G. asiatica* against bacterial strains

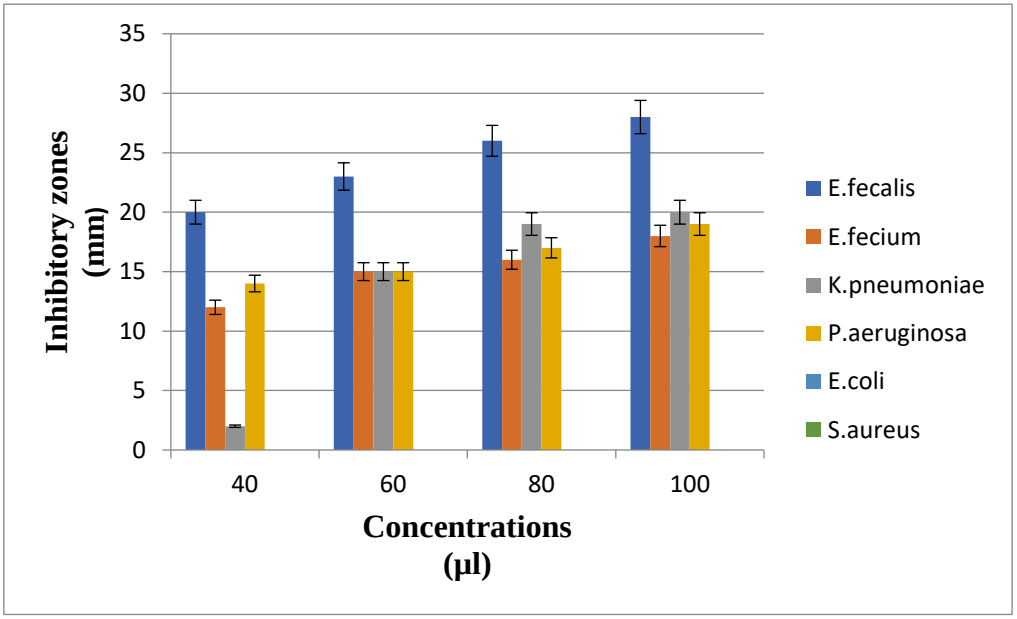

Fig.03: Zone of inhibition of aqueous extract of *J. regia* against bacterial strains

5. Conclusions

The current study was conducted with five bacterial strains including both gram negative and gram positive and their susceptibility was checked against aqueous extract of three medicinal plants. The results showed good antibacterial activity of the extracts against different strains in dose dependent manners. The aqueous extract of *Z. alatum* was the most effective compared to others and *E. fecalis* and *E. fecium* have the least resistance against these extracts.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, RN.; methodology, RK and RN; validation, RTM and KH.; formal analysis, RK.; investigation, RN and AAA.; resources, AAA.; data curation, RK and RN: writing—original draft preparation, RK and MM; writing—review and editing, RTM and KH; visualization, RTM; supervision, RN; project administration, RN.

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