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ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS FROM BALOCHISTAN AGAINST MULTIDRUG RESISTANCE BACTERIA

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Abstract

The emergence of antibiotic resistance bacteria and diseases caused becoming the major health concern globally. The effective treatment for bacterial infections includes a variety of drugs that makes it necessary to know about the potential source of these drugs. The medicinal herbs used by local community of Quetta Balochistan, can be explored as a source to fight against disease caused by bacteria. This study aims to identify antibacterial activity of: Berberis baluchistanica, Solanum nigrum, Trachyspermum ammi, Trigonella foenum-graecum, Anogeissus latifolia, E. coli, S. aureus, and P. aeruginosa (caused hospital acquired infection). Crude ethanolic plant extract was used by agar well diffusion method. The results showed T. Ammi, A. latifolia, B. Baluchistanica and S. Nigrum showed maximum zone of inhibition (ZOI) against E. coli (19.3mm, 18.3, 15.7mm, and 15.1mm), S. aureus (20.7mm, 18.3mm, 15.1mm and 12.3mm) while T. foenum-graecum showed minimum ZOI against E. coli (4.4mm) and S. aureus (3.8mm). Moreover, it was observed that extracts of B. Baluchistanica and A. latifolia were effective against P. aeruginosa with ZOI 8.6mm and 9.2mm, a multi drug resistance (MDR) bacterium, which suggested that these extracts contain some bioactive compound required to inhibit the growth of MDR bacterium. This study confirmed that the efficacy of selected medicinal plant extracts as natural antibiotic medicine and recommended the use of these plants to treat infectious diseases.

Keywords: *Antibacterial Activity, Medicinal Plants, Multidrug Resistance, Bacteria*

Introduction

From decades, human beings relying on medicinal plants (whole/plant parts), to treat different infectious disease caused by microorganism (Mohyuddin, et al., 2022). In today's world approximately more than half of the pharmaceutical drugs are directly or indirectly derived from medicinal plants because of their richness in phytochemicals compounds (secondary metabolites with disease preventing properties) such as phenolic, steroids, alkaloids, cardiac glycoside, saponins, flavonoids, terpenoids, anthraquinones, and phlobatannins (Mohyuddin, et al., 2022). The medicinal importance of plants based on which type of phytochemical compounds is presented with different physiological effects on human body (Adil, et al., 2024).

People from south Asian used medicinal plant commonly in traditional healthcare system to treat different infectious and noninfectious diseases. Among them Trachyspermum ammi (L. Family Apiaceae), also known as "ajwain" (Kumar & Singh, 2021). Traditionally, ajwain seeds are used as spices and flavoring agent. *Anogeissus latifolia* (DC. Family Combretaceae) also known as Axlewood or Dhawra, holds an important position in traditional medicine due to its diverse pharmacological applications. Berberis baluchistanica belongs to the family Berberidaceae and is commonly known as zarch or zaralg, native to mountainous regions of Mastung district Baluchistan Pakistan. Trigonella foenum-graceum (Fenugreek) is an herbaceous plant, used as spice in food and considered oldest medicinal plant to treat different infectious diseases. Solanum nigrum (L. Family Solanaceae), commonly known as "black night shade, makoi and makoh". The leaves and ripen seeds of *S. nigrum* are edible and used as vegetables in most parts of the world. Different parts (roots, stems, leaf, gum exudates) or whole plants are used to treat different aliments such as gastrointestinal disorder, respiratory disorder, urinary tract infection, wound healing, skin allergy, anti-inflammatory, antidiabetic and antioxidant (Kumar & Singh, 2021; Bibi, et al., 2014).

The inappropriate use of antibiotics led to the development of resistance in pathogens which play an important role in the death of million people around worldwide (Kumar & Singh, 2021). According to Global Burden of Disease (GBD) 2019 report, approximately 13.7 million people died from infectious disease caused by antibiotic resistance bacteria. Among the studied bacteria, five leading pathogen Escherichia coli (E. coli), Klebsiella pneumoniae, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa caused 54.9% mortality. Six leading resistance pathogens (E. coli, followed by S. aureus, K. pneumoniae, S. pneumoniae, A. baumannii, and P. aeruginosa) were responsible for 929,000 deaths in 2019 (Zhang, et al., 2024). In recent times, the occurrence of antibiotic-resistance bacteria has become a global concern (Saxena, et al., 2025). This alarming trend is further intensified by the absence of new classes of antibiotics being developed, eventually give rise a situation that is frequently referred as the "anti-bacterial crisis (Zhang, et al., 2024; Schcolnik, 2023). With an increase of this problem worldwide, medicinal plants have gained the attention of researcher as a potential source for the discovery of new anti-bacterial drugs (Saxena, et al., 2025).

Present study five plants were screened based on the ethnobotanical uses by rural communities of Pakistan to treat different bacterial disease. Ethanolic crude extract of T. ammi, A. latifolia, B. baluchistanica, T. foe. graceum and S. nigrum was used to evaluate antibacterial activity against i.e. E. coli, S. aureus and P. aeruginosa by agar well diffusion in the Microbiology laboratory of SBK Women University, Quetta. The implications of the findings are discussed.

Materials and methods

Plant material collection: During this study, T. ammi, A. latifolia, B. baluchistanica, T. foe. graceum and S. nigrum (common name, plant part and yield extract listed in Table 1) were collected from different fields of Hanna Urak and local market (pansar) Quetta, Baluchistan, and stored in sterile airtight plastic bags. The plant specimens were identified based on potential medicinal values as judged by local healers.

Extraction of plant materials: Collected plant materials were disinfected and washed three times with sterilized distilled water to clean all the dirt then dried at room temperature (Aliero, 2018). The dried plant parts of each species were grounded into fine powder by using an electric blender. 10 g of fine powder was soaked in ethanol with continuous shaking for 48 h maximum extraction of plant extract (Gul, Z., Akbar & Leghari, 2022). The extracts were filtered three times by using Whatman filter paper (No. 1) to get clear filtrate. The filtrates were evaporated and dried at 40 ^{o}C by using a rotary vacuum evaporator. The extracts yield was weighed according to the formula as described by (9) and were stored in a sterile small bottle at 4 ^{o}C until use.

Bacterial strains: Three bacterial strains were used in this study, E. coli, and P. aeruginosa (gram negative) and S. aureus (gram positive), provided by Center for Advanced studies in Vaccinology and Biotechnology (CASVAB) Quetta, Balochistan, Pakistan. The bacterial strains were kept in the refrigerator at 4oC until used.

Antibacterial activity of plants: Agar well diffusion assay was performed to investigate the antibacterial activity of each plant extract as described previously (10). Mueller Hinton Agar (MHA) plate was inoculated by spreading a volume of bacterial inoculum carefully on the agar surface. Wells of 6 mm have punched aseptically by using a sterile corn borer, and a volume (10-20 µl) of extract solution at a concentration 25-100mg/ml was added into the well. The plates were incubated under the sterile condition in the incubator at 37^{o}C for 18-24 hours. Thermo Scientific Oxoid Tetracycline antimicrobial disc 30µg (CT0054B) was used as a positive control. The diameter of the ZOI was measured and compared with positive control.

Statistical analysis: All the experiments were conducted in triplicates. The data were statistically analyzed and expressed as mean ± S.D.

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Results and Discussion

Antibacterial activity of *T. ammi, A. latifolia, B. baluchistanica, T. foe. graceum and S. nigrum* were investigated against *E. coli, P. aeruginosa* and *S.* aureus using the agar well diffusion method. The extract of 10 g of plant powder with ethanol yielded the plant extract residues that range from 3.22 to 7.12g. The highest yield (7.12g) of plant extract was obtained from A. latifolia and the lowest extract yield (3.22) from T. Ammi respectively listed in table1

Table 1: The ethnobotanical data of selected Medicinals plant species, their extract yield percentage and antimicrobial screening (10mg/ml)

					Inhibition zone (mm)		
Plant name	**Family**	**Local/common name**	**Part used**	**Yield extract**	***S. aureus***	***E. coli***	***P. aeruginosa***
Berberis baluchistanica	Berberidaceae	Zaralg/ Zarch	Bulbs	4.62	15.1±0.3	15.7±0.2	8.6±0.5
Trachyspermum ammi	Apiaceae	Ajowan/ Bishop's weed	Seeds	3.67	20.7±0.3	19.3±0.2	0.0±0.0
Anogeissus latifolia	Combretaceae	Dhawra/ Axlewood	Leaves	9.12	18.3±0.2	18.3±0.2	9.2±0.1
Solanum nigrum	Solanaceae	Petit morel/ Blacknight shade	Fruit	5.64	12.3±0.1	15.1±0.2	0.0±0.0
Trigonella foenum graceum	Fabaceae	Methi/Fenugreek	leaves	6.73	3.8±0.2	4.4±0.1	0.0±0.0
Tetracycline	--	--	--	--	9.3±0.1	12.7±0.2	12.5±0.1

Antibacterial activity of plant extract: The results of the Agar Well Diffusion method revealed that all plant extracts were potentially effective in suppressing the growth of studied bacteria strain with variable potency. In this study, ethanolic extracts of T. ammi showed the maximum ZOI 20.7 mm and 19.3mm against S. aureus (20.7 mm) and E. coli (19.3mm), among the plants studied and very little or no ZOI was observed against P. aeruginosa (Table1). These results are consistent with (11) showing that the extract of T. ammi was maximum effective against S. aureus and lessen effective against P. aeruginosa. Similarly, (Patil & Gaikwad, 2010) studied antibacterial activity of acetonic and ethanolic extract of T. ammi seeds. It was reported that S. aureus, E. coli, Pseudomonas sp., and Bacillus subtilis showed maximum inhibition against ethanolic extract while acetone extract showed no activity against S. aureus and Bacillus subtilis (Adil, et al., 2024). These findings are consistent with (Sunitha, et al., 2017) showing that T. ammi essential oil exhibits a broad spectrum of antimicrobial activities. The absence of activity against P. aeruginosa in this study might be due to the inherent resistance mechanisms for this Gram-negative bacterium, or differences in the composition of the extracts and the methods used to prepare them (Bibi, et al., 2014). Differences in the ZOI across different studies could also be due to differences in the plant's chemotype, solvent used, extraction methods, and the bacterial strain's susceptibility. In addition, crude extract of B. baluchistanica showed the maximum activity against E. coli (15.7mm), S. aureus (15.1mm), and the minimum activity against P. aeruginosa (8.6). very recently (14) reported similar antibacterial activity in methanolic extract. Further (15) reported that root extract of B. baluchistanica have immense effect on E. coli, S. aureus and least effect on P. aeruginosa. These findings confirmed by (16) and further enhance the prospects of B. baluchistanica as a potential bioactive compound containing components effective against both Gram-positive and Gram-negative bacteria (Lone, Ravindran & Jeandet, 2024). Furthermore, A. latifolia is one of the important medicinal plants and gave the highest quantity of extract (9.12 g) showed antibacterial activity against S. aureus (18.3mm), E. coli (18.3mm) and P. aeruginosa (9.2mm) indicated good extraction as well as rich phytochemical profile. (Hassanshahian, et al., 2014) reported strong antibacterial activity of A. latifolia against gram negative and gram-positive bacteria that further supported this study. In comparison, S. nigrum fruit extract was more sensitive against E. coli (15.1) and S. aureus (12.3). Previously, alcoholic extract of S. nigrum was reported to prevent the growth of caries causing bacteria (18). This study further supported by (Usha, Ragini, & Naqvi, 2012) suggested that ethanolic leaf extract of S. nigrum was more effective in inhibiting growth of E. coli than aqueous extract. Very recently, (Kumar & Singh, 2021; Valgas, et al., 2007) reported that antioxidant and antibacterial activity against seven tested bacteria and nutritional potential of S. nigrum.

The antibacterial activity observed might be due to the presence of bioactive phytochemicals and essential oils which may cause damage to the microbial cell wall, inhibit some enzyme activity, or hinder protein synthesis (Murthy, et al., 2009). The extracts, particularly at 50 mg/ml, were effective, which highlights their potential as herbal antimicrobial agents. Several researchers have proposed that antimicrobial agents of plant origin work by interacting with proteins and enzymes within the bacterial membrane, causing cell death. This method of action can be adopted to solve the problem of antibiotic resistance and over-dependence on modern antibiotics. This study aims to demonstrate the efficacy of selected medicinal plants for use as natural antibacterial agents. More detailed analyses of the plant's phytochemical constituents, toxicity, and the mechanism of actions can be done in future to use the plant extracts for safe and effective therapeutic methods.

Ethical Concern

In this study plant-based material was used only and did not include any animal or human. All the experiments were conducted in accordance with institution guidelines and biosafety procedures.

Declaration of Interest

The authors declare no conflict of interest

Submission declaration and verification

The authors declare that work done under this manuscript is original and has not been published previously and is not under consideration for publication elsewhere. All authors have read this manuscript and agree with its submission in “International Journal of Antimicrobial Agent”. All experiments were conducted in accordance with ethical research standards.

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