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Research Article

Antimicrobial activity of selected indigenous medicinal herbs against human pathogenic bacteria

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Abstract

The traditionally use of indigenous medicinal plants in the treatment of burn, dermatrophytes and human infectious diseases and also still essential part of primary public health care. Antimicrobial activities of nine medicinal plants were determined in vitro through agar well diffusion method against pathogenic microorganism species of gastrointestinal tract. Medicinal plants extract of *Cocculus pendulus*, *Malva neglecta*, *Rhazya stricta*, *Jaubertia aucheri*, *Corchorus depressus*, *Salvia bucharica*, *Microcephala lamellate*, *Berberis baluchistanica* and *Artemisa absinthium* were found sensitive to *Clostridium spp*. The extracts of *Malva neglecta*, *Jaubertia aucheri*, *Salvia bucharica* and *Berberis baluchistanica* were observed sensitive to *E. coli*. Similarly the extracts of *Malva neglecta*, *Jaubertia aucheri*, *Rhazya stricta*, *Corchorus depressus*, *and Artemisa absinthium* were found sensitive to *Salmonella spp*. The extracts of *Cocculus pendulus*, *Malva neglecta*, *Jaubertia aucheri*, *Corchorus depressus*, *Salvia bucharica*, *Microcephala lamellate*, *Berberis baluchistanica* and *Artemisa absinthium* were sensitive to *Shigella spp*. The extracts of *Cocculus pendulus*, *Jaubertia aucheri* and *Berberis baluchistanica* were found sensitive to *Klebsiella spp*. The extracts of *Cocculus pendulus*, *Rhazya stricta*, *Corchorus depressus*, *Microcephala lamellate* and *Artemisa absinthium* were revealed resistance to *E. coli*. The extracts of *Cocculus pendulus*, *Salvia bucharica*, *Microcephala lamellate* and *Berberis baluchistanica* were revealed resistance to *Salmonella spp*. The extract of *Rhazya stricta* was revealed resistance to *Shigella spp*. The extracts of *Malva neglecta*, *Rhazya stricta*, *Corchorus depressus*, *Salvia bucharica*, *Microcephala lamellate* and *Artemisa absinthium* were revealed to resistance to *Klebsiella spp*. The extract of *Jaubertia aucheri* was highly sensitivity against *E. coli*, *Salmonella spp*, *Shigella spp*, *Clostridium spp* and *Klebsiella spp*.

Keywords: Plants; Herbs, Bacteria; Gastrointestinal; Balochistan

Introduction

The herb meaning is grass which derived from the Latin word "*herba*", and primarily it was applied to a wide range of leafy vegetables. Herbs are seed plants that do not produce woody stems like a tree and live long enough to develop and produce flowers and seeds [1]. Many decades herbs have been used for their flavor, medicinal, antimicrobial and anti-oxidative properties. The most important part of herbs are considered as powerful tools to establish wellness such as stimulate production of enzymes that detoxify carcinogens, inhibit cholesterol synthesis, block estrogen, lower blood pressure and prevent blood clotting [2]. In addition herb plants providing nutrition and have an important source of chemical compounds, which could be used for medicinal purposes by human. According to the ancient human knowledge the importance of medicinal plants are date back to many centuries ago [3].

However, medicinal plants have been used as traditional medicine with excellent efficacious remedies and serve as dietary source to animals and humans. The plants provide sufficient nutrients to meet the metabolic requirements for their health, growth and productivity and also play essential role reducing the risk or delaying the onset of diseases and disorders [4], [5].

The world's population approximately 80% relies on traditional medicine for their primary health care. [6]. A various plants are being used as medicinal agents all over the world. It has been reported that many species possess medicinal use such as 1500 species in India [7], 5000 species in china [8] and 1600 species in north-west Amazonia [9]. Similarly it is noted that plants based medicine as a potential more effective limit the diseases and cheaper, therefore probably herbal medicines are responsible for the fast growing industry [10]. Currently traditional use of plants by indigenous communities many drugs in the market have come from folk medicine [11]. Moreover, medicinal plants played key roles in the health care for human and livestock. Therefore plants extracts and natural compounds are used controlling diverse diseases such as cough, inflammation and diarrhea in human and livestock. A large number of medicinal plants have been screened and validated for their ethno-pharmacological [12].

Pakistan has diverse weather condition and great biodiversity of medicinal plants. Generally medicinal plants are produced in large quantities in the mountainous area than in grasslands due to obviously favorable habitat and suitable climatic conditions [13]. Balochistan province is also natural home-producer of many medicinal plants and soled in native market place [14]. In Pakistan medicinal plants are naturally grown in ecological zones and rich flora of over 6000 plant species and more than 1000 species are identified as medicinal and aromatic plant species and also small scale of some species are cultivated. Temperature fluctuates below zero in the Northern mountainous area and 50 ^{0}C in the Southern plains in Pakistan. The usage of medicinal plants improved knowingly to abilities for healing purpose [15]. The demand of medicinal plants is increasing both in developed and developing countries and like Pakistan people depends on medicinal plants for health care, food, shelter and fodder for animals which plays a vital role against different bacterial infections [16].

There are definitely abundant clinical studies have not been reported for antimicrobial activity of local plants against pathogenic bacteria in Balochistan province. Hence, the present study attempt to determine the antimicrobial activity of local herbal medicinal plants against pathogenic bacteria such as *Salmonella spp, E. coli,*

Shigella spp, Klebsiella spp and *Clostridium spp.*

Materials and methods

Collection of plant samples

Nine medicinal plants species (*Cocculus pendulus, Malva neglecta, Jaubertia aucheri, Rhazya stricta, Corchorus depressus, Salvia bucharica, Microcephala lamellate, Berberis baluchistanica* and *Artemisa absinthium*) were obtained from different locations of Balochistan, Pakistan. Plants were washed with tap water in order to remove the dust and identified by a qualified plant taxonomist and labeled kept in the Center for Advanced Studies in Vaccinology and Biotechnology" (CASVAB) University of Balochistan and "Institute of Biochemistry" University of Balochistan, Quetta for extraction and further analysis.

Preparation of plants extract

The plant samples were dried and grind into fine powder by using a blender (ANEX-AG-179 GL). The samples were soaked in 50 ml methanol (LAB-SCAN ASIA Co., LTD) and shake twice a day, placed for two weeks at room temperature (25^0C). After two weeks, the mixture was twice filtered by Watt-man No-14 filter paper (Watt-man Grade 14), than methanol was completely evaporated by the help of rotary evaporator (Buchi Rotary – evaporator Model R-205). The obtained semisolid extracts were kept (open air) for two days and the extracts were stored at 8^0C for the further analysis [17].

Pathogenic microorganisms

The human pathogenic microorganisms such as *E. coli, Salmonella spp, Shigella spp, Clostridium spp* and *Klebsiella spp* were used for antibacterial activity. The bacteria were purified and biochemically characterized through different selective media (MacConkey Reinforced Clostridium and Salmonella- Shigella agar media), and tests (Indole, MR-VP and Sugar fermentation tests). The entire tests were performed three times at 37 ^{0}C and were incubated according to bacteria.

Preparation of medium

Brain heart infusion broth and agar (1.5%) were prepared by using distilled water for the growth of bacteria and pH of the medium was adjusted at 7.0 pH. The dissolved medium was autoclaved at 15 lbs pressure at 121^0C for 15 minutes. Three times experiments were performed to the confirmation of the sensitivity and resistance on herbal extracts for bacterial growth.

Agar well diffusion method

Agar diffusion method was used to determine the antimicrobial activity of medicinal plants. The 6 mm holes were made in agar plate and 50 ul extract solution was poured in it. After 24hrs the zone of inhibition was recorded.

Results

The antimicrobial activity of methanol extracts of 9 (nine) indigenous medicinal herbs were investigated by using agar well diffusion method against human pathogenic bacteria such as *E. coli, Salmonella spp, Shigella spp, Clostridium spp* and *Klebsiella spp*.

In the agar well diffusion method the *Cocculus pendulus* extract showed maximum zone of inhibition against *Clostridium spp, Klebsiella spp, Shigella spp* while *E. coli* and *Salmonella spp* were resistance to *Cocculus pendulus* extract as shown in Table 1.

Table 1. Zone of inhibition on *Cocculus pendulus* extract against pathogenic bacteria

Botanical Name	**Common Name**	***E. coli***	***Salmonella spp***	***Shigella spp***	***Clostridium spp***	***Klebsiella spp***
Cocculus pendulus	Zamur	–	–	17mm	26mm	20mm

The *Malva neglecta* extracts showed maximum zone of inhibition against *Shigella spp, E. coli, Clostridium spp, and Salmonella spp* while *Klebsiella spp* was resistance to *Malva neglecta* extracts shown in Table 2.

Table 2. Zone of inhibition on the *Malva neglecta* extract against pathogenic bacteria

Botanical Name	**Common Name**	***E. coli***	***Salmonella spp***	***Shigella spp***	***Clostridium spp***	***Klebsiella spp***
Malva neglecta	Pochko	21mm	14mm	31mm	16mm	_

The *Jaubertia aucheri* extract showed maximum zone of inhibition against *Clostridium spp, E. coli, Salmonella spp* and *Klebsiella spp* while *Shigella spp* was minimum *Jaubertia aucheri* extract as shown in Table 3.

Table 3. Zone of inhibition on the *Jaubertia aucheri* extract against pathogenic bacteria

Botanical Name	**Common Name**	***E. coli***	***Salmonella spp***	***Shigella spp***	***Clostridium spp***	***Klebsiella spp***
Jaubertia aucheri	Thusso	24mm	22mm	19mm	26mm	20mm

The *Rhazya stricta* extract showed maximum zone of inhibition against *Clostridium spp, Salmonella spp while Klebsiella spp, Shigella spp* and *E. coli* were resistance to *Rhazya stricta* extract as shown in Table 4.

Table 4. **Zone of inhibition on the *Rhazya stricta* extract against pathogenic bacteria**

Botanical Name	**Common Name**	***E. coli***	***Salmonella spp***	***Shigella spp***	***Clostridium spp***	***Klebsiella spp***
Rhazya stricta	Aeshark	_	24mm	_	31mm	_

The *Corchorus depressus* extract showed maximum zone of inhibition against *Clostridium spp, Shigella spp, Salmonella spp, Klebsiella spp* while *E. coli* was resistance to *Corchorus depressus* extract as shown in Table 5.

Table 5. Zone of inhibition on the *Corchorus depressus* extract against pathogenic bacteria

Botanical Name	**Common Name**	***E. coli***	***Salmonella spp***	***Shigella spp***	***Clostridium spp***	***Klebsiella spp***
Corchorus depressus	Bandary	_	16mm	17mm	22mm	_

The *Salvia bucharica* extracts showed maximum zone of inhibition against *Clostridium spp, E. coli, Shigella spp* while *Salmonella spp* and *Klebsiella spp* were resistance to *Salvia bucharica* extract as shown in Table 6.

Table 6. Zone of inhibition on the *Salvia bucharica* extract against pathogenic bacteria

Botanical Name	Common Name	*E. coli*	*Salmonella spp*	*Shigella spp*	*Clostridium spp*	*Klebsiella spp*
Salvia bucharica	Gul-e-kakar	21mm	_	16 mm	31mm	_

The *Microcephala lamellate* extract showed maximum zone of inhibition against *Clostridium spp, Shigella spp* while *Salmonella spp, E. coli* and *Klebsiella spp* were resistance to *Microcephala lamellate* extract as shown in Table 7.

Table 7. Zone of inhibition on the *Microcephala lamellate* extract against pathogenic bacteria

Botanical Name	Common Name	*E. coli*	*Salmonella spp*	*Shigella spp*	*Clostridium spp*	*Klebsiella spp*
Microcephala lamellate	Phenophulii	_	_	16mm	26mm	_

The *Berberis baluchistanica* extract showed maximum zone of inhibition against *E. coli, Clostridium spp, Shigella spp* and *Klebsiella spp* while *Salmonella spp* were resistance to *Berberis baluchistanica* extract as shown in Table 8.

Table 8. Zone of inhibition on the *Berberis baluchistanica* extract against pathogenic bacteria

Botanical Name	Common Name	*E. coli*	*Salmonella spp*	*Shigella spp*	*Clostridium spp*	*Klebsiella spp*
Berberis baluchistanica	Badrah / Zarch	19 mm	_	15mm	16mm	16mm

The *Artemisa absinthium* extracts showed maximum zone of inhibition against *Salmonella spp, Clostridium spp, Shigella spp* while *E. coli* and *Klebsiella spp* were resistance to *Artemisa absinthium* extract as shown in Table-9.

Table 9. Zone of inhibition on the *Artemisa absinthium* extract against pathogenic bacteria

Botanical Name	Common Name	*E. coli*	*Salmonella spp*	*Shigella spp*	*Clostridium spp*	*Klebsiella spp*
Artemisa absinthium	Aftasen	_	26mm	14mm	21mm	_

Biochemical tests

Different biochemical tests were performed for the confirmation of Gram negative and Gram positive bacteria as shown in Table 10.

Table 10. Biochemical test results for the gram positive bacteria and gram negative bacteria

S.No	Biochemical test	*E. coli*	*Salmonella spp*	*Shigella spp*	*Klebsiella spp*	*Clostridium spp*
1-	Methyl red	+	+	+	_	_
2-	Indole test	+	_	variable	_	_
3-	Voges-proskauer	_	_	_	+	_
4-	Lactose	+	_	_	+	+
5-	Maltose	+	+	variable	+	+
6-	Mannitol	+	+	variable	+	_
7-	Sucrose	D	_	_	+	+

d= 29-57% positive, V= reaction variable between species

Discussion

Medicinal plants comprise one of the major non wood forest practices and it has antimicrobial activity. The antimicrobial activities of methanol extracts of nine medicinal plants were determined in vitro through agar well diffusion method against pathogenic microorganism species and these caused different infection in human being. The antimicrobial activity result revealed that *Jaubertia aucheri* was highly sensitivity against *E. coli, Salmonella spp, Shigella spp, Klebsiella spp* and *Clostridium spp. Microcephala lamellate* showed sensitivity against *Shigella spp* and *Clostridium spp* while complete resistance towards the *E. coli, Salmonella spp* and *Klebsiella spp*. *Malva neglecta* showed sensitivity against *E. coli, Salmonella spp, Shigella spp* and *Clostridium spp* while show resistance towards the *Klebsiella spp.* The *Cocculus pendulus* have good medical values in traditional system and our results also showed that *Cocculus pendulus* was sensitivity against *Shigella spp, Clostridium spp* and *Klebsiella spp* while show resistance towards the *E. coli* and *Salmonella spp*. *Rhazya stricta* is used in various diseases and it have antimicrobial activity against *Salmonella spp* and *Clostridium spp* while show resistance towards the *E. coli ,Shigella spp* and *Klebsiella spp*. *Corchorus depressus* showed sensitivity against *Salmonella spp, Shigella spp* and *Clostridium spp* while show resistance towards the *E. coli* and *Klebsiella spp*. *Salvia bucharica* showed sensitivity against *E. coli, Shigella spp* and *Clostridium spp* while show resistance towards the *Salmonella spp* and *Klebsiella spp*. *Berberis baluchistanica* showed sensitivity against *E. coli, Shigella spp, Clostridium spp* and *Klebsiella spp* while show resistance towards *Salmonella spp*. *Artemisa absinthium* showed sensitivity against *Salmonella spp, Shigella spp* and *Clostridium spp* while show resistance towards the *E. coli* and *Klebsiella spp*. The Gram positive bacteria were more sensitive than Gram negative bacteria. This can be due to the variance in their cell wall composition as same as described by **[18].** Medicinal herbs are great significance used for different infectious diseases. *Jaubertia aucher* showed higher antimicrobial activity against *Clostridium spp, E. coli, Salmonella spp, Shigella spp* and *Klebsiella spp*. Pakistan is developing country and people of it have various diseases so it is necessary to search out good medicinal herb which will help for detection of active intergradient for the control of diseases.

Conclusion

Many diseases are common in the third world countries. It is essential to search out and stimulate medicines that are plant based. This work will support to isolate active intergradient for the treatment of bacterial diseases.

Authors' contributions

Conceived and designed the experiments: F Rehman, A Sajjid & MK Taj, Performed the Experiments: R Rehman, MK Taj, & MA Mengal, Analyzed the Data: F Rehman, H Mengal, A Mengal & S Azam, Contributed reagents/ materials/ analysis tools: F Rehman & A Sajjid, Wrote the paper: F Rehman & MK Taj

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