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Research Article

Analysis of antibacterial activity of indigenous *Mirabilis jalapa, Solanum nigrum* and *Aloe vera*

Rameez Nisar[1], Raja Tahir Mahmood[2*], Ansar Ahmed Abbasi[1], Khan Muhammad[3], Khalid Hameed[1], Muhammad Asghar Khan[4], Tauqeer Ahmed Mughal[1], Saiqa Khushal[1], Rabia Shaukat[1] and Maleeha Masood[2]

1. Department of Zoology, Mirpur University of Science and Technology (MUST), Mirpur (AJK) Pakistan
2. Department of Biotechnology, Mirpur University of Science and Technology (MUST), Mirpur (AJK) Pakistan
3. Azad Jammu and Kashmir Medical College, Muzaffarabad, Pakistan
4. National University of Medical Sciences, Rawalpindi, Pakistan

**Corresponding author's email: raja.tahir@must.edu.pk*

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Abstract

In this study, the antimicrobial potential of medicinal plants was determined against three gram-negative: *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*, and four gram-positive bacteria: *Streptococcus pyogenes, Bacillus subtilis*, *Enterococcus* and *Staphylococcus aureus*. Aqueous extracts of three medicinal plants namely *Mirabilis jalapa, Solanum nigrum* and *Aloe vera* were used to check the antimicrobial activity. The antimicrobial potential of these plants was determined by agar well diffusion method. The antimicrobial activity of aqueous extract of *Aloe vera* was better than *Solanum nigrum*, followed by *Mirabilis jalapa* which exhibited lesser activity. Maximum zone of inhibition was shown by *K. pneumonia* 22 mm at 100 µL concentration for *A. vera* extract. The was no antibacterial activity against *B. subtilis* and Enterococcus by *A. vera*. Maximum zone of inhibition was exhibited by *K. pneumoniae* and *B. subtilis* 20 mm at 100 µL concentrations of *M. jalapa*. The *Enterococcus*, *S. pyogenes* and *P. aeruginosa* did not show any result against *M. jalapa* extract. The highest zone of inhibition was exhibited by *S. pyogenes* 17 mm at 100 µL concentration. *E. coli* and *K. pneumoniae* did show any activity against plant.

Keywords: Antibacterial *activity; A. vera; B. subtilis;* Medicinal Plants; *S. nigrum*

Introduction

It has been reported that medicinal herbs would be the exceptional source to obtain numerous antibiotics. The majority of people in urbanized nations nearly 80% use traditional medicines that contain ingredients derived from medicinal herbs **[1]**. World Health Organization giving awareness to people about multidrug resistant microbes that are intensifying globally, which is a massive challenge to healthcare. Therefore, there is an urgent need to take action so that antibiotics might lose their power to cure maladies **[2]**. It is difficult to cure serious community and hospital-acquired infections caused by multidrug-resistant bacteria by taking the available antibiotics **[3]**.

Multiple factors such as specific nature of the connection between microorganisms and antibiotics, the use of antimicrobial agents, host features, and environmental conditions, all contribute to the spread of drug resistance. Due to this problem, researchers have been forced to hunt for novel antibacterial chemotherapeutic agents from a variety of sources; nevertheless, the manufacturing of artificial antibiotics is extremely expensive and has undesirable side effects when compared to antibiotics derived from plants [4].

Multi-drug resistance poses severe threat to the medicinal world and diseases brought about by multi-resistant microorganisms particularly in the serious consideration units create an enormous issue [5]. As a result, herbs are a priceless source of healing substances. Additionally, the main components of many antibiotics are still derived from natural sources, unless otherwise directed [6]. Man cannot live on our planet for a long, healthy, and generous existence without the help of the plant kingdom since herbal products and their dynamic components are essential for sustaining excellent health. The world is blessed with a wealth of restorative herbs [7]. In the last few decades, increased germ resistance to commonly used antibiotics has generated discussion on a global scale. As a result, demand from consumers for natural anti-microbial compounds is progressively rising. As an alternative therapeutic agent in the pharmaceutical sector, natural antibacterial agents have attracted a lot of attention [8].

These plants produce drugs that are easily accessible, affordable, safe, effective, and rarely come with adverse effects. The most obvious starting point for novel therapeutically effective medications, such as anticancer drugs and antibacterial pharmaceuticals, is plants that have been favoured for medical usage over thousands of years [9, 10]. Despite recent breakthroughs in the field of chemotherapy, the use of medicinal plants has grown. The explanations advanced include the use of medicinal herbs as sources for the extraction of potent pharmacological substances [11].

Therapeutic and scented herbs and their soul are enriched in antimicrobial components which could be a substitute to fight microbial infections even against few microscopic organisms that are becoming resistant to various artificial drugs [12-14]. The most significant constituents of herbs are alkaloids, flavonoids, tannins as well as phenolic components [15]. Due to the presence of significant levels of antioxidant and antibacterial phyto-constituents, many plant species have been ingested. As a result, a significant source of antioxidant and anti-aging characteristics can be found in the extracts of medicinal plants and natural goods [16]. Many human infections are caused by oxidative pressure that results from unevenness between the arrangement and neutralization of pro-oxidants [17].

Numerous secondary metabolites that are produced by plants are used in the pharmaceutical industry as lead molecules or as direct precursors. However, only a small portion of the 400,000 plant species on earth have had their antibacterial properties thoroughly researched [18].

Materials and Methods

Plant sample collection

The plant materials used in this study were obtained from different areas of Azad Jammu and Kashmir. *Solanum nigrum* was collected from Mirpur AJ&K. *Aloe vera* was collected from Forest office Mirpur AJ&K, while, *Mirabilis jalapa* was collected from Tatta pani District Kotli AJ&K.

Bacterial isolates

The bacterial isolates selected for this study were collected from Microbiology section of the Pathology Labs of following hospitals of Rawalpindi/Islamabad, Pakistan. *Staphalococus aureus* and *Enterococcus* were collected from PIMS Islamabad. The strains of *Bacillus subtilis* and *Streptococcus pyogenes* were obtained from Combined Military Hospital (CMH) Rawalpindi, while, *Psedomanas*

aeroginosa, *Escherichia coli* and *Klebisella pneumoniae* were collected from Al-shifa International hospital Islamabad. Bacterial isolates were stored in the refrigerator at 4 °C.

Culture media and chemicals

In this research, cultivation of cells was done by using nutrient agar (oxoid # CM003). The saline solution (0.9 % McFarland unit) was also used in this lab work.

Inoculum preparation

The stock of each of the microbes were revived and kept up on supplement agar plates. The cultures were prepared by streaking on sterile nutrient agar plates and kept in incubator at 37 °C for 24 hours and utilized as sub-cultures. Microbial cultures were revived after every 3 to 5 days to evade contamination.

Preparation of plant extract

Fresh extract

Before extraction, leaves of *Solanum nigrum* were washed with tap water and then, with distilled water thrice times to remove contamination or dust. Then leaves were crushed and ground with mortar pestle and was filtered through filter paper and kept in the refrigerator for the future use.

The *A. vera* juice and *M. jalapa* extracts were taken through filtration with the help of mesh cloth. The 40 mL of *A. vera* juice was soaked in 40 mL of distilled water and placed in water bath for 24 hours and placed in refrigerator.

Preparation of saline solution and McFarland solution

By mixing 0.9 g of NaCl with 100 mL of distilled water and sterilising it in an autoclave, saline solution was created and placed in an incubator at 37 °C for 24 hours. On next day, saline solution was inoculated with bacterial cultures and kept on shaking until the cultures were mixed in saline solution. Then, McFarland (0.5 McFarland turbidity standard) solution was used to adjust the turbidity of the strains by adding sterile distilled water. The McFarland standard solutions was prepared by adding 0.05 mL of barium chloride ($BaCl_2$) in 9.95 mL of 0.18 M H_2SO_4 with constant stirring. After mixing, solution was autoclaved and stored in refrigerator for 24 hours. After 24 hours, the turbidity of saline solution was adjusted for comparing the test and standard were compared against a white background with a contrasting black line.

Antibacterial activity testing by well diffusion method

For well diffusion method, nutrient agar was prepared and autoclaved. Then it was poured into sterilized plates and then let it solidify. And plates were incubated at 37 °C for 24 hours to check the sterility. Agar plates without any contamination were selected for inoculation. Pre-sterilized saline solution was inoculated with fresh cultures and the turbidity of the cultures was adjusted. Sanitized cotton swab was dipped into diluted cultures and microbial lawn was arranged over the nutrient agar plates. Wells having 6 mm diameter were prepared by the help of sterilized cork borer (dipped in spirit, followed by incineration) in the seeded agar using the previously marked places and the borer was used to remove the agar from the marked points. Wells were filled with 100 µL of each plant extract by the help of micropipette using sterilized tips and then incubated for 24 hours at 37 °C. The zones of inhibition were observed after 18 to 24 hours incubation. The same well diffusion method was used to check the minimum inhibitory concentration of plant extracts. Four wells of 100 µL, 40 µL, 20 µL and 10 µL were made in each agar plate. Each well of different concentration was filled with respective concentration by sterilized micro-pipettes and then incubated for 24 hours at 37 °C. The zones of inhibition were measured in millimeters after 18 to 24 hours.

Results

Antimicrobial activity of *A. vera*, *M. jalapa* and *S. nigrum* were determined against seven bacterial species namely: *S. pyogenes*, *P. aeruginosa*, *K. pneumonia*, *Enterococcus*, *E. coli*, *S. aureus* and *B. subtilis.* All these herbs showed effective

inhibitory activity against some of the tested microbes and ineffective against others. The result of antimicrobial activity of aqueous extracts of three plants against seven bacterial isolates are presented in the (Fig. 1).

The antimicrobial activity of aqueous extract of *Aloe vera* was better than *S. nigrum*, followed by *M. jalapa*, which exhibited lesser activity.

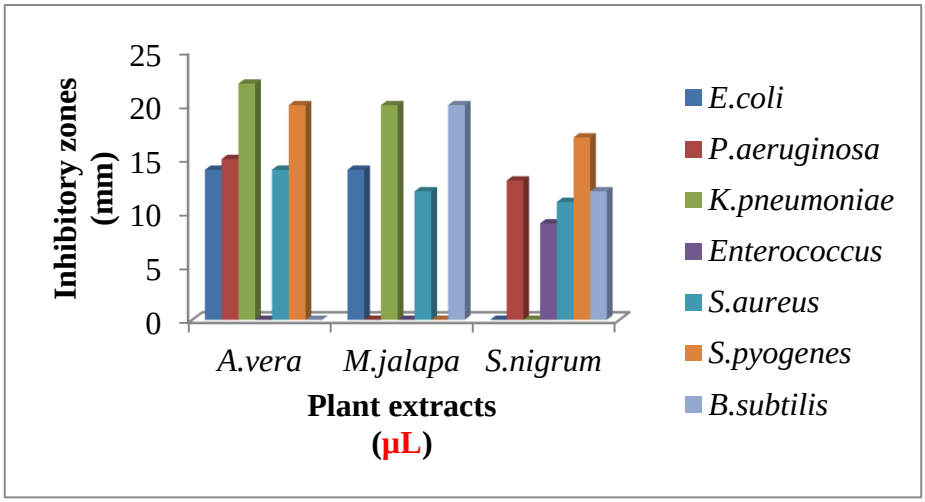

Figure 1. Zones of inhibition of three plant extracts against pathogenic bacteria in mm

A. vera plant showed antimicrobial activity against five pathogens and two pathogens showed resistance as shown in (Fig 2). Four different concentrations 10, 20, 40 and 100 µL concentrations were used against each tested pathogen. Maximum zone of inhibition was shown by *K. pneumonia* 22 mm at 100 µL concentration. Moderate level activity of activity was revealed by *S. pyogens*, *P. aeruginosa*, *E. coli* and *Enterococcus* with a zone of inhibition 20 mm, 15 mm, 14 mm, 14 mm, respectively at 100 µL concentration. The *B. subtilis* and Enterococcus did show any antimicrobial activity against *A. vera*.

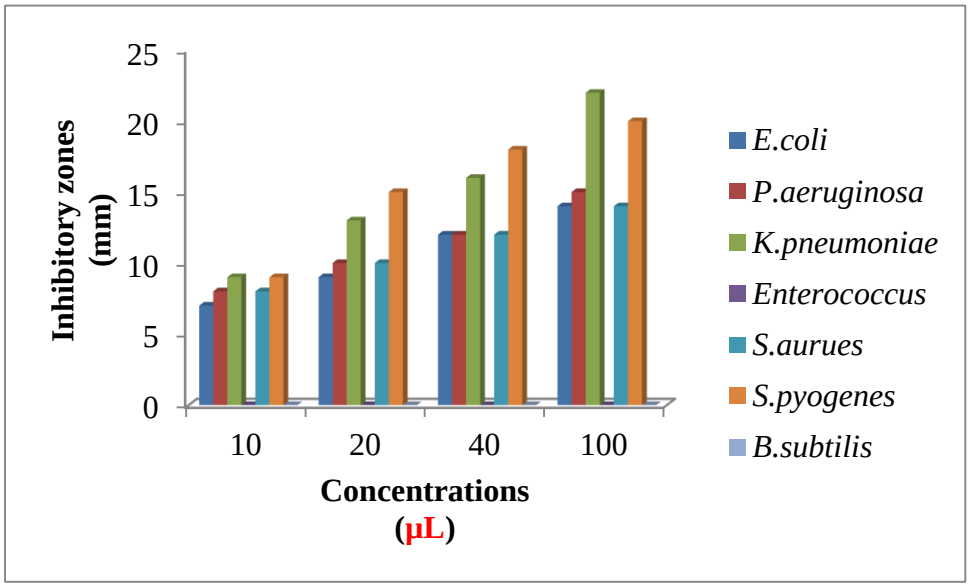

Figure 2. Zones of inhibition against seven bacterial strains by extract of *A. vera*

M. jalapa shown activity against four pathogens and three pathogens showed resistance as shown in (Fig. 3). Four different concentrations 10, 20, 40 and 100 µL concentrations were used against each tested pathogen Maximum zone of inhibition was exhibited by *K. pneumoniae* and *B. subtilis* 20 mm at 100 µL concentrations. Moderate level of activity was shown by *E. coli* and *S. aureus* 14 and 12 mm respectively at 100 µL concentrations. The *Enterococcus*, *S. pyogenes* and *P. aeruginosa* did not show any result against *M. jalapa*.

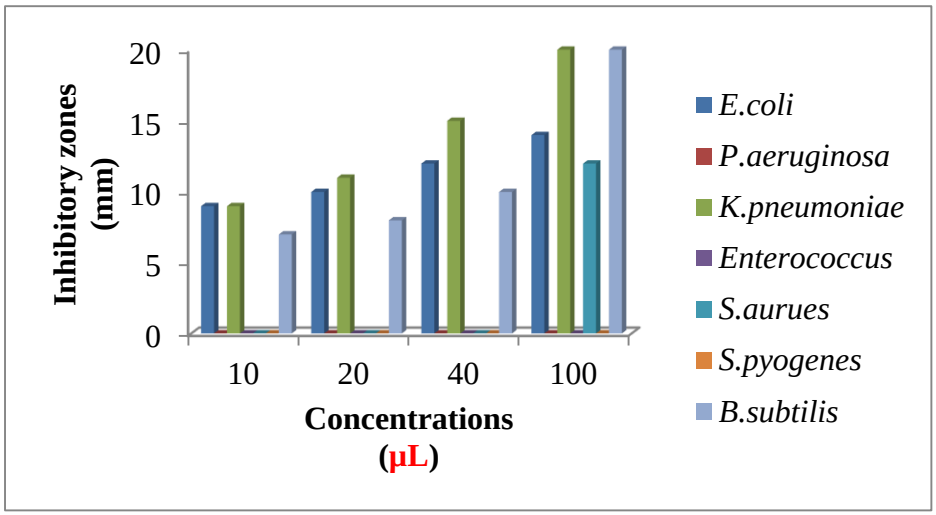

Figure 3. Zones of inhibition of *M. jalapa* against seven bacterial strains in µL

Antimicrobial activity of seven pathogens were checked against *S. nigrum* as shown in (Fig. 4). *E. coli* and *K. pneumoniae* did show any activity against plant. Four different concentrations 10, 20, 40 and 100 µL concentrations were used against each tested pathogen. The highest zone of inhibition was exhibited by *S. pyogenes* 17 mm at 100 µL concentration. Moderate level of activity was shown by *P. aeroginosa*, *S. aureus* and *B. subtilis* 13, 11 and 12 mm respectively at 100 µL concentration.

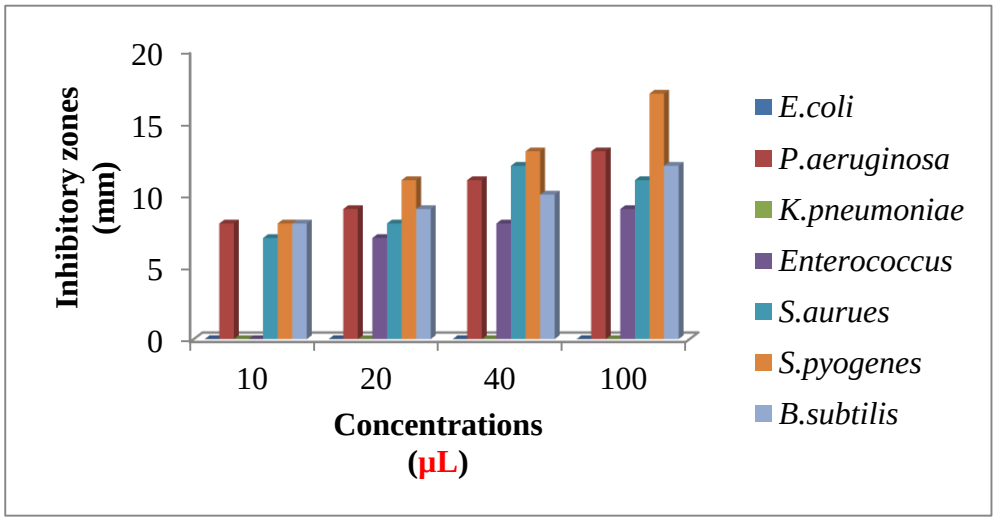

Figure 4. Zones of inhibition of *S. nigrum* against seven bacterial strains

Discussion

As herbal combinations have made significant pledges to human prosperity, the herbs have typically been a source of hope for novel antibiotic components. Utilizing plant extracts with well-known antibacterial properties can be extremely important for therapeutic purposes [19]. In the last decade, *Aloe vera* has been utilized broadly in health-care products like; ointments, cosmetics and health drinks, with its antimicrobial properties [20].

In this study, it was observed that aqueous extract of *A. vera* exhibited excellent antibacterial action against the selected bacterial species: *P. aeruginosa, K. pneumoniae, E. coli, S. aureus* and *S. pyogenes*. Results of the conducted research work were similar to that of Kaithwas *et al*. [21] and also to the studies conducted by Mangena, 1999 in which it was found out that *A. vera* gel was rich in various types of secondary metabolites like, tannins, polysaccharides, anthraquinone, glycosides, enzymes, sterols, vitamins, minerals and organic acids [22, 23].

The antimicrobial activity of *A. vera* extracts containing anthraquinone aloe emodin [24]. Selvamohan *et al.* [25], found the aqueous extract of *A.vera* exhibited moderate antibacterial activity against *E. coli, Klebisella* sp., *Pseudomonas* sp. and *Staphylococcus* sp., which was similar to our present work.

In the previous research, it was shown that *A. vera* exhibited inhibitory activity against *S. aureus K. pneumoniae* and *E. coli*, which was in accordance to our work [21, 26, 27]. The present study was in accordance with the results stated by Johnson *et al.*, 2011, who found aqueous extract of *A. vera* had remarkable inhibitory activity against two bacterial species *i.e. E. coli* and *S. aureus* [28].

Arun kumar, *et al.* [29], reported the maximum inhibitory activity of *A. vera* extract against four bacterial species: *E. coli, S. pyogenes, S. aureus* and *P. aeruginosa*, which was similar to our present study. The aqueous extract of *A. vera* gel showed inhibition on *S. pyogenes, P. aeruginosa, E. coli* and *S. aureus*. Ferro *et al.* [30], found *A. vera* gel can inhibit gram positive bacteria *S. pyogenes*, which was not similar to our work.

A. vera exhibited minimum inhibitory activity against gram negative microbes due to the thick murein layer present in their structure, which inhibits the entry of growth inhibitors [31]. The MIC of *A. vera* gel for Gram-positive microbes, which has additionally been accounted for already, might be associated with the variation in cell wall structures of the microbes [30, 32].

In this study, the results depicted that aqueous abstract of *S. nigrum* showed antimicrobial action on *P. aeruginosa, Enterococcus, S. aureus, S. pyogenes* and *B. subtilis*, respectively. Results indicated that gram positive microorganisms were more sensitive to the aqueous extract of *S. nigrum* than gram negative microorganisms, which showed more resistance. The reason would be that gram positive bacteria have peptidoglycan in their cell wall and no outer membrane, which couldn't block the passage of hydrophobic molecules across the cell wall [33]. Whereas, gram negative microbes possess lippolysaccharides (LPS) layer in outer membrane presents as a strong barrier due to high hydrophobicity [34].

Chauhan, *et al.* [35], tested aqueous extract of *S.nigrum* against five bacterial species named as: *B. subtilis, P. aeruginosa, Enterobacter aerogenes, S. aureus,* and *E. coli* which is partially similar to our present research. In the present study, aqueous extract of *S. nigrum* prevented the development of *B. subtilis, P. aeruginosa* and *S. aureus* while exhibited no inhibitory activity against *E. coli*, respectively.

Aqueous extract of *S.nigrum* possess excellent hypoglycemic effect [36]. Abbas *et al.* [37] determined water extract of *S. nigrum* to be the most active candidate, possessing antimicrobial activity, which was in consistency to our present work. The reason would be that water is a polar compound and polar solvents possess

higher potential for antibacterial action than those extracts with lesser polarity. Also, the antibacterial activity always depends upon the concentration of extracted antibacterial secondary metabolites, which can be increased with the increase in polarity of solvents **[38]**.

Das *et al.* **[39]** tested the aqueous extract of *S. nigrum* against different pathogenic and food-borne microorganisms. From which the extract showed excellent inhibitory activity against *B. subtilis*, which was similar to the present study. *S. nigrum* contains phytochemical constituents including, alkaloids, saponins, tannins, flavinoids, proteins *etc*, which are responsible for the antimicrobial activity **[40]**.

Singh *et al.* **[41]** determined the antimicrobial potential of aqueous and alcoholic extract of *S. nigrum* by Agar diffusion method against *S. aureus, B. subtilis, E. coli, P. mirabilis, K. pneumonia* and *P. aeruginosa* and found out that *E. coli, P. mirabilis, K. pneumonia, B. subtilis* and *S. aureus* exhibited resistance to both the extracts of *S. nigrum*, while, *P. aeruginosa* showed sensitivity to both the extracts **[41]**. However, in this research, results revealed the antibacterial potential of aqueous extract of *S. nigrum* against *S. aureus, P. aeruginosa, Enterococcus, B. subtilis* and *S. pyogenes* whereas, *K. pneumoniae* and *E. coli* exhibited resistance, respectively.

Many studies were conducted which have been reported on antibacterial potential of *M. jalapa*. Akintobi *et al.* **[42]** studied the antibacterial activity of *M. jalapa* using four types of extracts against human pathogenic microorganisms and reported from the results that ethanol extract showed more inhibitory activity, followed by methanolic extract than aqueous and petroleum ether extracts, which was in accordance to the findings of Obi and Onuoha **[43]**, whereas, in our work aqueous extract exhibited inhibition on both gram positive as well as negative microorganisms with the lowest minimum inhibitory concentration (MIC). The observed variation in sensitivity testing pattern of pathogenic microorganisms utilized in this work might be related to the genetic diversity among the organisms, which played a major role in resistance to the effects of abstracts **[44]**.

Devi, *et al.* **[45]**, tested methanolic extract of *M. jalapa*, which showed potent antimicrobial action against two gram positive microbes *i.e. B. subtilis* and *S. aureus* and two gram negative microorganisms *i.e. P. aeruginosa* and *E. coli*, respectively **[45]**. Though, in this research, aqueous abstract of *M. jalapa* exhibited excellent antimicrobial action against three gram positive bacteria *i.e. Enterococcus, B. subtilis* and *S. aureus*) and gram negative bacteria *i.e. E. coli*, respectively. A variety of leaf extracts of *M. jalapa* have been shown to be extensively efficient against gram positive as well as negative microbes **[46]**.

Oladunmoye **[47]** and Kaladhar, *et al.* **[48]**, determined that methanolic extract of *M. jalapa* showed MIC against *S. aureus* and *Aspergillus flavus*, which was in contradiction to the present work. The aqueous extract of *M. jalapa* was used to check the MIC against different pathogenic and food-borne microorganisms, respectively.

The antimicrobial activity exhibited by *M. jalapa* tends to concur with earlier observations because of the presence of terpenoids, essential oils, tannins, flavonoids, and alkaloids **[49]**. Poovendran *et al.* **[50]** reported the aqueous extract of *M. jalapa* lack antimicrobial potential against uro-pathogenic *E. coli*, which was dissimilar to the present research work. The aqueous extract of *M. jalapa* exhibited antibacterial action against *E. coli* with zone of inhibition in the range of 14mm in diameter, respectively. The reason would be that the antibacterial potential of herbs varies greatly, depending on the type and kind of herbs, test medium and microbes. Oladunmoye **[47]** found that *M. jalapa* exhibited inhibitory potential against some

human food-borne and pathogenic microorganisms. These antibacterial activities explain many advantages of herbs in ethno-medicine

Aiyelaagbe *et al.* **[51]** found out in their study that gram positive bacteiria (GPB) used in the study were more sensitive to the herbal extracts as compared to the gram negative bacteria (GNB), which was similar to the prior studies that plant extracts exhibited excellent antimicrobial potential against gram positive and negative microorganisms **[52]**. The outer membrane of gram-negative microorganisms serves as a barrier to a variety of environmental factors, including medications, which could be the reason.

Muthumani *et al.* **[46]** examined the in-vitro antibacterial activity of aqueous extract of *M. jalapa* against *S. aureus* (23 mm), *B. subtilis* (20 mm), *P. aeruginosa* (23 mm), *K. pneumoniae* (21 mm) and *E. coli* (20 mm), with MIC values. This was in accordance to the present study, the inhibitory zones were in the range of *E. coli* (14 mm), *K. pneumoniae* (20 mm), *S. aureus* (12 mm) and *B. subtilis* (20 mm), with lowest MIC values, respectively.

The antimicrobial activity of aqueous extract of *M. jalapa* against *S. aureus*, *P. aeruginosa* and *E. coli* with inhibitory zones in the range of (11-12 mm) in diameter which was in consistency to our present work **[53]**. Results showed remarkable inhibitory activity against *E. coli* and *S. aureus* with zones of inhibition (12-14 mm), while, *P. aeruginosa* exhibited resistance. *M. jalapa* dried flower and leaf of the plants were used for medicinal treatment of gram negative microbial diseases **[54]**.

An important characteristic of herbal abstractions and their constituents is hydrophobicity, which makes it easier for them to split the lipids in the mitochondrial membrane and microbial cell membrane, upsetting the cell structures and making them more permeable. Death will result from widespread leakage from microbial cells or the escape of real particles and ions **[55]**. In the current study, the antibacterial potential of three indigenous plants was analyzed and this will help to develop drugs from the indigenous sources.

Authors' contributions

Conceived and designed the experiments: R Nisar & AA Abbasi, Performed the experiments: R Nisar, TA Mughal, S Khushal & R Shaukat, Analyzed the data: RT Mahmood, K Hameed, MA Khan & M Masood, Contributed materials/ analysis/ tools: RT Mahmood & AA Abbasi, Wrote the paper: R Nisar, K Muhammad & M Masood.

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