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Research Article

Efficacy of *Citrullus colocynthis* and *Solanum surattense* extracts against bacteria isolated from clinical environments

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ABSTRACT

Knowledge and awareness about the side effects of antibiotics have increased the use of traditional medicine. The search for new drugs with antimicrobial potential has increased the importance of medicinal plants worldwide. The present study investigated the antimicrobial potential of *Solanum surattense* (Spiny nightshade) and *Citrullus colocynthis* (Bitter apple) fruit extracts against the clinical isolates (n=50). The isolated bacteria were identified as Gram positive (*Staphylococcus aureus* (14), *Staphylococcus epidermidis* (3), *Bacillus subtilis* (13), *Micrococcus luteus* (4), *Streptococcus pyogenes* (1), *Enterococcus faecalis* (1) and Gram negative (*Escherichia coli* (11), *Klebsiella pneumoniae* (3)) by Gram staining and standard biochemical tests. Selected pathogens were tested against nine selected antibiotics by Kirby-Bauer disc diffusion method. Increased antimicrobial resistance was observed against most of the antibiotics. 87.5% *S. aureus* and *S. epidermidis* were found resistant to methicillin. *E. coli* and *K. pneumoniae* showed resistivity of 71.42% against ampicillin and penicillin while all the isolates of *Bacillus subtilis* (100%) were susceptible against chloramphenicol. Bacterial sensitivity was also accessed using the disc diffusion method against methanolic, ethanolic, acetonic, chloroform and aqueous fruit extracts of *Solanum surattense* and *Citrullus colocynthis*. Significant antimicrobial activity was observed in the aqueous extract of the *S. surattense* i.e., 18% of inhibition zones were greater than 20 mm and the highest zone of inhibition obtained from aqueous fruit extract was 25 mm against *S. aureus*. The methanolic fruit extract of *C. colocynthis* showed the highest inhibition zone, 21 mm, against *S. aureus*. Our findings concluded that pathogenic bacteria were mostly resistant to antibiotics. However, fruit extracts of *Solanum surattense* and *Citrullus colocynthis* exhibited notable antimicrobial activity. They may be explored for well-being and improved health standards by studying as antimicrobial agent against various diseases like cold cough, diabetes, diabetes, ulcer, respiratory and urinary diseases.

Keywords: *Citrullus colocynthis*; *Solanum surattense*; plant extracts; antibiotics; antimicrobial resistance; inhibition zone.

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INTRODUCTION

Pakistan ranks as the third largest consumer of antibiotics among Low- and Middle-Income Countries (LMICs), exhibiting a notable escalation in antibiotic usage from 2000 to 2015, characterized by a 103% increase in India and a 79% in China and 65% in Pakistan (Blaskovich, 2018). This phenomenon has precipitated a rise in antibiotic resistance, with the most diminished Drug Resistance Index (DRI) scores recorded in Higher-Income Countries (HICs). The underlying cause can be attributed to the restricted availability of novel, more effective antibiotics in LMICs, where broad-spectrum penicillins are used. The insufficiency of data regarding mortality and morbidity attributable to antibiotic resistance reflects an inadequate surveillance framework in Pakistan (Mirha et al., 2024).

In recent years, extensive and multidrug-resistant microorganisms have been reported in Pakistan. The emergence of multidrug-resistant *P. aeruginosa* infections poses significant therapeutic challenges, necessitating the exploration of novel treatment strategies (Yasmin et al., 2013). In 2016, a significant extensive drug-resistant *Salmonella* outbreak demonstrated complete fluoroquinolone resistance (Qamar et al., 2018). A prior investigation indicated that 93.7% of bloodstream bacteria exhibited resistance to third-generation cephalosporins (Latif et al. 2009). Shariati et al. (2020) found that the prevalence of Vancomycin-resistant *S. aureus* (VRSA) was 1% in Europe, 5% in Asia, 16% in Africa, 4% in America, and 3% in South America. According to Gogry et al. (2021) and Que et al. (2022), the prevalence of Colistin-resistant bacteria has climbed to 43% in Italy, 37.3–28.8% in China, 31% in Spain, and 20.8% in Greece. In conclusion, bacterial antibiotic resistance has increased to alarming proportions in a number of nations around the world (Li et al., 2023).

The rising problem of antibiotic resistance in the world's health demands novel strategies to combat harmful bacteria. Scientists are thus always searching for novel antimicrobial drugs that aid in treating infectious diseases. The prevalence of multidrug-resistant microbes has grown, necessitating the use of novel antibiotic sources (Naik et al., 2022). Natural compounds have drawn interest as possible substitutes or supplements to traditional antibiotics, especially plant extracts. According to reports from the World Health Organization, 80% of people worldwide rely on traditional medicine. Herbs are used in traditional medicine to treat common illnesses and encourage a healthy lifestyle. Plant-derived medications have several benefits, including cultural acceptability, safety, effectiveness, and fewer side effects. Because they interact with particular chemical receptors in the body and, in a pharmacodynamic sense, with the drugs themselves, plant extracts are more effective than other forms of medicine (Kumar, 2021).

Citrullus colocynthis belongs to the family *Cucurbitaceae* and genus *Citrullus*. The common name of *C. colocynthis* is Bitter Apple, Bitter Cucumber, and Colocynth while the vernacular name is Hanjal, Indrayan, Kattu vellari, and Rakha. *C. colocynthis* is a native viny plant of the desert that is distributed throughout Asia and the Mediterranean region. It is a perennial creeping herb with a climbing stem, possessing smooth spherical fruits which are flecked green when young and yellowish when ripe. The fruit is pulpy, and several fruits are used as vegetable or nutritious fruits. *C. colocynthis* has long been used to treat various illnesses, such as diabetes, bacterial infections, constipation, and many more (Shah et al., 2022; Jabeen et al., 2017). *C. colocynthis* has long been used to treat various illnesses, such as diabetes, bacterial infections, constipation, and many more. Each plant part exhibited unique antimicrobial and antioxidant properties (Ghani, 2016). *C. colocynthis* has been found to have antimicrobial activity against multi-drug resistant-pathogens due to a variety of compounds isolated from the plant using organic solvents. Butanol extract demonstrated the highest activity level against multi-drug-resistant pathogens, including *Pseudomonas aeruginosa*, methicillin-resistant *Staphyllococcus aureus* (MRSA), *Kleibsella pneumoniae*, *Salmonella typhi*, *Acinetobacter baumannii*, and *Enterococcus faecalis*. Chloroform, acetone, ethanol, hexane, and DMSO extract showed the greatest level of activity against these MDR pathogens (Nadeem et al., 2024).

Solanum surattense belongs to the family Solanaceae and genus *Solanum*. The common name is Spiny Nightshade and Yellow Fruited Nightshade while the vernacular name of *Solanum surattense* is Kundiari, Momoli and Dhatura. It is a perennial herb which is widely distributed in sub-tropical and tropical regions of Southeast Asia. When the fruit is young, it is a spherical berry with green markings that turns light yellow or whitish when it is ripe (Hasan et al., 2024; Yousaf et al., 2009). It is utilized medicinally to cure cough, asthma and stiffness. *Solanum surattense* is a medicinally essential prostrate herb with scattered stellate hairs and thorny stems. This plant has a high concentration of solasodine, a beginning material for the manufacture of cortisone. It grows widely in Pakistan up to 1300m and in different parts of world similar to climatic and geological conditions (Sudhanshu et al., 2012). *Solanum surattense* is useful ethnomedically for treating toothaches, piles and skin conditions. The most popular part of this plant was found to be fruit (25%), followed by the whole plant (22%), which was used to treat various illnesses. The best method for making herbal drugs was found to be decocting the fruit (Hasan et al., 2024). The present study was planned to investigate antibiotic resistance among the clinical isolates and to evaluate the potential of fruit extracts of *C. colocynthis* and *S. surattense* against the bacteria.

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MATERIALS AND METHODS

Fresh fruit of selected plants, i.e., *C. colocynthis* and *S. surattense* were collected from Hassan Abdal, District Attock. Fruits were carefully placed in labeled polythene bags and bought to the laboratory in September. The botanical identification of each plant was done based on indigenous information by Dr. Muhammad Idrees, Assistant Professor in Department of Biosciences, University of Wah, before preparing the plant extract.

Table 1.1. Botanical description of the selected plants: *Citrullus colocynthis* and *Solanum surattense.*

Scientific Name	Family	Genus	Leaves	Flower	Fruit	Seeds	Plant type	Habitat	References	Picture
Citrullus Colocynthis	*Cucurbitaceae*	*Citrullus*	Triangular, 5-10cm long, rough and hairy texture, upper surface is green and lower is pale yellow	Yellow, usually solitary, Monoecious	Spherical, flecked green when young and yellowish when ripe	Smooth, ovoid-shaped, compressed and 6 mm in size, usually dark brown	Perennial creeping herb	Desert Areas	Shah et al. 2022; Jabeen et al. 2017	
Solanum surattense	*Solanaceae*	*Solanum*	Ovate to lanceolate, 5-15cm long, smooth or slightly hairy texture	Blue or bluish purple, Star shaped with 5 petals	Spherical berry with green markings that turns light yellow or whitish when ripe	Brown, circular, smooth, numerous	Perennial herb	Sub-tropical and tropical regions	Hasan et. al. 2024; Tekuri et al. 2019	

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Preparation of Extracts

The fresh fruits underwent a comprehensive washing process, rinsed 2-3 times with tap water and subsequently once with distilled water, after which the materials were allowed to attain complete dedication. The fresh fruits were then ground in a mortar and pestle and used immediately for extraction. The pulp of each fruit weighed 20g was taken into 500 ml flask and mixed with 200 ml of each solvent. Five different solvents were used to prepare plant extract i.e., ethanol, methanol, chloroform, acetone, and distilled water.

The flask was then closed by using a cotton plug tightly wrapped with aluminum foil and placed in a shaking incubator at 37℃ for 48 hours. After 48 hours, the crude extract was filtered using Whatmann filter paper (Grade 42: 2.5 μm) and transferred to a pre-weighed beaker. The extract was concentrated by evaporating the solvent in a heating-drying oven.

Reconstitution of Plant Extracts

The solvent-free fruit extract was reconstituted using dimethyl sulphoxide (25 mg/ml DMSO). The fruit extract was then sterilized by filtering through the bacterial filter. The extracts were stored at 4℃ for further use.

Bacterial isolates

Sterile swabs and loops were used to collect and transfer the samples from clinical sources, i.e., medical devices and hospital waste, aseptically. Gram Staining and different biochemical tests were performed to identify the bacterial isolates. Bacterial isolates were identified as Gram positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Enterococcus faecalis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*).

Antibiotic Discs

The antimicrobial susceptibility of isolates was checked by selecting nine basic antibiotics i.e. Oxacillin (OX), Amoxicillin (AX), Ampicillin (AMP), Vancomycin (V), Methicillin (ME), Trimethoprim-Sulfamethoxazole (SXT), Rifampin (RA), Chloramphenicol (C) and Penicillin G (P).

The disc diffusion methodology was employed to assess the susceptibility of isolates against commercially available antibiotics. Kirby-Bauer disc diffusion approach was utilized to evaluate antibacterial drug susceptibility. Zones of inhibition were quantified in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2024).

Antibacterial Assay

Two different sets of experiments were conducted. In the initial series, solutions of predetermined concentrations (mg/ml) of the tested specimens were prepared by dissolving a measured quantity of the samples in a calculated volume of appropriate solvents. Subsequently, dried and sterilized filter paper discs (6mm in diameter) were impregnated using a micropipette with precise amounts of the tested substances. The discs containing the tested materials were uniformly placed upon a nutrient agar medium that had been inoculated with test microorganisms. The plates were then incubated at a temperature of 37°C for a duration of 24 hours to facilitate optimal growth of the organisms. The experimental substrates exhibiting antibacterial properties effectively suppressed the proliferation of microorganisms, resulting in a clearly defined zone of inhibition discernible around the medium. The antibacterial efficacy of the tested material was quantified by assessing the diameter of the zone of inhibition, expressed in millimeters. The antibacterial potency of methanol, ethanol, acetone, chloroform, and distilled water was evaluated at 50 μg/disc concentration. In the subsequent series of experiments, the commercially accessible standard antibiotic discs were meticulously positioned atop the medium within the petri dishes in accordance with the disc diffusion methodology (Scorzoni et al. 2007). The petri dishes containing bacterial cultures were subjected to incubation at a controlled temperature of 37ºC for a duration of 24 hours. Following the incubation period, all plates were systematically assessed for the manifestation of inhibition, which indicates antimicrobial activity.

Data Analysis

The data was processed by using Microsoft Excel (Aparna et al., 2024). The obtained zone of inhibition's frequency percentages was found using Excel.

$$\text{Frequency \%} = \frac{\text{Number of Sensitivity Responses}}{\text{Number of isolates}} \times 100$$

RESULTS AND DISCUSSION

The bacterial isolates exhibit different resistivity pattern against the selected antibiotics according to the CLSI. The resistivity of *Staphyllococcus* (*S. epidermidis* and *S. aureus*) against methicillin was 87.5% while 12.5% were susceptible. 93.75% and 81.25% *Staphyllococcus* were susceptible against chloramphenicol and trimethoprim-sulfamethoxazole respectively. *Staphyllococcus* were equally found susceptible and intermediate i.e 18.75% against rifampin and the resistant level is 62.5%. Similarly, half (50%) of this bacterial isolate are susceptible and resistant to ampicillin. The obtained pattern against penicillin showed 68.75% resistance and 31.25% susceptibility pattern. 56.25% of these were found susceptible to amoxicillin and 43.75% were resistant (Figure 2.1).

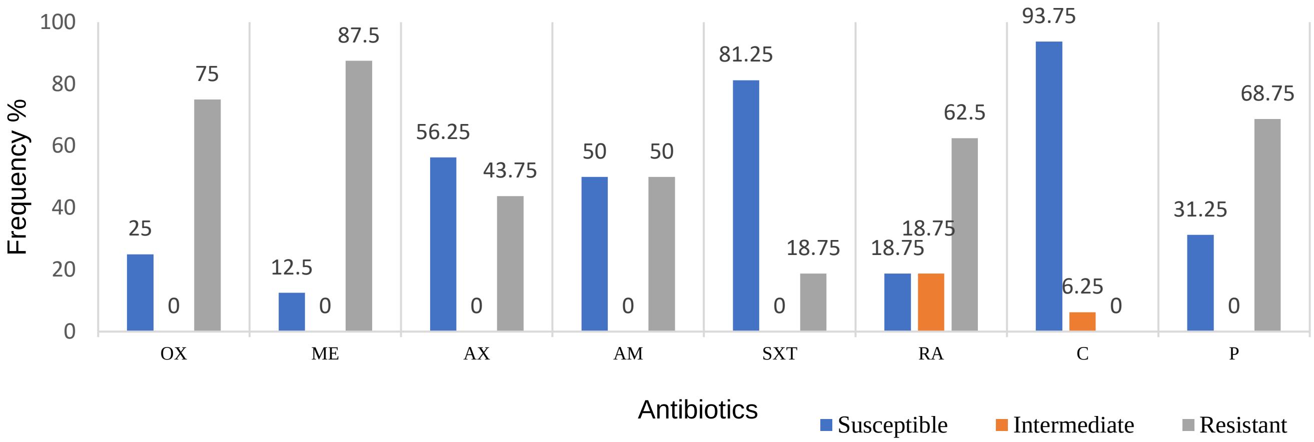

Figure 2.1. Antibacterial sensitivity pattern of *Staphyllococcus* (*S. epidermidis* and *S. aureus*) (n=16) against selected antibiotics.

All the isolates of *Bacillus subtilis* (100%) were susceptible to chloramphenicol while the resistivity was 92.3% and 84.61% against ampicillin and Vancomycin respectively. 69.23% of isolates were resistant against trimethoprim-sulfamethoxazole and penicillin each, and 30.76% were resistant. Against rifampin 61.53% susceptible, 23.07% intermediate and 15.38% of *B. subtilis* isolates were found resistant (Figure 2.2).

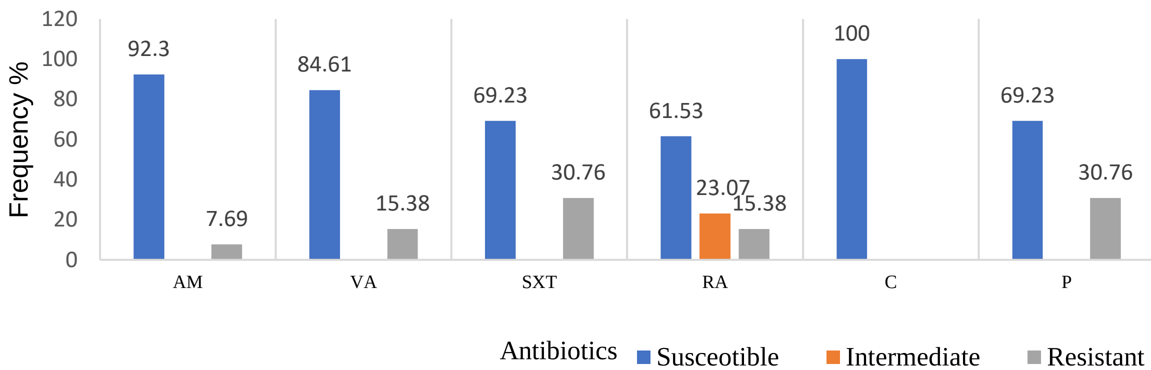

Figure 2.2. Antibacterial sensitivity pattern of *Bacillus subtilis* (n=13) against selected antibiotics.

Micrococcus luteus isolates showed higher sensitivity (75%) to vancomycin and 25% of isolates were resistant. While 50% isolates were found susceptible and 50% resistant against penicillin (Figure 2.3). On the other hand, isolate of *Streptococcus pyogenes* was found susceptible against ampicillin, vancomycin and chloramphenicol while it was found resistant to penicillin (Figure 2.4). The isolate of *Enterococcus faecalis* was resistant to all tested antibiotics i.e. amoxicillin, ampicillin, vancomycin, rifampin, chloramphenicol and penicillin (Figure 2.5). The isolates of *Enterobacteriacea* (*E. coli* and *K. pneumoniae*) showed resistivity of 71.42% against ampicillin and penicillin while 28.57% and 21.42% were found susceptible respectively. 7.14% of isolates showed intermediate pattern against penicillin. 64.28% of *Enterobacteriacea* isolates were resistant to trimethoprim-sulfamethoxazole and 35.74% were sensitive. The resistivity pattern of these isolates was same against amoxicillin and chloramphenicol i.e. 57.14% resistant and 42.85% susceptible (Figure 2.6). Figure 2.7 exhibits the antibacterial activity patterns of fruit extracts of *Citrullus colocynthis* against different bacterial isolates. The highest zone of inhibition observed was 21mm against S.

aureus, which is obtained from the methanolic fruit extract, while in case of antibiotics the highest zone was 35mm against chloramphenicol. The frequency% of aqueous fruit extract showed that 56% zones of inhibition were obtained

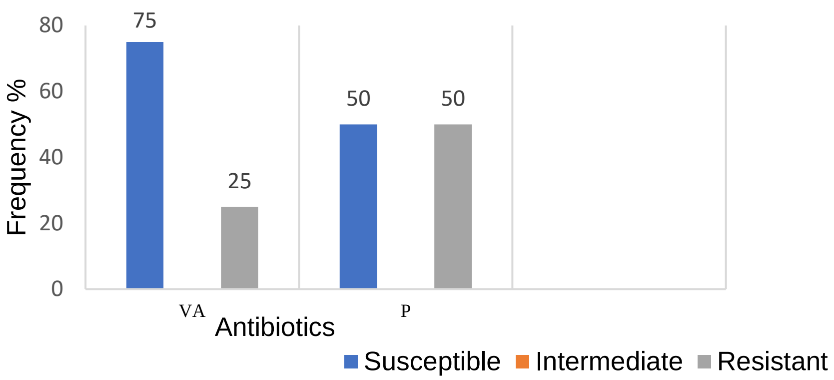

Figure 2.3. Antibacterial sensitivity pattern of *Micrococcus luteus* (n=4) against selected antibiotics.

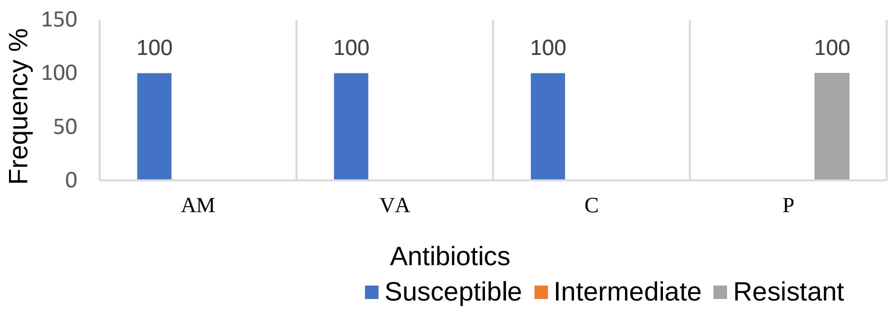

Figure 2.4. Antibacterial sensitivity pattern of *Streptococcus pyogenes* (n=1) against selected antibiotics.

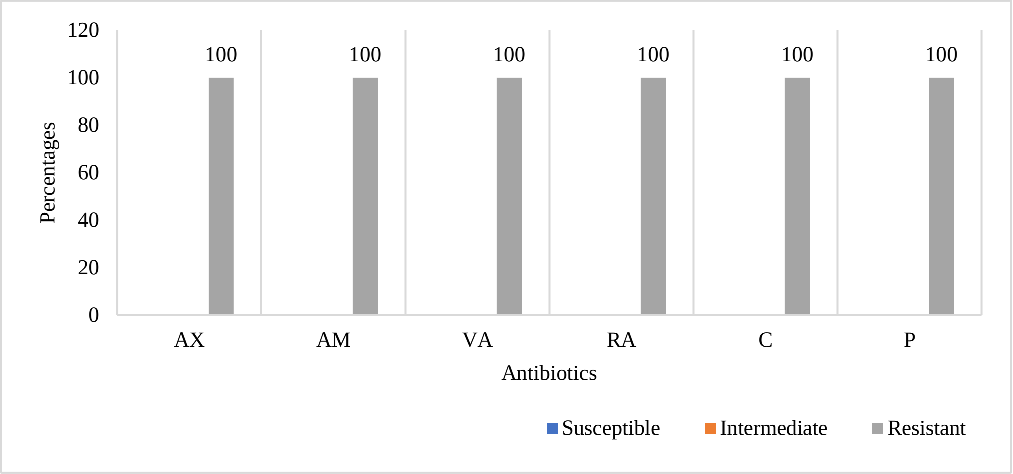

Figure 2.5. Antibacterial sensitivity pattern of *Enterococcus faecalis* (n=1) against selected antibiotics.

from 11-19 mm, and the frequency% of ethanolic extract showed that 22% inhibition zones are measured between 11-19mm. It was observed that the methanolic fruit extract showed 2% zones of inhibition ≥ 20 mm and 22% between 11-19 mm. The frequency% of chloroform and acetonic fruit extract showed that the inhibition zones between 11-19 mm were 28% and 26% respectively. The antibacterial activity patterns of fruit extracts of *Solanum surattense* against different bacterial isolates were shown in Figure (2.8). The highest zone of inhibition was 25 mm against S. aureus, obtained from the aqueous extract of *Solanum surattense*. The frequency% of aqueous fruit extract showed that 18% of inhibition zones were ≥ 20 mm, and 50% of inhibition zones were obtained from 11-19 mm. The frequency% of ethanolic extract showed that 8% of inhibition zones were ≥ 20 mm, and 56% were measured between 11-19mm. It was shown that the frequency% of methanolic extract, according to which 10% of the inhibition zones were ≥ 20 mm and 27% were 11-19 mm. According to the frequency% of chloroform extract, it was observed that 8% of zones of inhibition were ≥ 20 mm and 58 % were 11-19%, and in case of acetonic fruit extract it was found that 10% zones of inhibition were ≥ 20 mm and 54% were between 11-19 mm. Antibiotics were considered the magic bullets for treating diseases caused by bacteria. With the increase in the use of antibiotics, bacterial resistance increases over time.

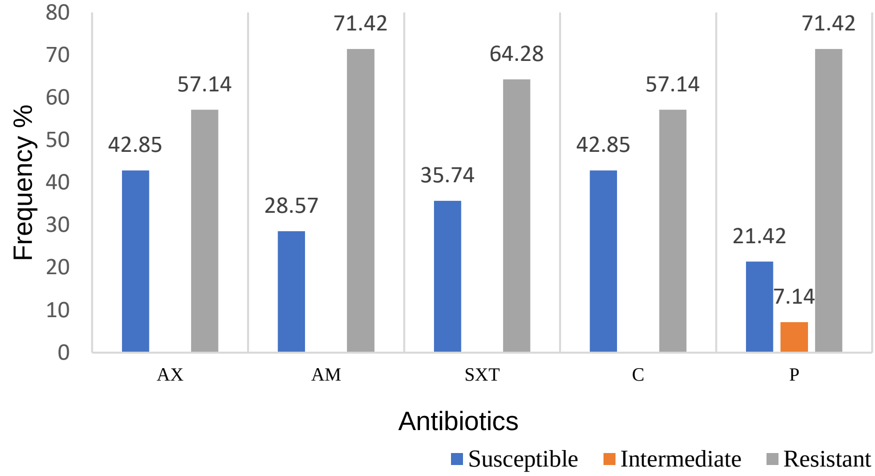

Figure 2.6. Antibacterial sensitivity pattern of *Enterobacteriacea* (*E. coli* and *K. pneumoniae*) (n=14) against selected antibiotics.

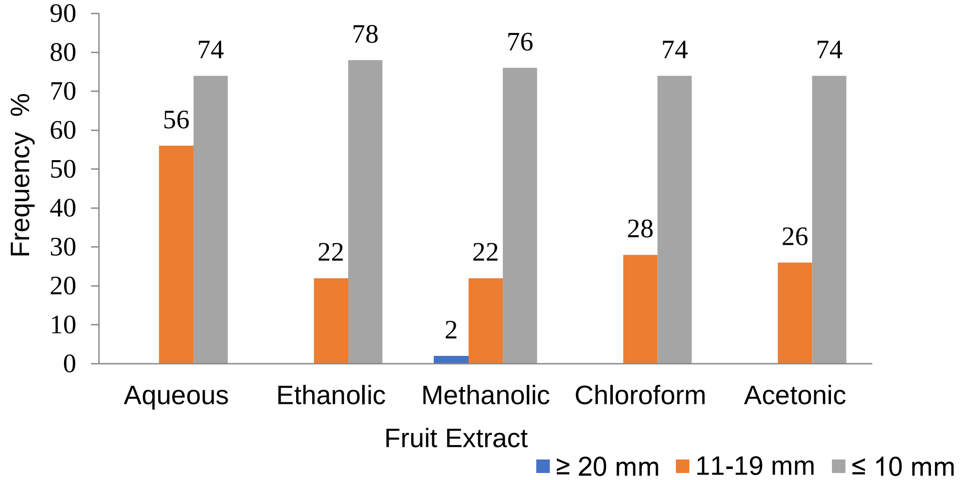

Figure 2.7. Antibacterial sensitivity patterns of all selected bacterial isolates (n=50) against fruit extracts of *Citrullus colocynthis.*

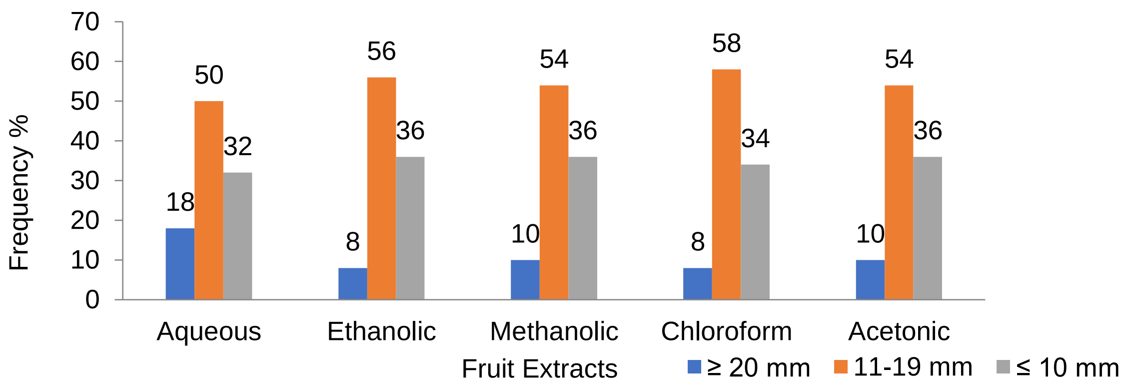

Figure 2.8. Frequency% of Fruit Extracts of *Solanum surattense* against Different Bacterial Isolates (n=50).

Improper use of antibiotics is the main cause of the emergence of resistance in microorganisms (Nadgir & Biswas, 2023). As a result of increasing bacterial halting against antibiotics, there is a crucial need to thrive novel and ingenious antibacterial factors. Among the potential wellspring of new factors, plants have long been researched because they consist of numerous bioactive compounds that may be therapeutic (Vaou et al., 2021). The findings of the present study concluded that *S. aureus* shows resistance against common antibiotics, which is in accordance with the findings of Ezeh et al. (2023) and Manzoor et al. (2024). According to Zia et al. (2023), *E. coli* showed the highest resistivity against Penicillin, which is in line with the results of the current study. *B. subtilis* is resistant to Rifampin, ampicillin, and amoxicillin while found susceptible to Chloramphenicol, and these findings are also supported by Van et al. (2022). He et al. (2024) reported 13.8% resistance rate of *M. luteus* against penicillin. Zhu et al. (2021) also reported the low resistance of *M. luteus* against penicillin. The present study found that 50% of isolates are resistant to penicillin. The survival strategies of bacterial species against antibiotics may include reducing antibiotic penetration into the microbial cell, utilizing efflux pumps to expel antibacterial agents, inactivating antibiotics through modification or degradation and altering the antimicrobial target within the bacteria (Chinemerem et al., 2022). The present findings are supported by the previous literature mentioning the significant antimicrobial activities of *C. colocynthis* and *S. surattense*. The research study exhibited the antibacterial capability of *S. surattense* and demonstrated strong activity against a range of pathogenic bacteria.

Plant extracts, particularly those derived from fruits and roots, have shown promise in effectively inhibiting both Gram-positive and Gram-negative bacteria, including *P. aeruginosa*, *S. aureus*, and *E. coli*. These results validate the plant's historic use in treating bacterial infections like skin and respiratory disorders. According to phytochemical studies, the plant's bioactive substances, such as flavonoids, saponins, and alkaloids, are responsible for its antibacterial activity. These substances function as a natural substitute for antibiotics by rupturing bacterial cell membranes and interfering with bacterial metabolic activities (Tekuri et al., 2019). *Citrullus colocynthis* has strong antibacterial activity, especially against *S. aureus* and *E. coli*, furthermore ethanolic extracts of the plant show more inhibition than aqueous extracts (Prashant et al., 2017).

In accordance with these findings, the present study also observed significant activity of *C. colocynthis*. Methanolic fruit extract of *C. colocynthis* demonstrated a maximum inhibition zone of 21 mm against *S. aureus*. Against all the selected isolates, aqueous fruit extract exhibited a 56% occurrence of inhibition zones ranging from 11-19 mm, while the chloroform, acetonic and ethanolic extracts displayed 28%, 26%, and 22%, respectively in the same range. The substantial antibacterial activity of *C. colocynthis* fruit coatings were also corroborated by Elansary et al. (2018), highlighting the plant's broad-spectrum antimicrobial potential. Moreover, inhibitory zones for different bacterial isolates were observed by Idan et al. (2015) to range from 7 mm to 23 mm, suggesting that *C. colocynthis* extracts had strong antibacterial effects. Hameed et al. (2020) provided additional support for the possibility of *C. colocynthis* as a substitute therapeutic drug by comparing its antibacterial efficacy against a range of clinical pathogens with tetracycline.

Solanum surattense yielded 338 metabolites of different chemical classes, including 52 (15.38%) lipids, 56 (16.56%) phenol derivatives, and 137 (40.53%) terpenoids. Water-ethanol mixtures of this plant's various parts have demonstrated larvicidal, hepatoprotective, anti-inflammatory, antimicrobial, anti-tumoral, and antioxidant properties both in vitro and in vivo. The most commonly reported activities among the 51 metabolites that were identified and biologically tested were antitumoral, anti-inflammatory, and antioxidant (Hasan et al., 2024). According to the study by Sheeba (2010) ethanol extracts of *Solanum surattense* leaf have antibacterial activity against *Staphyllococcus aureus, Streptococcus sp., Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae* and *Vibrio cholera*. A maximum zone of inhibition of 25 mm was observed for *Bacillus subtilis*, 23 mm against *Staphllococcus aureus*. The present study also observed remarkable activity of *Solanum surattense* against the selected isolates. The maximum zone of inhibition recorded was 25 mm against *S. aureus* from the aqueous extract of *Solanum surattense*. The ethanolic extract revealed 8% of inhibition zones were ≥ 20 mm, while 56% were measured between 11-19 mm.

LIMITATIONS

Only a small number of isolates were tested for each species in this study. We anticipate that CLSI will release the breakpoint of the disc diffusion method for Infrequently Isolated Bacteria.

CONCLUSION

The results of the present study concluded that the bacterial isolates were mostly resistant to antibiotics; however, fruit extracts of *Solanum surattense* and *Citrullus colocynthis* exhibited antimicrobial activity, and from a future perspective, they can be further explored as an antimicrobial agent against various diseases like cold cough, diabetes, ulcer, respiratory and urinary diseases. Further experimental and research efforts on these plants and their extracts are required, specifically regarding pharmacological applications.

AUTHOR'S CONTRIBUTIONS

Conceptualization: Iqra Suleman, Asma Noreen, Muhammad Idrees, Shumaila Naz. Project administration: Shumaila Naz, Muhammad Idrees. Supervision, Shumaila Naz, Muhammad Idrees. Formal analysis: Iqra Suleman, Asma Noreen, Shumaila Naz, Zeenat Haq, Muhammad Idrees. Investigation and Methodology: Iqra Suleman, Asma Noreen. Writing original draft: Iqra Suleman, Shumaila Naz. Writing and Reviewing: Shumaila Naz. Data curation: Iqra Suleman, Asma Noreen, Zeenat Haq. Validation: Iqra Suleman, Asma Noreen. All authors have read and agreed to the published version of the manuscript.

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