Research article
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Antimicrobial activities of extract of the two herbal *Antirrhinum majus, & Dodonaea viscosa* plants

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Abstract
In the current era of advancement in science and technology, the microbial resistance against synthetic medication is alarming due to its harmful effect on the human body; therefore, researchers are now focusing on plant-derived bioactive compounds or the use of their extracts. The extract of the plants contains diverse valuable bioactive compounds which found effective in controlling infectious diseases. The activities of these bioactive components are directly related to the antimicrobial and antioxidant activities of those bioactive compounds present in the extract of plant extract. For this purpose, two herbal plants *Antirrhinum majus,* & *Dodonaea viscosa* were collected from the Karachi Sea side region to investigate their antimicrobial activities in their methanolic extracts. The minimum inhibitory count was found effective in the determination of antimicrobial activities of extracts. Results showed that maximum inhibitory count was measured for *Staphylococcus aureus* and *Bacillus cereus* while lower for *Escherichia coli, Klebsiella numoneae,* and *Salmonella enterica.* It indicates that these plants contain bioactive compounds which can be extracted as future bio-medications.
***Keywords*:** phytochemicals, antibiotic drugs, resistant

Highlights:
- Phytochemicals of plants
- Antimicrobial activities of the extracts of plants
- Future safe herbal bioactive components for medication

1. Introduction
The lack of novel antimicrobial agents against the expansion of antibiotic resistance has been recognized in the last few decades (Walsh &Toleman 2012; Akram et al 2021). It is the main trial in worldwide healthiness that requires extra care; in innovative, operative, and inexpensive drugs to control microbial contagions, particularly in non-advanced countries of the world, wherever infectious illnesses result up to one-half of deaths (Awouafack, et al 2013). Respiratory and skin infections in human is related to a few microbial strains like some *Staphylococcus* species and *Streptococcus* species, followed by gastrointestinal, urogenital diseases and wound contamination by *Pseudomonads* and members of the Enterobacteriaceae, which are resilient or unaffected to entire old antibiotics (Neu, 1992). The antibiotic resistance in humans and animals is problematic and may continue for a long time. Therefore, there is a need to cover this drawback and the progress in formulating alternative medicines for the care of these virulent infections. Plants can manufacture an extensive diversity of secondary metabolites, like alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones, and coumarins (Das, 2010). These bioactive compounds are the cradle of plant-derived antimicrobial agents and are highly effective in treating bacterial infections. Biswas et al (2013) report extract efficiency of the guava of leaves in four different solvents like hexane, methanol, ethanol, and water). using a well-diffusion method in 50 μL leaf-extract solution per well and found that antimicrobial activities in the methanol and ethanol extracts were maximum
The current article investigates the antimicrobial activities of the extracts of two herbal plants *Antirrhinum majus,* & *Dodonaeaviscosa,* commonly found at Karachi Beach. The study reports the collection, transportation, and extract preparation followed by the determination of antimicrobial activities using the disc diffusion method: minimum inhibitory concentration (MBC) and combined fractional inhibitory concentration index (FICI index).

2. Material and Method
2.1 Collection of plants
The two herbal plants of medicinal significance were collected from that site of Karachi, where they were abundantly available. They were transported and preserved in Laboratory by the method described by Ahmed et al. (2018).
2.2 Transportations
These two herbal *Antirrhinum majus,* & *Dodonaeaviscosa* plants were transported to the Laboratory, where plants were washed with tap water and then kept for drying in shadow followed by heating in an oven. After the observing, the completions of dryness processes plant were crushed gently and grounded in a powder state like semi-amorphous.
2.3 Preparation of leaves extract

The extracts of leaves were prepared in several solvents like Methanol, Ethyl acetate, Chloroform, Hexane, Aqueous, and Ascorbic acid through crushing, where 100 g powered leave samples were mixed in 300 ml each of the above followed by filtration.

2.4 Antimicrobial Activity Test

2.4.1 Positive and Negative bacterial strains used in this study

In this study, three Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, and *Bacillus megaterium* DSM 32), four Gram-negative bacteria (*Enterobacter aerugenes* ATCC 13048, and *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027 and *Klebsiella pneumoniae* Antioxidants 2016, 5, 38 5 of 15 ATCC 13883), as well as three fungi species (Candida albicans ATCC 10231, Yarrowialipolytica, and Saccharomyces cerevisiae) were used as test microorganisms. In addition, erythromycin (E-15), ampicillin/sulbactam (SAM-20), amikacin (AK-30), and rifampicin (RD-5) were also used as a positive control.

2.4.2 Microbiological Assay

The antimicrobial activities of extracts were detected by the disc diffusion method. 30, 60, and 90 µL of each extract was absorbed onto sterile discs of 8 mm diameter. To inoculate the media for assay, a 1% rate of each microorganism from 106 to 107 CFU/mL suspensions was added to 15 mL sterile media (for bacteria Muller-Hintone agar, for yeast Sabouraud 2% glucose agar). Each inoculated medium was poured into a Petri dish (9 cm) and left at +4 ◦C for 1 h. Subsequently, discs prepared from samples were added to these inoculated media and left again at +4 ◦C for 1 h. Four standard antibiotic discs were used as the positive controls. Sensitivity was deduced by comparing the inhibition zone diameter produced by the erythromycin (E-15), ampicillin/sulbactam (SAM-20), amikacin (AK-30), and rifampicin (RD-5). The Petri dishes were incubated at 35 ◦C for 18–24 h, except for C. albicans ATCC 10231, Y. lipolytica, and S. cerevisiae which were incubated at 27 ◦C. Inhibition zones were measured, calibrated, and recorded as the mean diameter of three replications.

2.5 Agar diffusion method for minimum inhibitory concentration (MBC)

The minimum inhibitory concentration (MBC) was the method of distinct as the lowest concentration of the extract, which killed a particular microorganism during the exaction process. The MBC was determined through the agar test method by putting a spot on an agar plate. The 5 µl of aliquots were transferred into TSA plates and incubated for a period of 24 h, while the controls test was set with DMSO solution with amounts corresponding to the highest quantity present in the test solution where the appropriate reading was obtained in running off three replicates according to the reported method of Ahmed et al., (2018).

2.6 Combined fractional inhibitory concentration index (FICI index)

The combined effects of all combinations of extract investigated for the first time to evaluate the fractional inhibitory concentration index (FICI index) using FIC index = FICA + FICB, where FICA = (MICA in combination/MICA alone) and FICB = (MICB in combination/MICB alone) (Contreras et al. 2015). The trails were replicated at least twice application with the duplicate running of samples and analyzed per replicate of each sample.

3. Results and Discussion

3.1 Antimicrobial Activities of extracts of both herbal plants

Initial screening of the antimicrobial activities of the prepared extracts was tested against microorganisms, including two grams positive and three-gram negative microorganisms using the MBCs method. The antimicrobial properties of both herbal plants were measured in methanolic extracts by minimum inhibitory count, where the effective antimicrobial activity of extracts of both plants was observed. The MBC was evaluated by the presence and absence of inhibition zones. Results acquired by the MIC method are tabulated in Table (1), which shows that maximum inhibitory count was measured for *Staphylococcus aureus* and *Bacillus cereus* while lower for *Escherichia coli*, *Klebsiella numoneae*, and *Salmonella enterica.* The inhibition zones are related to plants' medicinal value, which indicates the presence of the bioactive phytocomponents existing in the plants that dissolve in different solvent systems.

The advancement of the current era leads to new inventions for a safe, healthy, and luxurious life coupled with side effects in relation to damaging the existing natural environment that appeared in the form of diseases. A significant part of this advancement appears in the form of resistant microbes against the newly developed synthetic antibiotic. The resistance of microbes against infectious diseases is problematic and challenging in the healthcare sector in the overall world, including both advanced and under-developed countries. The advent and extent of multidrug-resistant bacteria have considerably imperiled the current antibacterial medication. Maximum antibacterial activity in the present search suggests the significance of these plants as a practical herbal medicinal resource. This has required exploration for a new source of antimicrobial substances like plants, where numerous bioactive compounds synthesized during secondary metabolic reactions familiar for therapeutic properties. The present findings are similar to the outcome of Nascimento et al., (2000), where it was observed that extracts of herbal plants possess great potential as antimicrobial compounds against

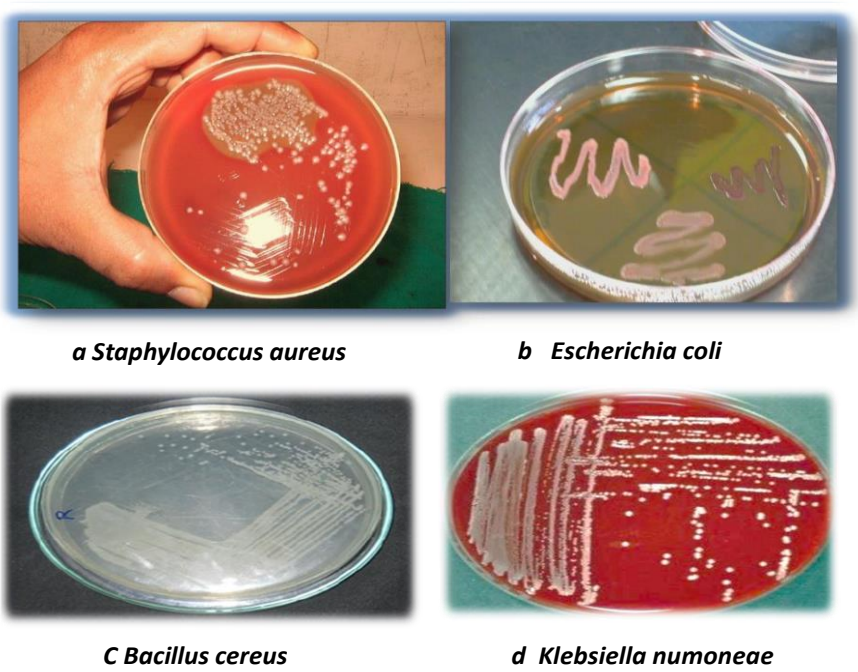
microorganisms and proved to be the best replacement for existing antibiotics for the treatment of infectious diseases caused by resistant microbes. The extract activities of both herbal species showed that they might contain phytochemicals like flavonoids, terpenoids, tannins, and alkaloids.

The extract of both herbal species showed a substantial MIC value against *Staphylococcus aureus*, signifying that they could be a potential alternative to contest it (Hiremath, et al., 2011). Interestingly, cell wall structure is related to bacterial action, making gram-positive bacteria more susceptible to different compounds than gram-negative bacteria.Thus the variation in the inhibitory effect may be due to the difference in the amount or type of biomolecules in the extracts.

Although both species showed excellent antibacterial activities in methanolic extract, contrary to this MIC, limited MIC against *Salmonella enterica* suggested that it is not necessary for herbal plants to show similar behavior as an effective remedy for infectious diseases. Furthermore, the study is still reasonable to discover their efficacy in preventing the progress of parasites, viruses, or fungi. The additional prospect of the limited antibacterial activity of extract may be linked to the cold percolation extraction method and the use of crude extracts.

Table 1: Minimum bacterial concentrations (MBCs) of Antirrhinum majus (Snapdragon)s and DodonaeaViscosa (Hopbush)s against the bacterial disease

Isolates of bacterial strains	**(MBCs) (µg/mL)**	
Bacterial Strain	***Antirrhinum majus* (Snapdragon)s**	***DodonaeaViscosa* (Hopbush)**
	Gram positive bacteria	
Staphylococcus aureus	520	432
Bacillus cereus	230	203
	Gram negative bacteria	
Escherichia coli	335	331
Klebsiella numoneae	272	266
Salmonella enterica	150	100



a Staphylococcus aureus ***b Escherichia coli***

C Bacillus cereus ***d Klebsiella numoneae***

Figure. 1 Shows the antimicrobial activities of herbal plants

3.2 Fractional inhibitory concentration (FIC) of two herbal *species* for bacterial strains

The Fractional inhibitory concentration (FIC) indices of ethanolic extracts display antimicrobial properties (Table 2). The *S. aureus* showed maximum FIC values (515 (µg/mL) in negative bacteria like *E-coil*, containing FIC (333µg/mL) from*Antirrhinum majus* (Snapdragon)s. The extraction from *Dodonaea viscosa* (Hopbush) including FIC from the active

bacterial strain of *S. aureus* was 432 (µg/mL) when compared with *B. cereus* contained 203 (µg/mL). The comparison of a negative bacterial strain of *E-coil* and *K. numoneae* showed (333 and 265 µg/mL), which was in accordance with reports that herbal extracts have a more significant activity towards various microbiological strains (Roy et al., 2010). These depend on chemical composition, lipophilic properties, and solubility to show an additive effect (Faulds et al., 1995; Ahmed et al., 2019) and higher methanolic extract. The gram-positive and negative bacteria were only controlled by the help of the cell membrane, which was blocked due to the presence of these phytochemicals in vast quantities. The fractional inhibitory concentration (FIC) index range of 0.5 to 4 is commonly applied to express additivity resulting in no interactions in maximum combination studies of antimicrobial and antifungal agents.

Results showed that the plant extracts are an essential indicator for the drug interactions; assessed using a checkerboard micro-dilution method. The concentrations of the antimicrobial agent typically ranged from four or five below the expected MIC to twice the anticipated MIC as the 45-degree line (each square represents one plate). The two-fold dilutions of each antimicrobial agent were used to assess the FIC interactions, the concentration of MIC point, and dilution lower than it for each antimicrobial agent alone (Ahmed et al.,2018). The Inocula were prepared spectrophotometrically and diluted to obtain final concentrations of 0.5 × 106 CFU/mL. Each microdilution well included 100 µL of the diluted (two times) the FIC was indictor of drug concentrations of both antimicrobials. The trays were incubated at 37°C, and the results were read at 24 hours visually using an ELISA reader system (statfax-2100, Awareness Technology Inc., USA). The Fractional inhibitory concentration (FIC) indices of ethanolic extracts display antimicrobial properties (Table 2). The *S. aureus* showed maximum FIC values (515 (µg/mL), in negative bacteria like *E-coil*,which contained FIC (333µg/mL) from *Antirrhinum majus* (Snapdragon)s. The extraction from *DodonaeaViscosa* (Hopbush),including FIC from activebacterial strain of *S. aureus* was 432 (µg/mL) when compared with *B. cereus*contained 203 (µg/mL). The comparison of a negative bacterial strain of *E-coil* and *K. numoneae* showed (333 and 265 µg/mL), which was in accordance with reports that herbal extracts have a greater activity towards various microbiological strains (Roy et al., 2010). These depend on chemical composition, lipophilic properties, and solubility to show an additive stabilized effect (Faulds et al., 1995, 1995; Ahmed et al., 2019) higher methanolic extract (Table 2. Fig1).

Table 2: Fractional inhibitory concentration (FIC) of two herbal *species* for bacterial strains

Isolates of bacterial strains	**(FIC) (µg/mL)**	
Bacterial Strain	**Antirrhinum majus (Snapdragon)s**	**DodonaeaViscosa (Hopbush)**
	Gram positive bacteria	
Staphylococcus aureus	515	432
Bacillus cereus	229	203
	Gram negative bacteria	
Escherichia coli	333	330
Klebsiella numoneae	271	265

4. Conclusion

It was concluded that both herbal plants extract contains significant ingredient for drug discovery in the current investigation, and the bioactive compounds present in plant extract consist of multi-component mixtures, while their extraction, separation, and isolation need an effective separating technique to avoid problems of decomposition and other quantitative matters required during purification

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Conflict of interest:

Authors declare that there is no conflict of interest among authors

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