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Demonstration of drug-resistant bacteria among commonly available flowers within Dhaka Metropolis and assessment of their anti-bacterial properties

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Present study attempted to emphasize on the drug-resistance attribute of the previously isolated microorganisms within the commonly available flowers including *Hibiscus rosa-sinensis*, *Ixora coccinea*, *Ipomoea digitata*, *Allamanda cathartica*, *Nymphaea nouchali*, *Vinca rosea*, *Rosa kordesii*, *Gladiolus hybrid*, *Acmella oleracea*, *Nyctanthes arbor-tristis* and *Pseudomussaenda flava*, randomly collected from different areas in Dhaka city, Bangladesh. Conventional agar well diffusion method was applied to examine the antibiogram of the isolates (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus* spp.) against different drugs. Subsequently the anti-bacterial traits of 6 flowers such as *Rosa kordesii*, *Gladiolus hybrid*, *Acmella oleracea*, *Nymphaea nouchali* (Water lily), *Hibiscus rosa- sinensis* (Jaba), *Ixora coccinea* (Rangan) were demonstrated through broth micro-dilution methods. *Pseudomonas* spp. showed sensitivity against almost 42% tested drugs. Conversely, *Staphylococcus* spp. (83%) and *Klebsiella* spp. (75%) revealed resistance against highest number of drugs. Among all the pathogens, *E coli* showed the highest sensitivity (50%) against all the tested antibiotics. All the isolated bacteria were found to be multi-drug resistant against commonly used antibiotics. Finally six (6) samples unveiled anti-bacterial features against 8 laboratory strains with their MIC concentrations up to 0.2 mg/mL.

Key words: Flowers; Microorganisms; Drug-resistance; Anti-bacterial properties

Currently a remarkable number of antibiotic have become non functional or outdated or primitive due to the rapid provenance of resistant or even the multi-resistant strains and their use also have been reported to be related with several adverse side effects (such as hypersensitivity) (1-3). Leading the herbal medication could be a choice to treat diseases caused by multi-drug resistant bacteria (4-7). Now a day a significant fraction (~10%) of flowering plants are being used to prepare a number of herbal medicines for disease medication since they have been reported to possess the antioxidant activity with wound-healing effects (5, 8-10). Consequently the world wide use of flowers to treat diseases is not new with few or no side effects during medication (11-15).

However, apart the effectiveness of flowers of having the anti-bacterial activity, a diverse number of bacteria and fungi has been reported to populate the floral nectar [16]. Knowledge on the microorganisms prevailing within flowers could reinforce the hygiene consciousness and to diminish the infections acquired from environment (4, 17). Along these lines, present study aimed to identify the drug-resistance traits of the pathogenic isolates as well as the anti-bacterial attributes of the samples.

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MATERIALS AND METHODS

Study area, sampling and sample processing. As described earlier, samples of 11 categories of flowers including *Hibiscus rosa-sinensis* (Jaba), *Ixora coccinea* (Rangan), *Ipomoea digitata* (Giant potato), *Allamanda cathartica* (Allamanda), *Nymphaea nouchali* (Water Lily), *Vinca rosea* (Nayantara), *Rosa kordesii, Gladiolus hybrid, Acmella oleracea* (Toothache plant), *Nyctanthes arbor-tristis* (Shiuli) and *Pseudomussaenda flava* were randomly collected during August 2013-October 2013 following standard protocol (18). Microorganisms were isolated and identified as published by Sharmin et al. (4).

Antibiotic susceptibility test for detection of resistant bacteria. In the current study, the pathogenic isolates were examined for their antibiotic susceptibility traits by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used antibiotics following the standard protocol (19, 20). Antibiotics used in the study included erythromycin (ERY, 15 µg), amoxicillin (AMO, 30 µg), ceftriaxone (CEF, 30 µg), ciprofloxacin (CIP, 5 µg), streptomycin (SET, 10 µg), ampicillin (AMP, 10 µg), tetracycline (TE, 30 µg), chloramphenicol (CHL, 30 µg), cefixime (CFM, 5 µg), gentamycine (GEN, 10 µg), azythromycin (AZI, 15 µg) and cefalexim (CFL, 30 µg).

Assay of anti-bacterial properties of flower samples through broth micro-dilution method (assessment of the minimal inhibitory concentration, MIC). According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the anti-bacterial activity of flower samples was studied through broth micro-dilution method (5, 21, 22). In order to determine the MIC of the flower samples, 100 µL of bacterial inoculam was prepared with final concentration of 10^5 cfu/mL and introduced into appropriately labeled sterile tubes containing MH broth. In current study, different volume of samples ware employed (16 µL, 32 µL, 64 µL, 128 µL, 256 µL, 512 µL, 1024 µL and 2048 µL) into the MH broth to find out the lowest concentration, whch can trim down the maximum number of bacterial cell (5).

RESULTS AND DISCUSSION

In developing countries like Bangladesh, the possibility of existence of contaminating microorganisms is very high in food and water as well as other environmental means (23-29). Such a situation further tends to the probable onset of contagious diseases caused by the pathogenic bacteria most of which have been found to be drug-resistant (30-31). Based on these evidences on the existence of drug-resistance features of the food and water contaminating microorganisms together with the demonstration of the antibiotic resistant pathogenic bacteria in various clinical samples, current study further pondered to the microbiological complications raised by flowers which are indeed very affirm part of the environment.

In the study conducted by Sharmin et al. (4), almost all samples have been found to be populated by a huge range of bacterial and fungal flora. No growth of *E. coli* was detected in *Hibiscus rosa-sinensis* and *Vinca rosea*. *Pseudomonas* spp. and *Klebsiella* spp. were only found in *Ixora coccinea* and *Hibiscus rosa-sinensis*. *Staphylococcus* spp. was the most prevalent among four types of bacteria. *Actinomycetes* were also present in almost all flower samples except *Ipomoea digitata*. Growth of *E. coli* was significant in *Nymphaea nouchali*, *Rosa kordesii*, *Gladiolus hybrid*, *Acmella oleracea*, *Nyctanthes arbor-tristis* and *Pseudomussaenda flava*. *Pseudomonas* spp. was found in *Gladiolus hybrid*, *Acmella oleracea*, *Pseudomussaenda flava*. *Staphylococcus* spp. was the most prevalent among all types of bacteria. *Nymphaea nouchali* was found to harbor actinomycetes (4).

In the aspect of the public health significance, an important attention has to be arisen on the drugs resistance trait of pathogens proliferated in flowers, which may create serious problem in course of diseases medication (1, 33). In developing countries, the problem of resistance may arise from the extensive misuse of antibiotics and for the use of antibiotics during fish harvesting and processing which may decrease the effectiveness of drugs eliminating bacterial infections (1, 34, 35). Therefore, determination of the complete profile of pathogenic load and their antibiotic resistance in flowers is necessary to estimate the associated public health risk.

In our study *E. coli* exhibited sensitivity against almost 50% tested drugs. *Pseudomonas* exhibited sensitivity against 42% among all tested antibiotics (Table 1). However, *Staphylococcus* spp. and *Klebsiella* spp. exhibited highest multi-drug resistance against 83% and 75% of drugs, respectively. Interestingly ampicillin and cefalexim was found to be ineffective against all four types of isolated microbes. In contrast, streptomycin and tetracycline exhibited zone of inhibition against all the pathogens. Ciprofloxacin (CIP, 5 µg) and ceftriaxone (CEF, 30 µg) exhibited highest zone of inhibition (31 mm and 33 mm) (data not shown) against *Pseudomonas* and *E. coli*, respectively.

Anti-bacterial activity of flower samples. Previous reports showed the herbs or plants have the potential anti-bacterial agent which can retard the rapid propagation of microbial population [4-6]. Present study successfully claim that all the tested flower samples unveiled *in vitro* anti-bacterial activity against *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Bacillus* spp., *Salmonella* spp., *Listeria* spp., *Staphylococcus* spp., and *Vibrio* spp. through broth micro-dilution procedure or the MIC assay (Table 2). The MIC value was recorded for all samples within the range of 0.05 mg/mL (lowest)-0.2mg/mL (highest) (Table 2). The ideal concentrations of the samples were recorded at 0.2 mg/mL against all the tested bacteria. The MIC was recorded 0.1mg/mL against *E. coli*, *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus* spp., and 0.2 mg/mL recorded against *Klebsiella* spp., *Vibrio* spp., *Bacillus* spp., and *Listeria* spp. in case of

TABLE 1. Antimicrobial susceptibility patterns of different pathogenic isolates in flower samples

Organisms	*E. coli* (n=9)		*Klebsiella* spp. (n=2)		*Pseudomonas* spp. (n=5)		*Staphylococcus* spp. (n=11)	
Antibiotics	R	S	R	S	R	S	R	S
AMP (10µg)	77.77%	22.22%	100%	0%	80%	20%	72%	28%
CIP (5µg)	33.33%	66.66%	100%	0%	20%	80%	81.81%	18.18%
SET(10 µg)	11.11%	88.88%	0%	100%	0%	100%	9.09%	90.90%
CEF (5µg)	88.88%	11.11%	100%	0%	100%	0%	72.72%	27.27%
AMO (10µg)	100%	0%	100%	100%	80%	20%	54.54%	45.45%
TE(30µg)	33.33%	66.66%	0%	100%	20%	80%	18.18%	81.81%
CHL (10µg)	33.33%	66.66%	100%	0%	80%	20%	100%	0%
GEN(10µg)	33.33%	66.66%	50%	50%	80%	20%	100%	0%
AZI (15µg)	88.88%	11.11%	100%	0%	20%	80%	100%	0%
CFL(30µg)	88.88%	11.11%	100%	0%	100%	0%	72.72%	27.27%
ERY (15 µg)	88.88%	11.11%	100%	0%	20%	80%	100%	0%
CFM (5µg)	0%	100%	100%	0%	80%	20%	100%	0%

AMP= Ampicillin, AMO= Amoxicillin, CIP= Ciprofloxacin, CEF= Ceftriaxone, NALI= Nalidixic acid, IPM= Imipenem, ERY= Erythromycin, CHL= Chloramphenicol, TMP/SUL= Trimethoprime-sulfomethoxazole, GEN= Gentamicin, PIP= Piperaciline.
Sensitive- S; Registrant- R; not done- ND

TABLE 2. Minimum Inhibitory Concentration (MIC) of the samples

Sample	Organisms							
	E. coli	*Klebsiella* spp.	*Salmonella* spp.	*Vibrio* spp.	*Pseudomoas* spp.	*Bacillus* spp.	*Staphylococcus* spp.	*Listeria* spp.
Rosa kordesii	0.1 mg/mL	0.2 mg/mL	0.1 mg/mL	0.2 mg/mL	0.1 mg/ mL	0.2 mg/mL	0.1 mg/mL	0.2 mg/mL
Gladiolus hybrid	0.2 mg/mL	0.2 mg/mL	0.1 mg/mL	0.2 mg/mL	0.05 mg/ mL	0.2 mg/mL	0.1 mg/mL	0.1 mg/mL
Acmella oleracea	0.2 mg/mL	0.1 mg/mL	0.2 mg/mL	0.2 mg/mL	0.2 mg/ mL	0.05 mg/mL	0.2 mg/mL	0.2 mg/mL
Nymphaea nouchali (Water lily)	0.1mg/mL	0.05 mg/mL	0.1 mg/mL	0.1 mg/mL	0.2 mg/ mL	0.2 mg/mL	0.1 mg/mL	0.1 mg/mL
Hibiscus rosa-sinensis (Jaba)	0.2 mg/mL	0.2 mg/mL	0.2 mg/mL	0.2 mg/mL	0.1 mg /mL	0.1 mg mL	0.05 mg/mL	0.2 mg/mL
Ixora coccinea (Rangan)	0.05 mg/mL	0.1 mg/mL	0.2 mg/mL	0.1 mg/mL	0.2 mg/ mL	0.2 mg/mL	0.2 mg/mL	0.2 mg/mL

The experiment was conducted three times independently, and the results were found to be reproducible. One representative data has been shown.

mg/mL against *E. coli*, *Klebieilla* spp., *Vibrio* spp., and *Bacillus* spp., while the endurance of *Salmonella* spp., *Staphylococcus* spp., and *Listeria* spp. were found to be inhibited at 0.1 mg/mL and 0.05 mg/mL. recorded for *Pseudomonas* spp. The growth of *E. coli*, *Pseudomonas* spp., *Salmonella* spp., *Listeria* spp., *Staphylococcus* spp., and *Vibrio* spp. were found to be stalled at 0.2 mg/mL even as the MIC was noticed at 0.1 mg/mL and 0.05 mg/mL for *Klebsiella* spp., and *Bacillus* spp. respectively in case of *Acmella oleracea* sample. The MIC was recorded for *Nymphaea nouchali* at 0.1 mg/mL against *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Vibrio* spp., *Listeria* spp. and 0.2 mg/mL against *Pseudomonas* spp. and *Bacillus* spp. while 0.05 mg/mL was recorded against *Klebseilla* spp. For Hibiscus rosa-sinensis, MIC was noticed at 0.2 mg/mL for *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Listeria* spp., and *Vibrio* spp., 0.05 mg/mL recorded for *Staphylococcus* spp. and 0.1 mg/mL recorded for *Pseudomonas* spp. and *Bacillus* spp. For sample *Ixora coccinea*, MIC was noticed at 0.2 mg/mL for *Pseudomonas* spp., *Bacillus* spp., *Salmonella* spp., *Listeria* spp., *Staphylococcus* spp., 0.05 mg/mL recorded for *E. coli* and 0.1 mg/mL recorded for *Klebsiella* spp. and *Vibrio* spp. (Table 2).

CONCLUSION

Overall, the result of the present study revealed that the extracts of 6 flower samples were found to have potential anti-bacterial activity which can effectively reduce the growth of pathogenic isolates including multi-drug resistant strain. The extraction of pilot ingredients from such flower would largely aid to plot the template for the production of new antibiotics.

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