Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2021.019

SHORT COMMUNICATION

Antibacterial Activity of Medicinal Flowers against Multi Drug Resistant *E. coli*

Iqra Nazir[1], Masood Rabbani[1*], Ali Ahmad Sheikh[1], Sehrish Firyal[2], Sohail Raza[1] and Muhammad Ilyas Riaz[1]

[1]Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: mrabbani@uvas.edu.pk

ARTICLE HISTORY (20-522)

Received: October 12, 2020
Revised: December 23, 2020
Accepted: January 08, 2021
Published online: January 26, 2021

Key words:
Antibacterial activity
E. coli
Medicinal flowers
Multidrug resistant (MDR)

A B S T R A C T

The antibiotic resistance is creating a serious challenge to treat diseases especially against multidrug resistant (MDR) *E. coli*. The present study was conducted to evaluate the antibacterial activity of seven different flowers ethanolic extracts named as *Achillea millefolium*, *Bombax ceiba*, *Chrysanthemum cinerarifolium*, *Hyssopus officinalis*, *Rosa damascena Miller*, *Taraxacum officinale Weber* and *Woodfordia fruticosa* against MDR *E. coli* through spot test and minimum inhibitory concentrations (MIC) test. The results showed all the seven extracts have significant antibacterial activity against MDR *E. coli* and control. The MIC of *Rosa damascena Miller*, *Bombax ceiba* was 3.125 mg/ml and *Taraxacum officinale Weber* was 12.5 mg/ml for MDR *E. coli*. Similarly, *Achillea millefolium, Hyssopus officinalis*, *Chrysanthemum cinerarifolium* MIC value was 25 mg/ml and 12.5 mg/ml for *Woodfordia fruticosa Kurtz.* The current study reveals that these flowers possess strong antibacterial activity and thus can be used as potential antimicrobial agent against various resistant bacterial pathogens.

To Cite This Article: Nazir I, Rabbani M, Sheikh AA, Firyal S, Raza S and Riaz MI, 2021. Antibacterial activity of medicinal flowers against multi drug resistant *E. Coli*. Pak Vet J, 41(1): 166-168. http://dx.doi.org/10.29261/pakvetj/2021.019

INTRODUCTION

In recent years, antimicrobial resistance (AMR) has become one of the most important public health concern of this century. The infectious diseases are caused by bacteria, viruses, parasites and fungi. Among these, bacteria are a major problem of some common and severe infections. The irrational uses of antibiotics have developed resistance against the drugs that are commonly used to treat bacterial infections. The bacteria causing the common or lethal infections have developed resistance to existing and new antibiotics coming to the market that leads to a global health crisis (Prestinaci *et al*., 2015). Antibiotics put a selective pressure on gut microflora and kills selectively susceptible bacterial cells and allowing resistant form to survive. The surviving resistant bacterial grow continuously producing a large population of resistant cells and became predominant in the gut. Such resistant form when release through fecal material in the environment, spreads the resistance genes to other bacterial genera (Shecho *et al*., 2017). Among the pathogens, *E. coli* is the most lethal pathogen that can be transmitted to humans directly through sewage contamination of food chain system. Being present as natural intestinal flora of almost all warm blooded animal, carry drug resistance genes due to irrational use of antibiotics. With increasing trend of AMR, experiments are being under way to use alternatives to antibiotics. The natural ingredients have least side effects and are more effective against pathogens as compared to chemosynthetic agents. The plant extracts have wide spectrum of active compounds like terpenoids, flavonoid, coumarin, tannin, saponin, alkaloids, glucosides, essential oils and polysaccharides. The natural constituents can be derived from any part of the plants are a gift to cure diseases and have vital role in health development (Karole *et al*., 2017). It has been found that natural products are more effective and should be used in healthcare system as having least side effects.

MATERIALS AND METHODS

Samples: One *E. coli* isolate recovered from morbid chicken was processed at the Institute of Microbiology (IOM), University of Veterinary and Animal Sciences (UVAS), Lahore. Reference bacterial strain of *E. coli* (ATCC 25922) was obtained from the IOM, UVAS, Lahore.

Identification of *E. coli*: The *E. coli* isolated from bird sample was initially identified based on culturing in tryptone soya broth (TSB) which is an enrichment media, EMB agar plates and biochemical test viz IMViC test (Cappuccino and Sherman, 1999).

Confirmation of *E. coli* by PCR: Confirmation of *E. coli* was done through PCR. Extracted DNA was subjected to PCR by targeting *E. coli* specific *uspA* gene using PCR condition as described by (Shaheen *et al*., 2015).

Multi-drug resistance (MDR) testing: The *E. coli* isolated from the bird was subjected for antibiotic sensitivity test by Kirby-Bauer method. Fresh bacterial colonies were suspended in normal saline to produce turbidity equal to 0.5 McFarland units. The suspension was swabbed on Muller Hinton agar (MHA) using a sterile cotton swab. Antibiotic discs were placed on MHA plates and incubated for 24 hrs at 37°C. After incubation, zones of inhibition were measured (mm).

Antibacterial activity of herbal extracts against bacterial pathogens

Flowers collection: 100g of dried flowers named as *Achillea millefolium*, *Bombax ceiba*, *Chrysanthemum cinerarifolium*, *Hyssopus officinalis*, *Rosa damascena Miller*, *Taraxacum officinale Weber*, *Woodfordia fruticosa* were purchased from local herbs market and transferred to IOM, UVAS, Lahore.

Preparation of Ethanolic flowers extracts: Ethanolic extracts of collected flowers were prepared by grinding the flowers into fine amorphous powder. Briefly, 10 g of powder was soaked into 90 ml of 80 percent ethanol (1:10 w/v). After 2 days of incubation in shaking incubator at 150 rpm at 37ºC incubation, the extracts were filtered through filter paper No.1 (Whatman, USA). The extracts were poured into glass petri plates to dry at 40°C. The stock solution of dried extracts were prepared by using 10% dimethyl sulfoxide (DMSO) (Obeidat, 2011; Shaheen *et al*., 2015).

Spot test: Antibacterial activity of ethanolic extracts were determined by spot test. By internal study, the concentration of each extract was optimized. Initially, 200mg/ml extract concentration was used and performed repeatedly with increased concentration until get clear zones. After that, 0.5 McFarland's bacterial suspensions were used for the preparation of bacterial lawn on MHA plates. A total of 10µl of each extract, having concentration of 200mg/ml, 250mg/ml, 300mg/ml, 350mg/ml and 400mg/ml were placed in the center of the plate and the plate was incubated at 37ºC for 24hrs. After incubation, the spots showed bacterial inhibition considered having antibacterial potential and the concentration at which all extracts showed antibacterial activity was selected for further processing.

Minimum inhibitory concentration (MIC) Determination: For MIC, broth microdilution method was used. Briefly, Muller Hinton broth (50 µl) was added in all wells of microtitration plate, from well 1 to 12. Then in first well, 50µl of stock solution of the extract in DMSO was added and 2-fold serial dilutions were made up to 10th well. After that, 50µl of 0.5 Mcfarland bacterial suspension was added in all wells from 1 to 11, while 12th well was considered as a negative control. Microtitration plate was then incubated at 37ºC for 24 hrs. Bacterial growth was determined by turbidity in negative control well. The MIC values were determined by observing the wells with no bacterial turbidity comparing with negative control well (Shaheen *et al*., 2015).

RESULTS AND DISCUSSION

In the present study, ethanolic extracts of seven different flowers were evaluated against *E. coli* (ATCC 25922) and MDR *E. coli*. The isolate recovered from morbid chicken showed green metallic sheen on EMB plates and PCR exhibited corrected band size of 884 bp similar to the study conducted on *E. coli* isolated from chicken (Sheikh *et al*., 2012). Results of antibiotic sensitivity test revealed that *E. coli* showed resistance against more than three different classes of antibiotics used i.e Ceftazidime, Ofloxacin, Erythromycin, Doxycycline, Ampicillin, and Augmentin (Fig. 2). In order to observe the antimicrobial potential of flowers, initially, various concentrations of ethanolic extracts, ranging from 50mg/ml to 400mg/ml, were used as pilot study. Out of which concentration of (400mg/ml) was selected for further testing as at this concentration all extracts showed bacterial inhibition by spot test as well as MIC as shown in Table 1.

The MIC of *Rosa damascena Miller* against *E. coli* (ATCC 25922) and MDR *E. coli* was 12.5mg/ml and 6.125mg/ml similar to the previous study (Halawani, 2014). The antimicrobial effect of *Achillea millefolium* was achieved at 50mg/ml and 25mg/ml MIC values against MDR *E. coli* and the control, respectively. Analyzing further, flowers of *Bombax ceiba* inhibit *E. coli* (ATCC) at 50mg/ml concentration and MDR *E. coli* at 3.125mg/ml comparable to the study on methanolic extracts of *B. ceiba* stem bark (100µg/disc) which also possess strong antibacterial activity against *E. coli* (Akhtar and Mustafa, 2017).

The ethanolic extract of *Woodfordia fruticose Kurtz* flowers inhibit *E. coli* and MDR *E. coli* at MIC value of 12.5 mg/ml and 6.25 mg/ml, respectively. Similarly, its acetone extract exhibited antimicrobial activity against *E. coli* at 300µg/ml concentration. Furthermore, essential oils of *Chrysanthemum coronarium* found inhibitory effect against *E. coli* ATCC 29425 by disc diffusion method in contrast to the present study where its flowers had MIC value at 50mg/ml and 25mg/ml against *E. coli* (ATCC) and MDR *E. coli*, respectively.

The flower extract of *Hyssopus officialnalis* showed activity against *E. coli* ATCC and MDR *E. coli* at MIC 50mg/ml and 25mg/ml concentration In a recent study, nanoparticles of *Taxacum officianale* leaves also revealed strong antibacterial activity against gram negative phytopathogens at various concentrations ranged from 10ug/ml to 30ug/ml (Saratale *et al*., 2018) similar to the present study where *Taxacum officinale* flowers exhibited antibacterial activity with 25 mg/ml MIC against *E. coli* ATCC and 12.5mg/ml against pathogenic *E. coli*.

Table 1: Spot Test and Minimum inhibitory concentration values of herbal extracts

SR. No	Herbal Extracts	Concentration	Spot Test		MIC	
			E. coli (Control)	MDR *E. coli*	*E. coli* (Control)	MDR *E. coli*
1.	*Rosa damascena Miller*	400mg/ml	+ve	+ve	1:32	1:128
2.	*Achilleamillefolium*	400mg/ml	+ve	+ve	1:8	1:16
3.	*Bombax ceiba*	400mg/ml	+ve	+ve	1:8	1:128
4.	*Taxacumofficianale weber*	400mg/ml	+ve	+ve	1:16	1:32
5.	*Woodfordiafruticosakurtz*	400mg/ml	+ve	+ve	1:32	1:64
6.	*Hyssopusofficianalis L*	400mg/ml	+ve	+ve	1:8	1:16
7.	*Chrysanthemum cinerafolium*	400mg/ml	+ve	+ve	1:8	1:16

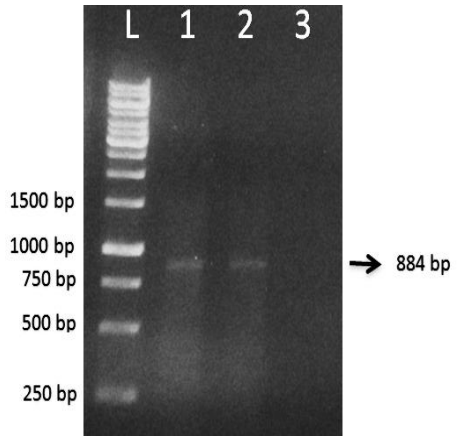

Fig. 1: PCR results for *usp A* gene for amplification of *E. coli* isolates. L: Ladder, 1: Sample, 2: *E. coli ATCC, 3: Negative control.*

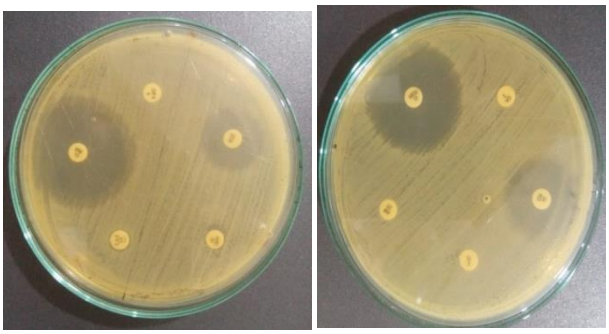

Fig. 2: Multidrug resistance monitoring by disc diffusion assay. Antibiotic discs of Chloramphenicol, Nalidixic acid, Norfloxacin, Clarithromycin, Clindamycin, Meropenem, Azithromycin, Erythromycin, Ampicillin, Ciprofloxacin, Imipenem, Levofloxacin, Tetracyclin, Ceftazidime, Augmentin, Ofloxacin, Colistine, Gentamycin and Moxifloxacin were placed on MHA plates. Bacterial isolate resistant to three or more than three drugs of various classes considered as MDR.

Statistical analysis by one way ANOVA for multiple comparisons showed the significant results of all extracts with P-value $\leq$0.05 for MIC of *E. coli* ATCC and MDR *E. coli* except *Hyssopus officianalis* and *Chrysanthemum cinerafolium* with each other. In multiple comparisons, independent (I. flower) were compared with dependent (J. flowers). The mean difference showed that the results were also significant (P-value $\leq$0.05). The current results reveal that these flowers possess strong antibacterial activity and thus can be used for treatment purpose.

Authors contribution: Conceive and designed the experiments: MR, AAS, SF Performed experiments: IN Analyzed the data: IN, AAS, MIR Wrote paper: MR, IN, AAS, SR.

REFERENCES

Akhtar N and Mustafa R, 2017. Antibacterial and antioxidant potential of stem bark extract of *Bombax ceiba* collected locally from south Punjab Area of Pakistan. Afr J Trad Compl Altern Med 14:9-15.

Cappuccino J and Sherman N, 1999. A laboratory Manual of Microbiology. Rock land Community College, Suffern, New York.

Halawani EM, 2014. Antimicrobial activity of *Rosa damascena* petals extracts and chemical composition by gas chromatography-mass spectrometry (GC/MS) analysis. Afr J Microbiol Res 8:2359-67.

Karole S, Gautam G and Gupta S, 2017. Pharmacognostic and pharmacological profile of *Bombax ceiba.* Asian J Pharma Ed Res 6:16-27.

Obeidat M, 2011. Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens. J Med Plant Res 5:3856-60.

Prestinaci F, Pezzotti P and Pantosti A, 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109:309-18.

Saratale RG, Benelli G, Kumar G, *et al*., 2018. Bio-fabrication of silver nanoparticles using the leaf extract of an ancient herbal medicine, dandelion (*Taraxacum officinale*), evaluation of their antioxidant, anticancer potential, and antimicrobial activity against phytopathogens. Environ Sci Pollut Res 25:10392-406.

Shaheen AY, Sheikh AA, Rabbani M, *et al.,* 2015. Antibacterial activity of herbal extracts against multi-drug resistant *Escherichia coli* recovered from retail chicken meat. Pak J Pharm Sci 28.

Shecho M, Thomas N, Kemal J, *et al*., 2017. Cloacael Carriage and Multidrug Resistance *Escherichia coli* O157:H7 from Poultry Farms, Eastern Ethiopia. J Vet Med 8264583.

Sheikh AA, Checkley S, Avery B, *et al*., 2012. Antimicrobial resistance and resistance genes in *Escherichia coli* isolated from retail meat purchased in Alberta, Canada. Foodborne Pathog Dis 9:625-31.