Research Article

Efficacy of Allicin against multi-drug resistant *Escherichia coli* recovered from potable water

Sehrish Nayab¹, Sajjad ur Rahman¹, Sanaullah Sajid^{1*}, Muhammad Ismail Abbas², Zia ud Din Sindhu³, Muhammad Idrees⁴, Muhammad Usama Tariq⁵, Rabia Kanwar¹ and Ifra Farzand¹

1. Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

2. Poultry Research Institute, Rawalpindi, Pakistan

3. Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

4. Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan

5. Department of Microbiology, Government College University, Faisalabad, Pakistan

*Corresponding author's email: Sanaullahsajid@gmail.com

Citation

Sehrish Nayab, Sajjad ur Rahman, Sanaullah Sajid, Muhammad Ismail Abbas, Zia ud Din Sindhu, Muhammad Idrees, Muhammad Usama Tariq, Rabia Kanwar and Ifra Farzand. Efficacy of Allicin against multi-drug resistant *Escherichia coli* recovered from potable water. Pure and Applied Biology. Vol. 11, Issue 2, pp509-517.

<http://dx.doi.org/10.19045/bspab.2022.110050>

Received: 11/05/2021

Revised: 16/07/2021

Accepted: 03/08/2021

Online First: 26/08/2021

Abstract

The increased prevalence of *E. coli*, especially the multidrug resistant *E. coli* recovered from drinking water supplies, demands a new substance, allicin that may potentially be effective and decrease the spread of these microorganisms. Allicin is an antibacterial agent derived from garlic to test its efficiency against multi-drug resistant (MDR) *E. coli*. The presumptive coliforms were isolated followed by confirmation of pathogenic strains from potable water supply samples received during 2018-2019. Isolated *E. coli* were found resistant against multiple drugs including Amikacin, Ampicillin, Vancomycin, Oxacillin, Fusidic acid, Gentamycin, Oxytetracycline and Moxifloxacin. The methanolic and ethanolic extracts of garlic were prepared and the allicin compounds were analysed quantitatively through HPLC. The standard concentration of *E. coli* (0.5 McFarland) was maintained to determine the MIC₅₀ through microdilution technique for both allicin extracts separately. The minimum inhibitory concentration (MIC₅₀) of methanolic and ethanolic derivatives of allicin was found at 28.64 µg/ml and 7.57 µg/ml of crude allicin extracts respectively. Out of three consecutive concentrations of ethanolic allicin extracts in the experimental wash water containing MDR *E. coli* was capable to clean the water at 8.00 µg/ml in 10 minutes followed by 10.00 µg/ml and 12.00 µg/ml in 5 minutes of treatment. These results suggested the application of ethanolic derivatives of allicin as potential biocide for cleaning water reservoirs particularly against MDR pathogenic *Escherichia coli* at dairy farms.

Keywords: Antibiotic resistance; Coliform count; *Escherichia coli*; Ethnomedicine; Herbal plants; MIC

Introduction

The availability of clean drinking water to its population is still a major problem being faced by many developing countries.

According to WHO (World Health Organization), biological contaminants in drinking water are responsible for about 80% of health problems [1, 2]. Every year,

over five million individuals die because of diseases associated with drinking water and out of this, more than 50% casualties occurred due to intestinal infections [3]. The food and waterborne pathogens cause a variety of intestinal diseases including diarrhea, cryptosporidiosis, giardiasis, shigellosis, gastroenteritis, typhoid fever, salmonellosis and cholera [4]. These pathogens are released in water through drainage from human sewage and animal husbandry facilities. Among different waterborne pathogens prevailing in developing countries, the biggest issue is the *E. coli* and its presence in drinking water indicates the contamination of fecal material. Though some strains of *E. coli* living in the colon of human and animals harmlessly, many strains induce severe extra-intestinal and intestinal diseases. So, for the availability of clean drinking water, removal of such waterborne pathogens is necessary [2].

Antibiotics are the main target drugs for the control of *E. coli* and other bacterial infections, but the emergence of resistance genes and enzymes render these therapies ineffective against such infections [5]. The ability of *E. coli* bacteria to transfer resistance genes horizontally by plasmid and production of Extended spectrum beta-lactamases (ESBL) enzymes renders the cephalosporins and penicillin languishing against it [6]. Several control strategies are suggested, including restricted use of antimicrobial agents in animal food, improve the mechanism of drugs to cope with the resistance genes and judicious use of antibiotics in veterinary and animal health [7].

Among highly populated and industrial cities of Pakistan, Faisalabad is the major city where the accessibility of clean drinking water is a notable issue. In Pakistan different research articles proclaimed the incidence of microbial presence in potable water, but no study represents the prevalence of *Escherichia coli* in potable drinking water in the district

Faisalabad. So, it's the need of time to estimate *Escherichia coli* in drinking water. The evolution of antibiotic resistance in bacteria is increasing due to the enormous usage of antibiotics that causes complications in the treatment. Another approach to treat or reduce *E. coli* associated diseases can be achieved by extracts of herbal plants. Several chemical components are present in herbal plants that are involved in biological activities [8-10]. Garlic (*Allium sativum*), a herbal plant, has shown exemplary antimicrobial properties belongs to *Amaryllidaceae* (family) and *Alloideae* (subfamily). Chester Cavallito in 1948 and his colleagues found an organosulfur component in garlic which has antimicrobial activity and is responsible for the pungent smell of garlic, later which was named allicin (thio-2-propene-1-sulfinic acid S-allyl ester). Allicin is produced when garlic cloves are crushed, (heat labile) alliinase converts alliin into allicin [11-13].

In history, Garlic has been used to cure different diseases. In ancient times, Egyptians treated diarrhea with garlic and Greeks used garlic to cure intestinal infections. Africans also used garlic to cure diarrhea, otitis media, abdominal pain and respiratory tract infections. Early Japanese and Chinese treated headache, fever, sore throat and flu with garlic. Therefore, garlic was being recognized as Russian penicillin or natural antibiotic [11].

The current study aimed to examine the drinking water in Faisalabad for microbial contamination and to provide an alternative source of treatment for multidrug resistant *E. coli* with allicin (a herbal drug).

Materials and Methods

Study area and sampling

We collected drinking water samples (n=150) from reverse osmosis (RO) plants tracked at various locations of District Faisalabad. Sterilized Falcon tubes of 50ml were utilized to collect water samples and shifted in a cold chain to the Microbiological Water Testing Laboratory

(MWTL) at Institute of Microbiology, University of Agriculture Faisalabad.

Isolation and characterization of *E. coli* from water

The total coliform count was determined through the MPN (Most Probable Number) method by inoculating the water samples in test tubes containing lactose broth. After 24 h of incubation at 37 °C, tubes were observed for the change in color (Yellow) and gas production (bubble formation in Durham tubes). To confirm the *Escherichia coli* a loop full from the positive tubes were streaked on MacConkey agar plates as the *E. coli* can ferment lactose. The plates were incubated at 37 °C for 8h in an incubator. Colonies of pink color appeared in MacConkey agar plates were streaked on Eosin Methylene Blue agar plates for confirmation. The typical colonies producing metallic sheen after 8h of incubation at 37 °C were checked through Gram staining for colony morphology. The biochemical profiling of *Escherichia coli* was done using the RapID ONE system (Remel) [14].

MDR (Multi drug Resistance) profiling of *Escherichia coli*

Multi drug resistance of *E. coli* was determined by disc diffusion method. Mueller-Hinton (MH) agar plates were prepared, *E. coli* cultures were streaked and Amikacin, Ciprofloxacin, Ampicillin, Vancomycin, Oxacillin, Fusidic acid, Gentamycin, Oxytetracycline and Moxifloxacin discs were employed for the antibiotic susceptibility testing. Zones of inhibition around the discs were determined and compared with the CLSI interpretative chart. Multi drug resistance was declared after the exhibition of resistance to three or more distinct classes of antibiotics [15].

Preparation of methanolic and ethanolic extracts garlic

Methanolic and ethanolic extracts were suggested for this study as both have a wide range of polarity as compared to water extract and can evaporate from the extract leaving a negligible amount remained in the extract. An equal volume of both was used

to compare the relative ability, as high extractability was reported with methanol [16]. Garlic was purchased from the local market of Faisalabad. Garlic cloves were washed in running tap water and then with distilled water. For preparing extract, 100g of the garlic cloves were crushed in mortar and pestle and homogenized 50g in 50ml of 20% ethanol while 50g in 50ml of 20% methanol. Both solutions were closed with aluminum foil and stored at 4°C for 48 hours. After storage of 48 hours, the solutions were centrifuged at 5500 rpm for 10 minutes in sterilized 50ml falcon tubes to separate the debris from the liquid. The supernatants were collected in a 50ml falcon tube and stored at 4°C [8].

Quantification of Allicin by HPLC (methanolic and ethanolic extracts)

Quantitative analysis of allicin (methanolic and ethanolic) extracted from garlic was determined by HPLC from High-Tech Laboratory, UAF. Internal standard (100µl) was added to 10µl of the extracts and final volume was adjusted to 1ml by the mobile phase. The solution was mixed and centrifuged at 15000 rpm for 5 minutes. The supernatant volume of 20µl was injected to HPLC system equipped with C18 and MG II analytical column with a size of 4.6 mm × 250 mm. The allicin extract applied to column with an isocratic solvent; it was eluted at the 0.5ml/min flow rate. Allicin was detected by the measurement of absorbance at 220 nm and quantified by measuring the peak produced by the fresh garlic extracts with the standard allicin [14].

Determination of the antimicrobial activity of allicin

Crude garlic extract contains allicin as an active ingredient that has the antibacterial potential because of its chemical reaction with thiol group of various enzymes e.g., Alcohol dehydrogenase, RNA polymerase and thioredoxin reductase, which can affect the metabolism of cysteine proteinase involved in the virulence of bacteria. The methanolic and ethanolic allicin extracts were tested for antimicrobial activity

(MIC₅₀) against MDR *Escherichia coli* using the microdilution method. *Escherichia coli* colonies were inoculated in test tubes containing nutrient broth and overnight incubated at 37°C. After incubation, cultures were examined for growth and used to determine the minimum bactericidal concentration (MBC) [17].

Results

Coliform count in potable water

Of total water samples (n=150), the highest coliform count was 1100/100ml in 11

samples, while 200-500 coliform was shown in 17 samples, 50-100 count was found in 23 water samples, 30-50 count was observed in 73 water samples, and less than 10 count was detected in 26 water samples. All the suspected samples were further inoculated on MacConkey agar and 57 samples were confirmed as *E. coli*. All cultures were found pathogenic as cultivated on Eosin Methylene Blue (EMB) agar with metallic sheen surface colonies (Fig. 1 & 2).

Figure 1. Pie graph represents of the coliform count and positive samples on media plates

Figure 2. Represents the coliform count method for the determination of most probable bacterial count per 100ml of the water sample

Characterization of *E. coli* using RapID ONE panel system

For the biochemical profiling of *Escherichia coli*, RapID ONE panel system from Remel was used. A single isolated *Escherichia coli* colony was dissolved in 2 ml fluid, mixed, and poured in the panel. After 24 hours, results were obtained and observed for the change in colour with the standard chart (Fig. 3).

Identification of multi-drug resistant *E. coli*

Antibiotic susceptibility testing was performed using Muller Hinton (MH) agar. The bacteria were inoculated on the plates with different antibiotics discs were submerged and incubated. Diameters of zones of inhibition were measured with the help of Vernier calliper for all the antibiotics. Zones were compared with the CLSI standard chart and the drugs Erythromycin, Amikacin and Ciprofloxacin were showed sensitive while Streptomycin, Ampicillin, Bacitracin, Vancomycin, Metronidazole and Tobramycin were found resistant to *Escherichia coli* (Fig. 4)

Quantification of Allicin

Using HPLC, the peak areas of standard and relevant analyte (allicin) in the samples were calculated. HPLC results indicated the quantity of allicin in methanolic extract contained 572.93 µg/ml of allicin and ethanolic extract was 151.40 µg/ml.

Determination of MIC₅₀ of Allicin

Methanolic extract of allicin showed the inhibition of *E. coli* in a microtitration plate with 50µl quantity containing 28.64µg of allicin while MIC₅₀ of ethanolic extract was 7.57µg of Allicin in 50µl dilution (Fig. 5 & 6).

Efficacy study of ethanolic Allicin extract

Three consecutive concentrations of ethanolic Allicin extracts in the experimental wash water from dairy farms containing MDR *E. coli* were found capable to clean up the water at 8.00 µg/ml in 10 minutes followed by 10.00 µg/ml and 12.00 µg/ml at 5 minutes of treatment compared to the negative control where no allicin extract was applied as detailed in (Table 1, 2 & 3).

Figure 3. Results on RapID ONE panel

Figure 4. Antibiotic resistance pattern of *E. coli* against different antibiotic disc on Muller Hinton agar plate

Figure 5. Representation of MIC₅₀ of allicin using microtitration plate method

Figure 6. a) HPLC analysis of methanolic extract b) represents the HPLC analysis of ethanol extract

Table 1. Antibiotic resistant profiling of *E. coli*

Antibiotic Name	Zone of Inhibition (mm)			Result
Amikacin (30µg)	<14	15-16	>17	Resistant
Ciprofloxacin (5µg)	<15	16-20	>21	Sensitive
Oxacillin (1µg)	<18	18-24	>24	Resistant
Ampicillin (10µg)	<27	27-35	>35	Resistant
Fusidic acid (10µg)	<24	24-32	>32	Resistant
Vancomycin (30µg)	<17	17-21	>21	Resistant
Gentamycin (30µg)	<12	13-14	>15	Resistant
Oxytetracycline (30µg)	<14	15-18	>19	Resistant
Moxifloxacin (5µg)	<15	16-20	>21	Resistant

Table 2. HPLC analysis of garlic extracts representing the quantification of Allicin

Analyte	Retention time (min)	Area (mV.s)	Area (%)	Concentration of allicin (µg/ml)
Methanolic extract of allicin	1.767	2491.102	60.4	572.93
Ethanollic extract of allicin	1.907	658.834	36.0	151.40

Table 3. Comparative efficacy of Ethanolic allicin extracts against MDR *E. coli* in water samples

<i>E. coli</i> Conc. µg/ml	Time in Minutes		
	5	10	15
8.00	++ --	----	----
10.00	----	----	----
12.00	----	----	----
Control negative	++++	++++	++++

Discussion

Water is an essential part of life for every human being, animals and plants. Bacterial contaminations in drinking water causing severe health issue all over the world especially in developing countries like Pakistan. Barely 25% community in Pakistan has an approach to safe potable water. Out of 25%, only 70% population belonging to urban areas and 30% community belonging to rural areas have access to drinking water. The remaining water is unhygienic because of the mixing of sewage water that contains harmful and disease-causing microorganisms in plenty [18]. Water is becoming a major threat to human health all over Pakistan, particularly in Faisalabad which is a densely populated area. In Faisalabad, mostly the water contamination is through municipal and

industrial wastes, which are the primary cause of waterborne illness. *E. coli* is the major trouble in drinking water gets mixed through sewerage water leading to diarrhea and other gastrointestinal infections [19]. The continuous use of antibiotics against *E. coli* infections leading to bacterial resistance against multiple antibiotics which ultimately causing complications in the treatment. The alternate therapy is to shift towards herbal medicines. The herbal plants including garlic possess many natural substances which have remarkable antibacterial effects on bacteria. Garlic is being used in ancient times to cure infections [16, 19].

The principal goal of this study was to explore the potential of allicin extracted from garlic to inhibit the activity of multidrug resistance *E. coli*. The

methanolic and ethanolic extracts of allicin were preferred to use as compared to water based extracts due to instability of allicin as hydrogen in water reacts with the oxygen atoms in allicin and destabilizes the molecule. Allicin reacts with water to form the diallyl disulphide, which does not exhibit the desired level of antimicrobial activity. In conclusion, out of three consecutive concentrations of ethanolic allicin extracts used in the experimental wash water containing MDR *E. coli* was capable to clean the water at 8.00 µg/ml of ethanolic extract of allicin in 10 minutes followed by 10.00 µg/ml and 12.00 µg/ml as effective at 5 minutes of treatment at 28°C. The ethanolic extract of allicin may be recommended as an excellent biocide for the elimination of multidrug resistant *E. coli* in water used for washing and drinking purposes particularly in the public hospital settings and at dairy farms.

Conclusion

The current study explored the potential of garlic extracted allicin used to inhibit the activity of multidrug resistance *E. coli*. Observing the potential bactericidal activity of ethanolic extract against *E. coli*, it may be recommended in water used for washing and drinking purposes particularly in the public hospital settings and at dairy farms.

Authors' contributions

Conceived and designed the experiments: S Nayab & SU Rahman, Performed the experiments: S Nayab & S Sajid, Analyzed the data: MI Abbas & M Idrees, Contributed materials/ analysis/ tools: MU Tariq, R Kanwar, I Farzand & ZUD Sindhu, Wrote the paper: S Nayab & S Sajid.

Acknowledgement

Authors appreciate the financial contribution of HEC/NRPU 4939 project for the completion of present study. Thanks are extended to ORIC, UAF for their technical support.

References

1. Farzand I, Rahman SU, Sajid S & Nayab S (2020). Evaluation of modified MRS media for the selective enumeration of *Lactobacillus casei*. *Pure Appl Biol* 10(1): 194-198.
2. Supong A, Bhomick PC & Sinha D (2017). Waterborne pathogens in drinking water-existing removal techniques and methods. *MOJ Toxicol* 3: 146-147.
3. Cabral JP (2010). Water microbiology. Bacterial pathogens and water. *Int J Environ Res Public Health* 7(10): 3657-3703.
4. Younas M, ur Rahman S, Shams S, Salman MM & Khan I (2019). Multidrug resistant carbapenemase-producing *Escherichia coli* from chicken meat reveals diversity and co-existence of carbapenemase encoding genes. *Pak Vet J* 39: 241-245.
5. Rahman SU & Mohsin M (2019). The under reported issue of antibiotic-resistance in food-producing animals in Pakistan. *Pak Vet J* 39: 323-328.
6. Ullah F, Malik S & Ahmed J (2009). Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. *African J Biotech* 8(16): 3921-3926.
7. Angulo FJ, Talbot EA, Gagnon ER & Greenblatt J (2006). Common ground for the control of multidrug-resistant *Salmonella* in ground beef. *Clinic Infect Dis* 42(10): 1455-1462.
8. Jarriyawattanachaikul W, Chaveerach P & Chokesajjawatee N (2016). Antimicrobial activity of Thai-herbal plants against food-borne pathogens *E. coli*, *S. aureus* and *C. jejuni*. *Agri Sci rocedia* 11: 20-24.
9. Bakr AF, Abdelgayed SS, EL-Tawil OS & Bakeer AM (2019). Assessment of ginger extract and ginger nanoparticles protective activity against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats. *Pak Vet J* 39: 479-486.
10. Afsheen N, Rehman KU, Jahan N, Khan KM & Zia MA (2019). Optimization of cardioprotective potential of various concentrations of

- medicinal plants by using response surface methodology. *Pak Vet J* 39: 13-18.
11. Abubakar EMM (2009). Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *J Med Plants Res* 3(4): 179-185.
 12. Belguith H, Kthiri F, Chati A, Sofah AA, Hamida JB & Landoulsi (2010). Study of the effect of aqueous garlic extract (*Allium sativum*) on some *Salmonella* serovars isolates. *Emir J Food Agric* 22(3): 189-206.
 13. Suvarna Y & Rajagopalan (2015). Garlic: nature's panacea. *Asian J Pharm Clin Res* 8: 8-13.
 14. Fujisawa H, Suma K, Origuchi K, Seki T & Ariga T (2008). Thermostability of allicin determined by chemical and biological assays. *Biosci Biotechnol Biochem* 72(11): 2877-2883.
 15. Ahmed S, Rahman Z, Khalid S & Iqbal A (2017). Bacterial Contamination of Drinking Water in Tehsil Oghi, Mansehra Pakistan. *Biologia* 63: 193-198.
 16. El-Astal ZY, Ashour AERA & Kerit AAM (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. *Pak J Med Sci* 21(2): 187-193.
 17. Ankri S & Mirelman D (1999). Antimicrobial properties of allicin from garlic. *Microbes Infect* 1(2): 125-129.
 18. Ww Nabeela F, Azizullah A, Bibi R, Uzma S, Murad W, Shakir SK & Häder DP (2014). Microbial contamination of drinking water in Pakistan—a review. *Environ Sci Pollut Res* 21(24): 13929-13942.
 19. Taj MK, Samreen Z, Ling JX, Taj I, Hassan TM & Yunlin W (2014). *Escherichia coli* as a model organism. *Int J Eng Res Sci Technol* 3(2): 1-8.