



Original Article

Comparative Study of the Combined Antibacterial Effects of Grape Seed and Cranberry Fruit Extracts on Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli*Ali Nawaz Bijarani^{1*}, Ulfat Sultana², Nasima Iqbal³, Nabeela Naeem⁴, Sidra Irshad⁵ and Momina Khadija Abbasi⁶¹Department of Pharmacology, Shaheed Mohtarma Benazir Bhutto Medical College Lyari, Karachi, Pakistan²Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan³Department of Pathology, Baqai Medical University, Karachi, Pakistan⁴Department of Pathology, Watim Medical and Dental College, Rawat, Pakistan⁵Department of Pharmacology, Muhammad College of Medicine, Peshawar, Pakistan⁶Department of Pathology, Watim Medical and Dental College, Rawat, Pakistan

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*Corresponding Author:

Ali Nawaz Bijarani
Department of Pharmacology, Shaheed Mohtarma Benazir Bhutto Medical College Lyari, Karachi, Pakistan
alibijrani@gmail.com

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ABSTRACT

Antimicrobial resistance is a threat to global healthcare system. Therefore, there has been growing interest towards the natural plants which possess antimicrobial properties. **Objective:** The key goal of the current research is to find out the combined effect of cranberry fruit and grape seed extracts against Extended-Spectrum Beta-Lactamase (ESBL) *Escherichia coli* strains. **Methods:** *E.coli* bacteria with ESBL resistance were collected from the patient's samples having bacterial infection. The extracts, grape seeds and cranberry fruits extract were prepared in different concentrations. Antibiotic susceptibility testing was done with different concentrations of the extracts, using agar dilution methods to assess their antibacterial efficacy against Meropenem and Linezolid. SPSS version 21.0 was used for data analysis. **Results:** The combined effect of cranberry fruit and grape seed extracts demonstrated significant antibacterial influence in counter to decrease ESBL *E.coli* strains. There were 40 specimens, with 45% female and 55% male, with an age range between 10 to 80 years old. Both the plant extract revealed high sensitivity against ESBL *E.coli*, with 95% sensitivity at the highest concentrations. The combination of CFE and GSE (50mg+60mg) showed more than 90% sensitivity, more than commonly used antibiotics like Meropenem and Linezolid. **Conclusions:** The combined effect of cranberry fruit and grape seed extracts showed excellent effect against antimicrobial resistance, particularly in ESBL-producing *E.coli* strains. The finding suggested the increase potential of natural products as alternative antimicrobial agents.

INTRODUCTION

Antibiotics, often referred to as "magic bullets," transformed 20th-century medicine by saving millions of individuals from bacterial infections [1]. The addition of antibiotic brought a positive change in the healthcare system, saving people from life threatening consequences. This plays a vital role in contemporary medicine [2]. However, the increased and excessive and inappropriate use of the antibiotics has directed to the development of

the drug resistance. Thus, this inclination has developed the resistance of the antibiotics and also reduced the possibilities for treating bacterial infections. In the 21st century, the prevalence of antimicrobial-resistance have reached to alarming levels, leading towards a silent and profound threat to worldwide healthcare system similar to a pandemic [3]. Antimicrobial resistance is the capability of bacteria to continue to multiply regardless of the drug

that was used to eradicate them. [4]. Antibiotic resistance presents a healthcare pandemic, after the appearance of ESBL-producing strains of (*E.coli*). The increase of ESBL has increases the rates of death, longer hospital stays, and increased expenses for treatment [5]. ESBLs are enzymes found in Gram-negative bacteria from the Enterobacteria ceae family, where they are encoded on plasmids or chromosomes. These enzymes degrade β -lactam antibiotics, making them ineffective. The World Health Organization (WHO) has recognized ESBL-producing Enterobacteriaceae (ESBL-E) as dangerous infectious agents which present major therapeutic problems [6]. Penicillin, Aztreonam, and first, second, and third generation Cephalosporins are among the antibiotics to which ESBL-E strains are resistant; however, they are still susceptible to cephamycin and carbapenems [7]. Natural products have received significant interest recently as possible sources of antimicrobial compounds because of their different chemical profiles and apparent reduced tendency to cause resistance when compared to conventional antibiotics [8]. These natural sources, cranberries and grape seeds have gained quite attention because of their well-established health benefits and high polyphenol content [9].

Therefore, the purpose of this study was to compare the antibacterial efficacy of grape seed and cranberry fruit extracts to ESBL *E.coli*.

METHODS

The *in vitro* research was done at a Tertiary care hospital from February 2021 to August 2022 to investigate the antibacterial efficacy of grape seed extract and cranberry fruit extract against ESBL *E.coli*. The participant involved in this study have an age range of 15 to 70 years old, showing symptoms of microbial infections. Samples of discharge, urine, body fluid, and tracheal aspirates were obtained for analysis at Clinical Microbiology Laboratory. Both were purchased from the marketplace in Karachi. The authentication of the grape seeds was conducted by the Botanical Department, GSE and CFE were extracted at the Department of Pharmacognosy, University of Karachi, and stored at room temperature for experiment. Patient of all ages and sample of blood, pus or urine in which ESBL *E.coli* were included whereas sample showing double growth or contamination on agar plates were excluded. Fresh grapes and cranberries purchased from Karachi markets underwent similar extraction processes. The grapes were crushed to separate the seeds, which were then carefully rinsed with fresh water and consequently dried-out at 60°C in an oven while cranberries were kept at 35°C in oven. The dried fruits were then powdered using an electric grinder. 20-gram powder of the grape seed and cranberry fruit extract was mixed with 100 ml of ethanol in a conical

flask separately. The mixtures were agitated for 48 hours; for grape seed extract, this was done by stirring, while for cranberry fruit extract, a rotary shaker was used. After the incubation period, the mixtures were filtered using Whatman filter paper (Whatman no. 1) to remove solid particles. The filtrates were then concentrated by evaporating the ethanol at 50°C in an oven. Finally, the concentrated extracts were mixed with 25% dimethyl sulfoxide (DMSO) to prepare solutions with concentrations ranging from 20 to 60 mg/ml. These solutions were stored in sealed vessels at 4°C for future analysis and use. Patient samples cultured on Mueller-Hinton agar plates were used to identify microorganisms using standard laboratory methods. Agar dilution techniques assessed the antibacterial effectiveness of GSE + CFE against Extended-Spectrum Beta-Lactamase-producing *Escherichia coli* (ESBL *E. coli*) isolated from patient specimens (urine, blood, pus). Test bacteria cultures grown in Nutrient broth for 24 hours were spread evenly on sterile Nutrient agar plates. Wells (8 mm diameter) created with a sterile cork borer in inoculated plates contained GSE (20 mg/ml in 25% Dimethyl sulfoxide), CFE (20 mg/ml in 25% Dimethyl sulfoxide), and positive controls Linezolid (10 μ g) and Meropenem (30 μ g) and plates were incubated for 24-48 hours at 37°C. Zones of inhibition was measured using a ruler to assess bacterial growth inhibition by extracts and antibiotics. The cranberry fruit and grape seed extracts were combined in equal ratios to maintain the desired concentrations. The concentrated extracts were diluted with final concentrations of 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, and 60 mg/ml. The susceptibility testing was performed using combined extract of both fruits against *E. coli* taken from patient samples. Agar dilution techniques were used for testing, where the bacteria cultured in nutrient broth were uniformly spread onto agar plates. Wells were prepared in the agar, and into these wells, various concentrations of GSE and CFE extracts were added, along with standard antibiotics Linezolid and Meropenem for comparison. Following incubation, the plates were examined to measure the zones of inhibition around the wells, indicating the extent to which the extracts and antibiotics inhibited bacterial growth. Data investigation was conducted using SPSS version 21.0. Frequencies and Percentages were calculated for categorical variables such as gender distribution (women vs. men). Mean and Standard Deviation were reported for continuous variables like participant age. Chi-square test was applied to determine whether there were significant differences in susceptibility rates between the combined CFE + GSE extract and the conventional antibiotics (Meropenem and Linezolid) against ESBL-producing *E. coli*. ($p < 0.05$).

RESULTS

The demographics of the studied partakers are brief in Table 1. Total 40 specimens were processed in the investigation. Amongst the partakers, there were 18 women, comprising 45% of the total, and 22 men, constituting 55% of the total cohort. The age range of the participants varied from 10 to 80 years. The average age of the partaker was 40.34 ± 11.45 years.

Table 1: Profile of the Investigation Participants

Variables	N (%) / Mean \pm SD
Samples Analyzed	40
Gender	
Women	18 (45%)
Men	22 (55%)
Age	
Age Distribution (Years)	10 to 80
Age	40.34 ± 11.45 Years

Table 2 showed the sensitivity of *Escherichia coli* to the extract across various concentrations. At the highest concentrations of 60 mg/ml, both CFE and GSE demonstrated 95% sensitivity. As the concentration levels decreased to 50mg/ml, sensitivity remained notably high, with 82.5% sensitivity observed at 40 mg/ml and 87.5% at 30 mg/ml. At the minimal dose tried, 20 mg/ml, a considerable sensitivity of 80% was still observed

Table 2: Evaluation of Efficacy of Cranberry Fruit and Grape Seed Extracts against ESBL *Escherichia coli*

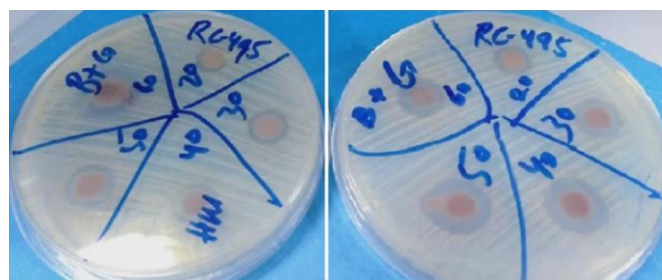
<i>Escherichia coli</i>	Cranberry Fruit and Grapes Seeds Extract Dose N (%)				
	20 mg /mL	30 mg /mL	40 mg /mL	50 mg /mL	60 mg /mL
Resistance	5 (12.5%)	4 (10%)	4 (10%)	3 (17.5%)	2 (5%)
Sensitivity	35 (87.5%)	36 (90%)	36 (90%)	37 (92.5%)	38 (95%)
Total	40 (100%)	40 (100%)	40 (100%)	40 (100%)	40 (100%)

At a concentration of 60 mg/ml, significant efficacy was observed, demonstrating a 95% sensitivity in inhibiting ESBL-producing *E.Coli*. Comparing the sensitivity rates, the combination of CFE + GSE 90% sensitivity was compared that exhibited a sensitivity of Meropenem at 75% (p-value <0.05) and Linezolid on the other hand, demonstrated a sensitivity of 0% (p-value = <0.05), as indicated in table 3.

Table 3: Assessment of Efficacy: CFE+GSE Blend versus Conventional Antibiotics against ESBL Producing *Escherichia coli*

Bacteria	Combined Extract	Antibiotic	Susceptibility Rate (%)	p-value
Extended-Spectrum Beta-Lactamase <i>E.Coli</i>	CFE and GSE (60mg/mL)	Meropenem	75%	0.032
	CFE and GSE (60mg/mL)	Linezolid	0%	0.001

The figure 1 illustrated that higher concentrations of combined extracts generally result in greater antibacterial activity, as indicated by larger inhibition zones or lower bacterial counts of ESBL producing *E.Coli*.



(a) MH Ager plate

(b) MH Ager plate

Figure 1: Antibacterial Activities of Different Concentration of Cranberry Fruit and Grapes Seed Extract against Pathogenic Bacteria

DISCUSSION

The rising incidence of anti-microbial resistance, coupled with recurring outbursts and worldwide epidemics, underscores the urgent necessity for ongoing investigation [10]. As concerns about antibiotic resistance continue to mount, there has been a surge in efforts to investigate natural alternatives to antibiotics. Cranberry fruit and grape seeds, commonly utilized in herbal drug and nutritional aid, have bring in attention for their possible anti-microbial, antioxidant, and health-enhancing properties [11, 12]. The current study used a combination of grape seed and cranberry fruit extracts to kill ESBL-producing *Escherichia coli* to validate their antibacterial properties. The study mentioned the positive antibacterial effect against *E.Coli* strains. The results obtained are consistent with previous research that demonstrated the antibacterial efficacy of both cranberry and grape seed extracts individually against a range of pathogens. [13, 14]. Grape seed extract has also earlier been recognized for its antibacterial activity against many pathogens, including ESBL-producing organisms [15]. Likewise, cranberry fruit extract has also shown antibacterial effects, particularly against *Escherichia coli* strains [16]. Therefore, it is predicted that the combination of these two extracts likely stated on their complementary mechanisms of action, subsequent in greater antibacterial efficacy. The increase in the dosage activity observed specifically against *E.coli* strains in our study mentioned the important role of concentration in determining the efficacy of the combination of cranberry and grape seed extract. The steady increase in Zone of Inhibition (ZOI) with increase concentrations of the extract proposes a concentration-response relationship, where higher concentrations lead to greater antimicrobial activity [12, 17]. Kandasamy M et al., conducted a study revealing that grape seed extract displayed bactericidal properties, yielding moderate zones of inhibition against common clinical isolates. Furthermore, it exhibited efficacy against drug-resistant strains, with inhibition zones ranging from 2 to 4 mm at extract concentrations between 2 mg/ml to 20 mg/ml. Notably, the extract demonstrated a more potent

bactericidal effect against *Escherichia coli* in comparison to other selected gram +ve and gram -ve bacteria [11]. Several investigations have suggested that Grape Seed Extract (GSE) harbors the most substantial levels of antioxidant and antimicrobial elements, including polyphenols [14, 18]. The finding of a study on cranberry fruit extract found that active compound successfully reduced the antibiotic resistance of the ESBL-producing *E.Coli* strain. The strain exhibited resistance to multiple drugs [19]. The other study similarly mentioned that cranberry juice decreased *E.Coli* colonization in the experimental mice in the bladder reducing urinary tract infection, with organic acids determined as the active agents. This demonstrated the possible benefit of cranberry products, especially their organic acid content, in avoiding and handling urinary tract infections caused by *E.Coli* [20]. As per the data researched and our knowledge, this is the first study to explore the combined effect of cranberry and grape seed extract against ESBL-producing *Escherichia coli*. There are, however, different studies that mentioned the synergistic potential of natural extracts against antibiotic-resistant bacteria [21, 22].

CONCLUSIONS

It was concluded that combination of grape seed and cranberry fruit extract showed promising results against ESBL-producing *Escherichia coli*, mentioning it a possible alternative against antibiotic resistance. The dosage adjustment emphasizes on the better efficacy and long term effectiveness. It also highlighted the growing synergistic interaction against antimicrobial resistance but also serve as safer option with minimal or no adverse effects.

Authors Contribution

Conceptualization: ANB

Methodology: ANB

Formal analysis: US

Writing, review and editing: US, NI, NN, SI, MKA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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