Detection of Antimicrobial Activity of Cranberry Fruit Extract against Methicillin Resistant Staphylococcus aureus and Extended Spectrum Beta Lactamase Escherichia coli

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors MOI and ANB were involved in conception of idea and study design. Author ANB did the data collection and performed bench work. Author FA supervise the microbiology part of the project. Authors MOI, ANB and ZM wrote the protocol of procedures and finalized the manuscript. Author AA performed the statistical analysis. Authors ANB and SQ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Antibacterial effects of Cranberry fruit extract on Methicillin Resistant Staphylococcus aureus and Extended Spectrum beta lactamase producing E. coli and its comparison with Linezolid and Meropenem.

Materials and Methods: It is a Pre Clinical (in-vitro) study conducted in Ziauddin University from January 2020 to October 2020. All samples were collected from Ziauddin University. All clinical

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1. INTRODUCTION

Bacteria and viruses stand first and most prevalent as the infectious causative agents making pneumonia and influenza the most common cause of death. The discovery of antibiotics in 1928 by Sir Alexander Fleming and then its commercial availability in 1945 provided a sigh of relief from the high mortality throughout the 20th century [1]. The total expectancy of life before antibiotic discovery is reported as forty years by some studies [1-2]. Antibiotics are used in almost all the medical facilities e.g. in surgery as prophylactic, intra-operative and postoperative shield for expected infections and seem to be one of the pillar in the uncomplicated outcome of surgery [3]. On the other hand misuse of antibiotics is also there due to prolonged, unnecessary and wrong usage giving rise to antimicrobial resistance (AMR) [4]. Lord O’Neill estimated in 2016 that by 2050 antimicrobial resistance will cause 10 million deaths and cost £66 trillion [5]. Keeping this aspect of antibiotics in mind the natural sources of medication has made their place to avoid the antimicrobial resistance and make the procedures successful according to some studies cranberry extract can alone be used rather than in combination with any other antibiotic [5-6]. Broadly the resistance being divided into acquired and intrinsic is gained by spontaneous gene mutations and by gene acquisition through horizontal transfer of gene and innate ability of resist against the antibiotic respectively [6]. Example of acquired resistance is Staphylococcus aureus which can mutate and become methicillin resistant Staphylococcus aureus (MRSA) [7]. An example of an intrinsically resistant bacteria is Pseudomonas aeruginosa. (P. aeruginosa) which has a high intrinsic resistance towards various classes of antibiotics such as aminoglycosides, quinolones and β-lactams [8-9]. Methicillin Resistant Staphylococcus aureus and Extended Spectrum beta lactamase producing E. coli are the two most common and most resistance developing strains. Staphylococcus aureus (S. aureus) is a Gram-positive cocci with high virulence causing multiple infections at the same time harmlessly colonizing 30% of human population [10]. In a study Fuches and colleagues (2018) reported that may be the use of certain old age plant based extracted medicinal can prove to be effective and can play a role for AMR [11]. Recently reported by Bazzaz and colleagues, the combination of verbascoside along with gentamicin against resistant Staph. aureus and E. coli proved to be effective [12]. The Antibiotic resistance breaker (ARB’s) is one possible technique to encounter AMR, in which the natural extract is used in combination of the resistant

Results: Out of 80 samples included in this study 46(57.5%) were female and 34(42.5%) samples were male; female to male samples ratio of 1.35:1. The mean age was 45.71±11.83 years. MRSA commonly found in pus swab 15(37.5%) and 21(52.5%) ESBL producing E. coli found in urine samples. 14(35%) samples were observed anti-bacterial activity of cranberry fruit extracts against Extended Spectrum Beta Lactamase Producing Escherichia coli at 50 mg/ml concentration, followed by 10(25%) and 9(22.5%) samples at 60 mg/ml, 40 mg/ml were respectively. While resistance of Extended Spectrum Beta Lactamase Producing Escherichia coli were observed high at different concentration level of cranberry fruit extracts. Good anti-bacterial activity of cranberry fruit extracts observed against Methicillin Resistant Staphylococcus aureus at different levels of concentration 20(50%), 23(57.5%), 21(52.5%), 26(65%), 29(72.5%) samples were 20 mg/ml, 30mg/ml, 40mg/ml, 50mg/ml, 60mg/ml respectively. Most superior and best dose of cranberry fruit extract against Staph Aureus in about 72.5% (29) at 60mg/ml and their Comparison with linezolid and meropenem against Methicillin Resistant Staphylococcus aureus and found best positive results as compared with Linezolid and found significant p value 0.005.

Conclusions: Cranberry extract has a lot of potential to prove itself to be a good antimicrobial agent. The cranberry fruit extract has high antimicrobial activity against methicillin resistant S.aureus and resistant strains of E. coli in comparison to linezolid and meropenem.

Keywords: Cranberry fruit extracts; methicillin resistant Staphylococcus aureus; extended spectrum beta lactamase producing E. coli.
antibiotic to increase the effectiveness of the therapy [13]. ARB's can be used alone or in combination of the respective drug e.g clavulanic acid which is often combined with amoxicillin and is a β-lactamase inhibitor. In this study we used cranberry fruit extract as ABR.

2. MATERIALS AND METHODS

It is a Pre Clinical (in-vitro) study conducted in Ziauddin University from January 2020 to October 2020. All samples were collected from Ziauddin University. Patients age were 10 to 80 years, both genders, showing signs and symptoms of bacterial infections were selected. All clinical samples were collected inform of pus, urine, blood, tracheal aspirations, patients admitted in surgical and medical wards, intensive care units and outdoor patients who were attending clinics. All these samples were transported to Clinical Microbiology Laboratory, Ziauddin hospital, North Nazimabad campus and then culture and sensitivity test were performed there. Sample showing double growth and contamination on agar plates were excluded from study.

2.1 Herbal Extraction and Authentication

Cranberry fruit was purchased from commercial market, Karachi and stored at room temperature. Authentication were done from Botany department, Karachi University. Extraction procedure were done at Pharmacognosy department, ZU Karachi. The final preparation was stored at normal room temperature and used for experimental work.

2.2 Preparation of Cranberry Fruit Extract (CFE)

Fresh cranberry fruits was purchased from commercial market, Karachi. Fruits were washed under tap water and dried in the oven at 35°C. The dried fruits were crushed to a fine powder by an electrical grinder. 20 g of dried fruit was placed in 100ml of ethanol in a conical flask, and then kept on a rotary shaker for 48 hours. After 48 hours, then filtered and centrifuged at 4500 rpm for 15 min. The content of flask was filtered through what man and evaporated to dryness in oven at 50°C. After the cranberry fruit powder had been obtained, different concentrations of cranberry fruit extract (20, 30, 40, 50 and 60 mg/ml) were prepared by mixing the cranberry fruit powder with dimethyl sulfoxide (DMSO) and stored at 4°C in air tight bottles [14].

2.3 Isolation of Bacteria from Clinical Specimens

The specimens were processed in culture plates and the pathogens were isolated and identified by standard biochemical tests. Mueller-Hinton agar [15] was used Agar dilution methods from low dose to high dose (20, 30, 40, 50, 60 mg) to determine the antibacterial effect of cranberry fruit extract and the routinely used antibiotics, Linezolid for MRSA strains and Meropenem for ESBL producing E. coli organisms were used for comparison [16].

2.4 Antibacterial Activity of CFE

Agar dilution methods were performed to investigate antibacterial activities of Cranberry fruit extract against MRSA and ESBL producing E. coli from specimen (urine, blood, pus, skin/oral/vaginal swabs, sputum / respiratory fluids, pleural and peritoneal secretions) of patients. 24 hours old Nutrient broth cultures of test bacteria was swabbed uniformly on sterile Nutrient agar plates. Using sterile cork borer, wells of 8mm diameter will be punched in the inoculated plates. Cranberry Fruit Extract (20mg/ml of 25% Dimethyl sulfoxide), Linezolid (10ug) and Meropenem (30ug) were added to labeled wells and the plates were incubated for 24 hours at 37°C. The zones of inhibition around the wells were measured using a ruler [17]. Zone diameter was measured and interpretation was done as per CLSI guidelines 2018.

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 21. Frequencies and percentages were calculated for categorical data like sensitivity and resistance. Chi-square tests were used to compare sensitivity between groups. P-value of less than 0.05 were considered as significant.

3. RESULTS

Total 80 samples were collected. 40 samples were Methicillin Resistant Staph Aureus (MRSA) and 40 samples were Extended Spectrum Beta Lactamase Producing Escherichia Coli (ESBL producing E. coli). The 80 samples of specimens were processed in culture plates and the pathogens were isolated and identified by standard biochemical tests. Out of 80 samples included in this study 46(57.5%) were female and 34(42.5%) samples were male; female to male samples ratio of 1.35:1. There was wide variation of age ranging from a minimum of 10 year to 79 years. The mean age was 45.71±11.83 years Table 2. In our study MRSA commonly found in
pus swab 15(37.5%) and 21(52.5%) ESBL E Coli found in urine samples Table 2. Most Commonly MRSA 13(32.5%) was found in surgical department, while ESBL producing E. coli 12(30%) were found in medical ward admitted patients Table 2.

In our study observed meropenem 100% sensitive against Extended Spectrum Beta Lactamase producing E. coli sample as compare to Methicillin Resistant Staphylococcus aureus 5(15%) samples. While resistance were observed in Methicillin Resistant Staphylococcus aureus 34 (85% samples), while Linezolid 37(92.5%) were sensitive in Methicillin Resistant Staphylococcus aureus. While resistance were observed in Extended Spectrum Beta Lactamase producing E. coli 40(100%) samples Chart 1.

In our study observed 14(35%) samples were observed anti-bacterial activity of cranberry fruit extracts against Extended Spectrum Beta Lactamase producing E. coli at 50 mg/ml concentration, followed by 10(25%) and 9(22.5%) samples at 60 mg/ml, 40 mg/ml were respectively. While resistance of Extended Spectrum Beta Lactamase producing E. coli were observed high at different concentration level of cranberry fruit extracts Chart 2. In our study good anti-bacterial activity of cranberry fruit extracts observed against Methicillin Resistant Staphylococcus aureus at different levels of concentration 20(50%), 23(57.5%), 21(52.5%), 26(65%), 29(72.5%) samples were 20 mg/ml,30mg/ml,40mg/ml, 50mg/ml, 60mg/ml respectively. While less resistance were observed of Methicillin Resistant Staphylococcus aureus at different concentration level of cranberry fruit extracts Chart. 3.

Best concentration of Cranberry fruit extract 14(35%) at 50mg/ml were observed anti-bacterial activity of against Extended Spectrum Beta Lactamase producing E. coli and their comparison with meropenem and linezolid, While high resistance of cranberry fruit extract were

| Table 1. Zone of inhibition found in different study |
|----------------|----------------|----------------|
| Discs          | Resistance     | Sensitive      |
| Linezolid (10μg) | ≤16 mm         | ≥21 mm         |
| Meropenem (30μg) | ≤15 mm         | ≥19 mm         |
| Plant extract   |                |                |
| Cranberry fruit extract | 12.1 ± 0.9 to 24.2 ±1.7 | Ibrahim et al., 2015 |

| Table 2. Variable |
|----------------|----------------|
| Variable        | MRSA           |
|                 | ESBL producing E. coli |
| Gender          |                |
| Male            | 21(52.5%)      |
| Female          | 19(47.5%)      |
| Age in years    |                |
| 10-30 years     | 7(17.5%)       |
| 31-50 years     | 11(27.5%)      |
| 51-70 years     | 16(40%)        |
| >71 years       | 6(15%)         |
| Samples Sources |                |
| Blood           | 9(22.5%)       |
| Pus             | 15(37.5%)      |
| Tracheal Asp    | 11(27.5%)      |
| Urine           | 5(12.5%)       |
| Department      |                |
| Surgical ward   | 13(32.5%)      |
| ICU/CCU         | 11(27.5%)      |
| Medical Ward    | 6(15%)         |
| Gynae/Obs       | 5(12.5%)       |
| Outpatients Department | 5(12.5%) |
observed against linezolid. Most superior and best dose of cranberry fruit extract against Methicillin Resistant Staph Aureus was about 72.5% (29) at 60mg/ml and their comparison with linezolid and meropenem and found best positive results as compared with Linezolid and found significant p value 0.005 Chart. 4.

4. DISCUSSION

Emergence antimicrobial resistance frequent emergence of epidemics and pandemics has raised the need for further research process in this regard. β-lactam resistant Enterobacteriaceae and Staphylococci has already negatively affected β-lactam-based therapy, so it is assumed that in near future a complete resistance to most effective β-lactam agents is going to surface [18-20]. In our study, we have tried to explore the effect of a natural derivative, cranberry fruit extract as it has adjuvant properties of interfering the resistance and can also enhance the efficacy of β-lactam agents. The main objective of this study was that wither the natural extracts can be used as ARB’S alone and its comparison with linezolid and meropenem against beta lactam resistant E. coli and MRSA.

Chart 1. Antibiotic susceptibility of MRSA and ESBL producing E. coli

Chart 2. Sensitivity of antibacterial activity of CFE against ESBL producing E. coli
In this study we extracted the cranberry fruit extract by standard method of extraction and used it in 80 patients with high female to male ratio as 1.35:1 which is almost similar to various previous studies [21-22]. The peak age of infection found in our study was 45.71±11.83 years from the range of 11-80 years as also reported by Dale, A., Pandey at al. [23]. Beytur, A., Yakupogullari at al. reported the more MRSA isolation from pus and wound samples while ESBL producing E. coli from the urine samples which is also supported by our study as MRSA most commonly found in pus swab as 15(37.5%) and 21(52.5%) respectively while ESBL E. coli found in urine samples [12]. Moreover MRSA 13(32.5%) was more prevalent in surgical patients, while ESBL producing E. coli (1230%) was found in medical patients mostly [12,23-24].

Meropenem is 100% sensitive in ESBL producing E. coli and more resistant to Methicillin Resistant Staphylococcus aureus 34(85% samples), while Linezolid 37(92.5%) were sensitive in Methicillin Resistant Staphylococcus aureus and more resistant to ESBL producing E. coli 40(100%) according to our study. The similar pattern of results were obtained by Oliva, A., Costantini at al. in their study [25].
The concentration of the natural product extract (cranberry extract) also matters a lot as according to this study 14(35%) samples were observed anti-bacterial activity of cranberry fruit extracts against ESBL producing E. coli at 50 mg/ml concentration, followed by 10(25%) and 9(22.5%) at 60 mg/ml, 40 mg/ml respectively and resistance of ESBL producing E. coli were observed high at different concentration levels. While good anti-bacterial activity of cranberry fruit extracts was elicited against Methicillin Resistant Staphylococcus aureus at different levels of concentration as 20(50%), 23(57.5%), 21(52.5%), 26(65%), 29(72.5%) in 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml respectively and comparatively less resistance were observed of Methicillin Resistant Staphylococcus aureus for different levels of concentrations [26-28].

According to this current study the maximum effective concentration of Cranberry fruit extract 14(35%) is at 50mg/ml showing its anti-bacterial activity against ESBL producing E. coli and its comparison with meropenem and linezolid. On the other hand high resistance of cranberry fruit extract were observed against linezolid, which is totally in contrary to some previous studies [28-30]. However most effective and best dosage of cranberry fruit extract against Methicillin Resistant Staph Aureus in almost 72.5% [27] is at 60mg/ml and is in Comparison with linezolid and meropenem against methicillin resistant staph aureus and also showing best positive results as compared with Linezolid which is somewhat in accordance with WHO 2008 [25].

The AMR is considered to be a potential cause of frequent outbreaks of epidemics and possibly pandemics and the further evolution in the field of antimicrobial agents is a necessity of time [27]. AMR and expensive antibiotics are becoming a significant economic burden [29-31]. The antibiotics should be prescribed and used according to the strict guidelines available [32]. The natural extracts (like cranberry) instead of expensive and resistance potential bearing drugs called antibiotics should be used and made common among the treatment of choice [33]. In our study we just studied the small number of natural products specifically cranberry fruit extract for sensitive antimicrobial activity and resistance in MRSA and resistant ESBL producing E. coli and we strongly and firmly endorse that the sole usage of cranberry extract instead of antibiotics but it further needs to be explored through more researches for further possibilities and prevention of the world to enter the pre antibiotic era [33-34].

5. CONCLUSION

It is to be concluded that the cranberry extract has a lot of potential to prove itself to be a good antimicrobial agent. The cranberry fruit extract has high antimicrobial activity against methicillin resistant Staph. aureus and resistant strains of ESBL producing E. coli in comparison to linezolid and meropenem. The cranberry fruit extract can be used alone as antibacterial agent against MRSA and resistant ESBL producing E. coli.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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