Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3473

EXPLORING THE SYNERGISTIC ANTIBACTERIAL POTENTIAL OF GRAPE SEED AND CRANBERRY FRUIT EXTRACT COMBINATION AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: The investigation is designed to assess the combination of grape seed and cranberry extracts against MRSA through a synergistic effect.

Methodology: This in vitro study used clinical MRSA isolates for analysis. Grape seed and cranberry extracts were prepared at different concentrations, and their antibacterial capabilities were assessed using agar dilution techniques. The resulting zones of inhibition were measured for analysis.

Results: The study revealed that both Cranberry Fruit Extract (CFE) and Grape Seed Extract (GSE) demonstrated significant antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) at various concentrations, even at the lowest concentration of 20mg/ml it showed a considerable 80% sensitivity rate and minimal resistance. The combination of CFE and GSE at different concentrations demonstrated outstanding antibacterial efficacy against MRSA, reaching 100% sensitivity at the highest concentration, surpassing common antibiotics like Meropenem. This combination exhibits great potential for clinical use in the fight against MRSA infections.

Conclusion: As antibiotic resistance continues its relentless ascent, the exploration of natural alternatives like grape seed and cranberry extracts becomes increasingly critical. These extracts not only demonstrate individual promise in combating MRSA but also exhibit the enticing potential for a synergistic effect when deployed together. Amid the pressing global concern over antibiotic

resistance, these natural alternatives emerge as promising weapons in our arsenal against bacterial infections.

Keywords: Grape seed extract, Cranberry fruit extract, Methicillin resistant *Staphylococcus aureus*, Antibiotic resistance, Meropenem, Linezolid.

INTRODUCTION:

Antibiotic resistance has been documented as a growing global health challenge, defined by bacteria and other microorganisms' ability to grow and develop resistance to drugs intended to kill or inhibit their growth (1). The occurrence showed decline in antibiotics effectiveness, and in some cases, completely ineffective, making it progressively challenging to treat bacterial infections (2).

The growing incidence of antibiotic-resistant strains, specifically Methicillin-Resistant *Staphylococcus aureus* (MRSA), offers a significant problem for modern healthcare systems(3). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen accountable for health care related infections, with an alarming rise in the prevalence of multidrug-resistant pathogens posturing a ample public health threat (4). According to the U.S. Centers for Disease Control and Prevention (CDC) report in 2007, MRSA caused 94,360 severe infections with 18,650 deaths in the United States in 2005 (5). The existing management options for MRSA infections are limited as MRSA strains displayed resistance to commonly prescribed antibiotics, including lactams, macrolides, aminoglycosides, and fluoroquinolones (6). Vancomycin, other glycopeptide and oxazolidinones antibiotics are currently employed to manage MRSA infections. Nevertheless, glycopeptides often lead to side effects, and instances of vancomycin-resistant S. aureus infections have been documented (7, 8).

There are multiple investigates that have highlighted the antimicrobial potential of different plant extracts, with the focus on the effectiveness of plants containing polyphenols against MRSA strains (9-11). Grape seed extract contain polyphenols, particularly proanthocyanidins, which are well-known for their antioxidative and antibacterial attributes (12). Contrariwise, cranberry fruit extract contains distinctive compounds, including proanthocyanidins and flavonoids, which have been experiential to exert antimicrobial effects against multiple pathogens (13). These two extracts have each independently displayed antibacterial effectiveness, prompting the investigation of their combined potential.

Particularly, a recent study conducted by Al-Habib et al. in 2010 revealed that at a concentration of 3 mg/mL, grape seed extract (GSE) was capable of fully inhibiting the growth of all 43 tested MRSA strains by causing disruption to the bacterial cell wall (14).

This study aims to explore the synergistic antibacterial potential of a grape seed and cranberry fruit extract combination against MRSA. Through comprehensive analyses, we seek to contribute valuable insights to the growing body of knowledge in the realm of natural antimicrobial agents, paving the way for innovative approaches to combat antibiotic resistance.

MATERIALS AND METHODS:

This in-vitro study, conducted at Ziauddin University between January 2020 and October 2020, aimed to investigate the antibacterial potential of Grape Seed Extract (GSE) and Cranberry Fruit Extract (CFE) against clinical isolates, including MRSA and ESBL E-coli. Patients aged 10 to 80, with signs of bacterial infections, provided clinical samples such as pus, urine, blood, and tracheal aspirates. These samples were processed in the Clinical Microbiology Laboratory at Ziauddin Hospital.

Grape seeds and cranberry fruits were sourced from a commercial market in Karachi. The grape seeds were authenticated by the Botany department at Karachi University. Extraction of GSE and CFE was carried out at Ziauddin University's Pharmacognosy department. Extracts were stored at room temperature for subsequent experiments.

Grape Seed Extract Preparation:

- Fully ripe grapes were obtained from a commercial market in Karachi.
- The grapes were crushed, and the seeds were separated.
- The seeds underwent a thorough washing with clean water and were subsequently dried in an oven at 60°C.
- The dried seeds were finely ground into a powder using a grinder.
- 20 grams of this grape seed powder were placed into a conical flask containing 100 ml of ethanol and stirred to ensure thorough mixing.
- The flask was left undisturbed for 48 hours, with periodic stirring.
- The content of the flask was filtered through Whatman filter paper and then evaporated to dryness in an oven at 50°C.
- Different concentrations of grape seed extract (ranging from 20 to 60 mg/ml) were prepared by blending the grape seed powder with dimethyl sulfoxide (DMSO), and these extracts were stored in airtight containers at 4°C for future research.

Cranberry Fruit Extract Preparation:

- Fresh cranberry fruits were purchased from a commercial market in Karachi.
- The fruits were washed under tap water and dried in an oven at 35°C.
- The dried fruits were ground into a fine powder using an electrical grinder.
- 20 grams of dried cranberry fruit powder were placed in 100 ml of ethanol in a conical flask, which was then kept on a rotary shaker for 48 hours.
- After 48 hours, the mixture was filtered and centrifuged at 4500 rpm for 15 minutes.
- The content of the flask was filtered through Whatman filter paper and evaporated to dryness in an oven at 50°C.
- 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 in airtight bottles at 4°C for further experiments.

Bacterial Isolation from Clinical Specimens:

- Clinical specimens were cultured on Mueller-Hinton agar plates.
- Pathogens were isolated and identified using standard biochemical tests.
- For antibiotic susceptibility testing, both grape seed extract and cranberry fruit extract were prepared at concentrations ranging from 20 to 60 mg/ml.

Antibacterial Activity Assessment:

- Agar dilution methods were employed to assess the antibacterial properties of Grape Seed Extract (GSE) and Cranberry Fruit Extract (CFE) against MRSA strains isolated from patient specimens (urine, blood, pus).
- Nutrient broth cultures of the test bacteria (24 hours old) were evenly spread on sterile Nutrient agar plates.
- Using a sterile cork borer, wells with an 8mm diameter were created in the inoculated plates.
- Grapes Seed Extract (20mg/ml in 25% Dimethyl sulfoxide), Cranberry Fruit Extract (20mg/ml in 25% Dimethyl sulfoxide), Linezolid (10ug), and Meropenem (30
- ug) were added to labeled wells.
- The plates were then incubated at 37°C for 24 to 48 hours.
- The zones of inhibition around the wells were measured using a ruler

Statistical Analysis: Data analysis utilized SPSS version 21, involving frequency and percentage calculations for categorical data like sensitivity and resistance. Chi-square tests assessed sensitivity differences among groups, with significance indicated by a p-value < 0.05.

RESULTS:

In this study, 40 specimens underwent processing on culture plates, and pathogens were subsequently identified through standard biochemical tests. Of the study participants, 19 (47.5%) were female, while 21 (52.5%) were male.

The age range of the participants exhibited significant variation, spanning from a minimum of 10 years to a maximum of 79 years, with an average age of 42.32±13.83 years.

Table 1: Demographics of the Studied Sample

PARAMETER	VALUE
Specimens Processed	40
Female Participants (n / %)	19 (47.5%)
Male Participants (n / %)	21 (52.5%)
Age Range (Years)	10 to 79
Average Age (Mean ± S.D)	42.32 ± 13.83 years

Table 2 showed that at the highest concentrations of 50mg/ml and 60mg/ml, both CFE and GSE attained 100% sensitivity, inhibiting the growth of *Staphylococcus aureus* with no resistant strain. As the concentration level is reduced, sensitivity remained higher, with 92.5% sensitivity at 40mg/ml and 87.5% at 30mg/ml. Notably, even at the lowest concentration of 20mg/ml, there was still a substantial sensitivity rate of 80%.

Table 2: Screening of Antibacterial Activity of Cranberry fruit and Grapes seeds extract against *Staphylococcus aureus*

Staphycoccus Aureus	Cranberry fruit and Grapes seeds extract concentration				
	20mg/ml	**30mg/ml**	**40mg/ml**	**50mg/ml**	**60mg/ml**
Sensitive	32 (80%)	35 (87.5%)	37 (92.5%)	40 (100%)	40 (100%)
Resistance	8 (20%)	5(12.5%)	3 (7.5%)	0 (0%)	0 (0%)
Total	40 (100%)	40 (100%)	40 (100%)	40 (100%)	40 (100%)

The combination of Cranberry Fruit Extract (CFE) and Grape Seed Extract (GSE) at a concentration of 50mg CFE and 60mg GSE (50mg+60mg) presented antibacterial efficacy, attaining 100% sensitivity in inhibiting Methicillin-Resistant *Staphylococcus aureus* (MRSA) growth.

The combination of CFE + GSE as compared to Meropenem showed a lower sensitivity rate of 16% (p-value <0.05) whereas Linezolid, showed a sensitivity rate of 92.5% (p-value = 0.415) as mentioned in Table 3.

Table 3: Antibacterial Activity Comparison of CFE+GSE Combination with Common Antibiotics Against MRSA

Agent	Combination of Plant Extract	Antibiotic	Sensitivity Rate	p-value
Methicillin-Resistant *Staphylococcus aureus*	CFE+GSE (50mg+60mg)	Meropenem	16%	<0.05
	CFE+GSE (50mg+60mg)	Linezolid	92.5%	0.415

DISCUSSION:

The increasing prevalence of antimicrobial resistance, together with the recurrent outbreaks and global epidemics, highlights the dire need for incessant research (15). With the escalating concern of antibiotic resistance, the exploration for natural alternatives to synthetic antibiotics has increased momentum. Cranberry fruit and Grape seeds, widely used in herbal medicine and dietary supplements, are being explored for their potential antimicrobial, antioxidant, and health-promoting properties (16,

17). It's notable that, at present, MRSA strains hold susceptibility to vancomycin, daptomycin, and linezolid, which are presently considered the ideal antimicrobial agents in clinical therapy (15).
Multiple research studies have established that grape seed extract is efficacious in reducing bacteria, including MRSA and other pathogens, and it is capable of generating inhibition zones (14, 18, 19). Additionally, research on cranberry fruit extract has unveiled antibacterial activity against MRSA, showing promising results (19-21).
According to Xiaowei et al., Grape seed extract (GSE) verified rapid antibacterial activity, considerably decreasing MRSA titers within just 2 hours at 37°C. Additionally, it also confirmed the beneficial antibacterial effects against cranberry (19). In present study, we witnessed that the combination of CBE and GSE showed antibacterial activity across a range of concentrations (20mg/ml, 30mg/ml, 40mg/ml, 50mg/ml, and 60mg/ml), with higher concentrations evidencing more effective. It's important to highlight that prior research on different pathogens has indicated that the combination of natural extracts can lead to a synergistic effect, enhancing their effectiveness beyond that of each individual extract (22, 23).
As the global concern over antibiotic resistance continues to rise, investigating natural alternatives like grape seed and cranberry extracts is a critical step toward addressing this public health challenge and expanding our antimicrobial arsenal.

CONCLUSION:

In conclusion, investigating the combined antibacterial potential of grape seed and cranberry fruit extracts against Methicillin-Resistant *Staphylococcus aureus* (MRSA) offers substantial potential. Research has shown that both grape seed and cranberry extracts exhibit antibacterial properties individually, with grape seed extract displaying rapid antibacterial activity. Moreover, combining these extracts may provide a synergistic effect, potentially enhancing their effectiveness in combating MRSA.
While these findings are encouraging, it's essential to consider safety, dosage, and potential resistance development. Further research is needed to explore the full clinical applications of this combination, including potential integration with conventional antibiotics for more effective MRSA treatment.

Conflict of Interest: There are no conflicts of interest to be disclosed by any of the authors in connection with this study

REFERENCES:

1. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MT, Chinedu Ikem J, Victor Chigozie U, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. Journal of clinical laboratory analysis. 2022;36(9):e24655.
2. Cella E, Giovanetti M, Benedetti F, Scarpa F, Johnston C, Borsetti A, et al. Joining Forces against Antibiotic Resistance: The One Health Solution. Pathogens. 2023;12(9):1074.
3. Elfadil A, Alzahrani AM, Abdullah H, Alsamhan H, Abujamel TS, Ahmed HE, et al. Evaluation of the Antibacterial Activity of Quinoxaline Derivative Compound Against Methicillin-Resistant *Staphylococcus aureus*. Infection and Drug Resistance. 2023:2291-6.
4. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE-S, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infection and Drug Resistance. 2020:3255-65.
5. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. Jama. 2007;298(15):1763-71.
6. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspectives in medicinal chemistry. 2014;6:PMC. S14459.

7. Riaz S, Hussain A, Sohail M, Rehman SU, Javed N, Abbas Z. Isolation and characterization of Vancomycin resistant *Staphylococcus aureus* (VRSA) from Intensive Care Units (ICU) of different hospitals in Lahore, Pakistan. Advancements in Life Sciences. 2021;8(4):339-44.
8. Pangercić A, Bukovski-Simonoski S, Barsić B. Lipopeptides and oxazolidinones--novel antibiotics in MRSA infection treatment. Lijecnicki Vjesnik. 2010;132:11-3.
9. Manso T, Lores M, de Miguel T. Antimicrobial activity of polyphenols and natural polyphenolic extracts on clinical isolates. Antibiotics. 2021;11(1):46.
10. Goñi P, López P, Sánchez C, Gómez-Lus R, Becerril R, Nerín C. Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food chemistry. 2009;116(4):982-9.
11. Becerril R, Gómez-Lus R, Goni P, López P, Nerín C. Combination of analytical and microbiological techniques to study the antimicrobial activity of a new active food packaging containing cinnamon or oregano against E. coli and S. aureus. Analytical and bioanalytical chemistry. 2007;388:1003-11.
12. Levy J, Boyer RR, Neilson AP, O'Keefe SF, Chu HSS, Williams RC, et al. Evaluation of peanut skin and grape seed extracts to inhibit growth of foodborne pathogens. Food Science & Nutrition. 2017;5(6):1130-8.
13. González de Llano D, Moreno-Arribas MV, Bartolomé B. Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. Molecules. 2020;25(15):3523.
14. Al-Habib A, Al-Saleh E, Safer A-M, Afzal M. Bactericidal effect of grape seed extract on methicillin-resistant *Staphylococcus aureus* (MRSA). The Journal of toxicological sciences. 2010;35(3):357-64.
15. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clinical microbiology reviews. 2010;23(1):99-139.
16. Kandasamy M, Nasimuddin S, Malayan J, Nithyalakshmi J, Gnanadesikan S, Chandrasekar M. A study on antibacterial effect of grape seed extracts in common clinical and drug resistant isolates. Int J Clin Trials. 2016;3(3):165-8.
17. Daoutidou M, Plessas S, Alexopoulos A, Mantzourani I. Assessment of antimicrobial activity of pomegranate, cranberry, and black chokeberry extracts against foodborne pathogens. Foods. 2021;10(3):486.
18. Silván JM, Mingo E, Hidalgo M, de Pascual-Teresa S, Carrascosa AV, Martinez-Rodriguez AJ. Antibacterial activity of a grape seed extract and its fractions against Campylobacter spp. Food control. 2013;29(1):25-31.
19. Su X, Howell AB, D'Souza DH. Antibacterial effects of plant-derived extracts on methicillin-resistant *Staphylococcus aureus*. Foodborne pathogens and disease. 2012;9(6):573-8.
20. Sarhan SR, Mohammed HA. Isolation and Identification of mecA gene from MRSA isolated from Local Sheep and Evaluate the Inhibitory effect of Cranberry Leaves extract In-Vitro. Research Journal of Pharmacy and Technology. 2019;12(5):2131-6.
21. Bijarani AN, Ismail MO, Memon Z, Afridi F, Qabulio S, Ali A. Detection of Antimicrobial Activity of Cranberry Fruit Extract against Methicillin Resistant *Staphylococcus aureus* and Extended Spectrum Beta Lactamase Escherichia coli. Journal of Pharmaceutical Research International. 2021;33(23B):44-52.
22. Haroun MF, Al-Kayali RS. Synergistic effect of Thymbra spicata L. extracts with antibiotics against multidrug-resistant *Staphylococcus aureus* and Klebsiella pneumoniae strains. Iranian Journal of Basic Medical Sciences. 2016;19(11):1193.
23. Atta S, Waseem D, Fatima H, Naz I, Rasheed F, Kanwal N. Antibacterial potential and synergistic interaction between natural polyphenolic extracts and synthetic antibiotic on clinical isolates. Saudi Journal of Biological Sciences. 2023;30(3):103576.