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(RESEARCH ARTICLE)

Evaluation of potential Synergistic effect of Cranberry extract and Amoxicillin-Clavulanic acid using Checkerboard method

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Abstract

This study investigates the potential synergistic effect of Cranberry (*Vaccinium oxycoccos*) extract and Amoxicillin-Clavulanic acid in enhancing antibacterial effectiveness and combating resistance. The main objectives of this study are do determine the MIC and FIC values for Amoxicillin-Clavulanic Acid and *Vaccinium oxycoccos* extract on different bacterial strains, and therefore to evaluate the synergistic effect of compounds combined.

Vaccinium oxycoccos extract is rich in proanthocyanidins, phenolic acids, anthocyanins, vitamin C, and triterpene compounds, and has demonstrated antibacterial, antifungal, and anti-inflammatory properties. This study specifically evaluates its potential to inhibit biofilm formation by gram-negative bacteria.

Amoxicillin, a semi-synthetic penicillin, is combined with Clavulanic acid, a β-lactamase inhibitor, to treat various bacterial infections. However, the rising antimicrobial resistance challenges the efficacy of this antibiotic combination.

The effectiveness of combining antibiotics and natural substances in inhibiting bacterial growth can vary among different bacteria. This research emphasizes the importance of personalized treatment for each bacterial strain to effectively combat antibiotic-resistant infections.

Keywords: *Vaccinium oxycoccos*; Amoxicillin-Clavulanic acid; Minimal Inhibitory Concentration; Checkerboard method; Synergy

1. Introduction

The *Vaccinium* genus, belonging to the Ericaceae family, includes around 4,250 species within 125 genera, found across Europe, Africa, Asia, and North and Central America. Research on the European cranberry (*Vaccinium oxycoccos*) is relatively limited, even though it is commercially cultivated in Russia, Estonia, and Lithuania [1].

Vaccinium oxycoccos plant lives in peat soils with high water levels and is rich in proanthocyanidins, phenolic acids, anthocyanins, vitamin C, and triterpene compounds. Proanthocyanidins are known for preventing *Escherichia coli* from adhering to the urinary tract [2].The phenolic acids in cranberries, such as p-coumaric, caffeic, ferulic, and chlorogenic acids, vary with ripeness. Anthocyanins, which give cranberries their red color, and flavonoids, acting as antioxidants, are also present. Triterpenoids like ursolic acid contribute to anti-inflammatory and anticancer properties. Additionally, vitamin C provides antioxidant benefits and supports immunity [3].

Cranberries have been traditionally used to prevent and treat various ailments, particularly urinary tract infections (UTIs). Clinical evidence supports their effectiveness in preventing recurrent UTIs, mainly due to fructose and A-type proanthocyanidins that inhibit *E. coli* adhesion to uroepithelial cells. Ursolic acid prevents *E. coli* biofilm formation,

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while quinic acid helps excrete hippuric acid, which acidifies urine. Sialic acid offers anti-inflammatory and analgesic effects [4]. Cranberries also prevent *Helicobacter pylori* adhesion, which is linked to ulcers, and are recommended for kidney and bladder stones, incontinence, and prostate [3].

Amoxicillin, a penicillin derivative introduced in the 1970s, is widely used alone or in combination with clavulanic acid, a β-lactamase inhibitor (BLI) This combination, known as amoxicillin-clavulanic acid (AMC), is extensively prescribed in Europe and recognized by the WHO. It is effective in treating paediatric infections such as respiratory and urinary tract infections [8, 5]. Amoxicillin binds to penicillin-binding protein 1A, disrupting cell wall synthesis and causing bacterial cell death. Clavulanic acid, derived from *Streptomyces clavuligerus*, inhibits β-lactamase enzymes, protecting penicillins from degradation [8, 6]. In recent years, the incidence of infections caused by multi-drug-resistant strains has surged, exacerbating this significant concern. The increasing microbial resistance and the negative side effects associated with synthetic antimicrobials have prompted many researchers to investigate natural alternative treatments for various infectious diseases [7].

Combining antibiotics with plant-derived antimicrobials, such as essential oils and extracts can enhance antibiotic efficacy. Although plant-derived antimicrobials may be less potent, this highlights the need to explore synergistic interactions between plant compounds and antimicrobial agents. The combination of plant extracts with synthetic medications offers a new approach to enhancing antibiotic efficacy [7]. Antibiotics containing β-lactams have demonstrated promising results in overcoming resistance when combined with natural compounds. Such combinations can enhance antimicrobial effectiveness while potentially reducing side effects [7, 9].

The main objectives of this study are to determine the MIC and FIC values for Amoxicillin-Clavulanic Acid and *Vaccinium oxycoccos* extract on different bacterial strains. Also, to evaluate their potential synergistic effect, using checkerboard method.

2. Materials and methods

2.1. Tested samples

- Panklav® 2x - Ingredients (1 oral tablet): 875 mg Amoxicillin + 125 mg Clavulanic Acid. Drug manufacturer: HEMOFARM d.o.o. Banja Luka, Bosnia and Herzegovina.
- Vaccinium oxycoccos extract- Liquid Extract of *Vaccinium oxycoccos* (pure extract -100%). Extract manufacturer: SEMIKEM d.o.o. Sarajevo, Bosnia and Herzegovina.

2.2. Bacterial strains

The used bacterial strains are: *Escherichia coli ATCC 14169, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC BAA-1706 and Klebsiella pneumoniae ATCC 700603.*

2.3. Chemicals

- Tryptic Soy Broth (TSB)
- Mueller Hinton Agar (MHA)
- Distilled water

2.4. Equipment

- Analytical balance
- Glass bottles
- Vortex
- Mortar and pestle
- Autoclave
- Autoclave tape
- Autoclave bags
- Micropipette
- Micropipette tips
- Inoculation loop
- Petri dishes
- Sterile swabs

- Plastic test tubes
- Incubator
- 96 well plates
- ELISA plate reader
- Densitometer
- Magnetic stirrer and magnet

2.5. Minimal Inhibitory Concentration

The microbroth dilution method was employed to determine the Minimal Inhibitory Concentration. This method is crucial for performing serial dilutions of both the antibiotic and the extract. It serves the dual purpose of checking for any contamination throughout the experiment and figuring out the dosage of drug needed.

In this method, 96-well plates were used. Initially, each well, except those in the third column, was filled with 100 µL of Tryptic Soy Broth (TSB). The first column was used as a negative control to monitor contamination. The subsequent column contained 100 µL of TSB and 20 µL of microbial inoculum specific to each bacterial strain, serving as the positive control to observe bacterial growth without any treatment.

In the third column, 200 µL of the prepared drug solution was pipetted. We did this for both, antibiotic and extract, for all tested bacterial strains. Serial dilutions of the drug were initiated by transferring 100 µL to subsequent wells, resulting in two-fold dilutions. Finally, 20 µL of microbial inoculum was added to all wells except those in the first column. The primary objective of this method was to evaluate how the decreasing concentrations of treatment affect bacterial growth and to determine the Minimal Inhibitory Concentration (MIC).

Subsequently, the 96-well plates were incubated for 24 hours at 37°C in an incubator. Following incubation, the plates were analyzed using an ELISA plate reader (Biochrom EZ Read 400) at a wavelength of 595 nm to measure bacterial growth and determine the MIC. Checkerboard method

2.6. Checkerboard method

The checkerboard method is an experimental approach employed to assess the interaction between two antibacterial agents: a plant extract (*Vaccinium oxycoccos*) and an antibiotic (Amoxicillin-Clavulanic acid). This method allows for the evaluation of synergistic effects by comparing the antibacterial efficacy of antibiotic combinations with their individual activities. The extent of this interaction is quantified using a fractional inhibitory concentration (FIC) index.

In this procedure, two concentrations of the antibiotic (based on their MIC values against different bacteria) and a 100% concentration of the plant extract were utilized. Initially, each well of a microplate was filled with 100 µL of Tryptic Soy Broth (TSB). The first column received 200 µL of the antibiotic solution, which was then subjected to serial dilutions across subsequent columns up to the 11th column. The 12th column remained antibiotic-free to serve as a control.

For the plant extract, 200 µL of pure extract was added to the first row of the microplate, followed by serial dilutions down to the second-to-last row (G row). The last row (H) was left without extract, except for the last well in the last column, which contained only TSB to serve as the negative control, ensuring no contamination occurred.

Subsequently, 20 µL of a 0.5 McFarland bacterial inoculum was added to each well, excluding the negative control well (12H). The FIC index was calculated using the formula:

Formula used for calculation of FIC index: A /MIC A + B/MIC B = FIC A + FIC B= FIC Index.

A and B represent the concentrations of the antibiotic and extract, and MIC A and MIC B indicate their respective MIC values against the tested bacteria.

3. Results

3.1. Minimal Inhibitory Concentration results

Table 1 Effect of Amoxicillin-Clavulanic acid on planktonic growth of *Escherichia coli* ATCC 14169

EC1	**NC**	**PC**	**Concentration (μg/ml)**									
			800	**400**	**200**	**100**	**50**	**25**	**12.5**	**(MIC) 6.25**	**3.125**	**1.56**
OD values	0.056	1.118	0.06	0.052	0.057	0.045	0.047	0.053	0.08	0.054	0.448	0.992
	0.044	1.009	0.058	0.062	0.051	0.046	0.046	0.047	0.047	0.049	0.298	0.79
	0.042	0.989	0.065	0.053	0.05	0.048	0.047	0.047	0.047	0.047	0.468	0.895
	0.046	1.22	0.063	0.054	0.05	0.047	0.047	0.047	0.046	0.049	0.582	0.983
Average	0.047	1.084	0.061	0.055	0.052	0.046	0.046	0.048	0.055	0.049	0.449	0.915

Table 1. Shows the optical density values at 595 nm for *Escherichia coli* ATCC 14169, indicating the effect of different concentrations of Amoxicillin-Clavulanic Acid on its planktonic growth.

Table 2 Effect of Amoxicillin-Clavulanic acid on planktonic growth of *Escherichia coli* ATCC 25922

EC2	**NC**	**PC**	**Concentration (μg/ml)**									
			800	**400**	**200**	**100**	**50**	**25**	**12.5**	**(MIC) 6.25**	**3.125**	**1.56**
OD values	0.057	0.816	0.064	0.055	0.055	0.049	0.048	0.059	0.05	0.057	0.208	0.47
	0.045	0.904	0.064	0.063	0.051	0.05	0.047	0.054	0.053	0.051	0.295	0.371
	0.045	0.742	0.067	0.056	0.053	0.048	0.048	0.053	0.051	0.05	0.472	0.459
	0.047	0.748	0.069	0.054	0.051	0.05	0.048	0.058	0.051	0.055	0.22	0.5
Average	0.048	0.802	0.066	0.057	0.052	0.049	0.047	0.056	0.051	0.053	0.298	0.462

Table 2. Shows the optical density values at 595 nm for *Escherichia coli* ATCC 25922, indicating the effect of different concentrations of Amoxicillin-Clavulanic Acid on its planktonic growth.

Table 3 Effect of Amoxicillin-Clavulanic acid on planktonic growth of *Klebsiella pneumoniae* ATCC BAA-1706

KP1	**NC**	**PC**	**Concentration (μg/ml)**									
			800	**400**	**200**	**100**	**50**	**25**	**(MIC) 12.5**	**6.25**	**3.125**	**1.56**
OD values	0.053	0.683	0.057	0.057	0.049	0.082	0.05	0.052	0.055	0.352	0.238	0.655
	0.044	0.586	0.058	0.052	0.049	0.048	0.101	0.057	0.054	0.194	0.241	0.635
	0.045	0.571	0.055	0.056	0.05	0.05	0.049	0.05	0.054	0.295	0.203	0.664
	0.045	0.621	0.057	0.053	0.05	0.048	0.048	0.048	0.051	0.42	0.258	0.665
Average	0.0468	0.6152	0.0568	0.0545	0.0495	0.057	0.062	0.051	0.053	0.3153	0.235	0.654

Table 3. Shows the optical density values at 595 nm for *Klebsiella pneumoniae* ATCC BAA-1706 indicating the effect of different concentrations of Amoxicillin-Clavulanic Acid on its planktonic growth.

Table 4 Effect of Amoxicillin-Clavulanic acid on planktonic growth of *Klebsiella pneumoniae* ATCC 700603

KP7	**NC**	**PC**	**Concentration (µg/ml)**									
			800	**400**	**200**	**100**	**50**	**(MIC) 25**	**12.5**	**6.25**	**3.125**	**1.56**
OD values	0.052	0.734	0.06	0.056	0.049	0.05	0.05	0.051	0.511	0.688	0.764	0.816
	0.043	0.694	0.064	0.057	0.052	0.05	0.051	0.052	0.463	0.662	0.694	0.76
	0.044	0.706	0.059	0.056	0.053	0.05	0.051	0.052	0.477	0.663	0.684	0.765
	0.045	0.748	0.064	0.056	0.052	0.051	0.05	0.05	0.558	0.772	0.765	0.819
Average	0.046	0.7205	0.0618	0.0563	0.0515	0.0503	0.0505	0.0513	0.5023	0.6963	0.7268	0.79

Table 4. Shows the optical density values at 595 nm for *Klebsiella pneumoniae* ATCC 700603 indicating the effect of different concentrations of Amoxicillin-Clavulanic Acid on its planktonic growth.

Table 5 Effect of *Vaccinium oxycoccos* extract on planktonic growth of *Escherichia coli* ATCC 14169

EC1	**NC**	**PC**	**Concentration (µg/ml)**									
			800	**400**	**(MIC) 200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
OD values	0.044	0.609	0.194	0.059	0.039	0.385	0.487	0.799	0.917	1.126	0.985	1.023
	0.046	0.528	0.099	0.071	0.041	0.267	0.357	0.618	0.795	0.97	0.934	0.959
	0.065	0.587	0.052	0.08	0.04	0.324	0.36	0.569	0.784	0.912	0.911	0.975
	0.045	0.551	0.039	0.052	0.043	0.266	0.324	0.478	0.739	0.904	0.894	1.012
Average	0.05	0.5687	0.096	0.0655	0.04075	0.3105	0.382	0.616	0.80875	0.978	0.931	0.99225

Table 5. Shows the optical density values at 595 nm for *Escherichia coli* ATCC 14169 indicating the effect of different concentrations of *Vaccinium oxycoccos* on its planktonic growth.

Table 6 Effect of *Vaccinium oxycoccos* extract on planktonic growth of *Escherichia coli* ATCC 25922

EC2	**NC**	**PC**	**Concentration (µg/ml)**									
			800	**400**	**(MIC) 200**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
OD values	0.048	0.842	0.112	0.064	0.048	0.309	0.966	1.024	0.94	0.938	1.012	0.902
	0.047	0.531	0.09	0.07	0.059	0.315	0.769	1.026	0.967	0.948	0.975	0.931
	0.047	0.283	0.098	0.074	0.048	0.335	0.601	0.96	1.013	0.93	0.911	0.962
	0.055	0.496	0.157	0.068	0.048	0.291	0.669	0.974	0.998	0.944	0.922	1.035
Average	0.04925	0.538	0.11425	0.069	0.05075	0.3125	0.75125	0.996	0.9795	0.94	0.955	0.9575

Table 6. Shows the optical density values at 595 nm for *Escherichia coli* ATCC 25922 indicating the effect of different concentrations of *Vaccinium oxycoccos* on its planktonic growth.

Table 7 Effect of *Vaccinium oxycoccos* extract on planktonic growth of *Klebsiella pneumoniae* ATCC BAA-1706

KP1	NC	PC	Concentration (µg/ml)									
			800	400	(MIC) 200	100	50	25	12.5	6.25	3.125	1.56
OD values	0.045	0.239	0.184	0.064	0.037	0.305	0.511	0.528	0.561	0.576	0.581	0.615
	0.046	0.359	0.095	0.047	0.038	0.277	0.477	0.536	0.55	0.611	0.567	0.55
	0.046	0.308	0.127	0.061	0.044	0.265	0.452	0.51	0.549	0.562	0.583	0.565
	0.046	0.361	0.136	0.061	0.045	0.198	0.425	0.488	0.54	0.582	0.613	0.572
Average	0.04575	0.31675	0.1355	0.05825	0.041	0.26125	0.46625	0.5155	0.55	0.58275	0.586	0.5755

Table 7. Shows the optical density values at 595 nm for *Klebsiella pneumoniae* ATCC BAA-1706 indicating the effect of different concentrations of *Vaccinium oxycoccos* on its planktonic growth.

Table 8 Effect of *Vaccinium oxycoccos* extract on planktonic growth of *Klebsiella pneumoniae* ATCC 700603

KP7	NC	PC	Concentration (µg/ml)									
			800	400	(MIC) 200	100	50	25	12.5	6.25	3.125	1.56
OD values	0.047	0.191	0.167	0.071	0.053	0.396	0.564	0.599	0.663	0.78	0.969	0.743
	0.048	0.048	0.18	0.062	0.06	0.361	0.607	0.546	0.566	0.771	0.854	0.624
	0.046	0.091	0.185	0.082	0.065	0.393	0.545	0.5	0.583	0.804	0.815	0.617
	0.052	0.073	0.19	0.084	0.067	0.367	0.441	0.467	0.644	0.833	0.835	0.638
Average	0.0482	0.1007	0.1805	0.0747	0.0612	0.3792	0.5392	0.528	0.614	0.797	0.8682	0.6555

Table 8. Shows the optical density values at 595 nm for *Klebsiella pneumoniae* ATCC 700603 indicating the effect of different concentrations of *Vaccinium oxycoccos* on its planktonic growth.

3.2. Checkerboard results

Table 9 Synergistic effect of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract on *Escherichia coli* ATCC 14169

	EC1		Amoxicillin-Clavulanic acid (µg/ml)											
Vaccinium oxycoccos extract (%)			50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049	0.024
	A	100	0.078	0.081	0.083	0.081	0.082	0.083	0.081	0.081	0.078	0.08	0.081	0.82
	B	50	0.082	0.083	0.082	0.082	0.083	0.082	0.082	81	0.08	0.081	0.082	0.081
	C	25	0.084	0.081	0.081	0.083	0.084	0.084	0.078	0.083	0.078	0.08	0.081	0.08
	D	12.5	0.087	0.073	0.075	0.077	0.08	0.188	0.26	0.238	0.238	0.345	0.567	0.731
	E	6.25	0.08	0.066	0.072	0.068	0.077	0.374	0.866	1.006	0.786	0.783	0.723	0.829
	F	3.13	0.067	0.06	0.062	0.063	0.209	0.444	1.07	1.13	0.945	0.817	0.856	0.903
	G	1.56	0.054	0.059	0.06	0.059	0.199	0.53	1.022	1.087	1.013	0.907	0.928	1.061
	H	0.78	0.051	0.051	0.045	0.046	0.243	0.662	1.066	1.008	1.008	0.981	0.943	0.044

Table 9. shows serial dilutions of both of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract and their synergistic effect on *Escherichia coli* ATCC 14169 at the absorbance of 595nm. Negative control is marked in red, and

the low value is suggesting that no contamination occurred. Amoxicillin-Clavulanic acid was added in the first column and diluted, while *Vaccinium oxycoccos* extract was added in the first row and diluted.

Calculation:
A /MIC A + B/MIC B = FIC A + FIC B= FIC Index

6.25/6.25 + 25/200 = 1 + 0.125 = 1.125

FIC index is interpreted as indifferent or additive.

Table 10 Synergistic effect of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract on *Escherichia coli* ATCC 25922

EC2			Amoxicillin-Clavulanic acid (µg/ml)											
Vaccinium oxycoccos extract (%)			50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049	0.024
	A	100	0.081	0.082	0.085	0.086	0.087	0.089	0.091	0.093	0.094	0.097	0.099	0.098
	B	50	0.087	0.086	0.089	0.091	0.093	0.093	0.097	0.098	0.099	0.099	0.097	0.098
	C	25	0.097	0.101	0.086	0.09	0.126	0.131	0.197	0.22	0.252	0.223	0.212	0.269
	D	12.5	0.081	0.073	0.076	0.08	0.11	0.28	0.28	0.402	0.445	0.503	0.543	0.614
	E	6.25	0.068	0.068	0.08	0.085	0.088	0.292	0.566	0.778	0.81	0.756	0.882	1.052
	F	3.13	0.062	0.06	0.062	0.067	0.076	0.392	0.588	0.822	0.861	0.922	0.915	0.858
	G	1.56	0.054	0.063	0.059	0.063	0.073	0.231	0.66	0.76	0.804	0.802	0.812	0.834
	H	0.78	0.051	0.046	0.048	0.049	0.055	0.272	0.62	0.793	0.82	0.836	0.856	**0.042**

Table 10. shows serial dilutions of both of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract and their synergistic effect on *Escherichia coli* ATCC 25922 at the absorbance of 595nm. Negative control is marked in red, and the low value is suggesting that no contamination occurred. Amoxicillin-Clavulanic acid was added in the first column and diluted, while *Vaccinium oxycoccos* extract was added in the first row and diluted.

Calculation:
A /MIC A + B/MIC B = FIC A + FIC B= FIC Index

6.25/6.25 + 50/200 = 1+ 0.25 = 1.25

FIC index is interpreted as indifferent or additive.

Table 11 Synergistic effect of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract on *Klebsiella pneumoniae* ATCC BAA-1706

KP1			Amoxicilclin-Clavulanic acid (µg/ml)											
Vaccinium oxycoccos extract (%)			200	100	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098
	A	100	0.081	0.083	0.084	0.083	0.085	0.086	0.084	0.082	0.083	0.085	0.085	0.084
	B	50	0.087	0.089	0.086	0.085	0.085	0.087	0.084	0.085	0.084	0.087	0.086	0.085
	C	25	0.101	0.095	0.086	0.086	0.087	0.087	0.086	0.084	0.084	0.086	0.086	0.087
	D	12.5	0.139	0.083	0.082	0.079	0.101	0.186	0.291	0.279	0.269	0.327	0.321	0.352
	E	6.25	0.074	0.07	0.073	0.077	0.132	0.191	0.276	0.42	0.442	0.441	0.427	0.477
	F	3.13	0.067	0.064	0.065	0.071	0.089	0.159	0.239	0.429	0.47	0.48	0.484	0.523
	G	1.56	0.058	0.079	0.062	0.064	0.078	0.129	0.211	0.422	0.482	0.499	0.493	0.523
	H	0.78	0.054	0.045	0.05	0.054	0.059	0.152	0.222	0.519	0.544	0.549	0.529	**0.045**

Table 11. shows serial dilutions of both of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract and their synergistic effect on *Klebsiella pneumoniae* ATCC BAA-1706 at the absorbance of 595nm. Negative control is marked in

red, and the low value is suggesting that no contamination occurred. Amoxicillin-Clavulanic acid was added in the first column and diluted, while *Vaccinium oxycoccos* extract was added in the first row and diluted.

Calculation:
A /MIC A + B/MIC B = FIC A + FIC B= FIC Index

6.25/12.5 + 50/200 = 0.5 + 0.25 = 0.75

FIC index is interpreted as indifferent or additive.

Table 12 Synergistic effect of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract on *Klebsiella pneumoniae* ATCC 700603

KP7			Amoxicillin-Clavulanic acid (µg/ml)											
Vaccinium oxycoccos extract (%)			200	100	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098
	A	100	0.081	0.083	0.085	0.084	0.088	0.081	0.084	0.082	0.082	0.085	0.082	0.084
	B	50	0.093	0.084	0.087	0.085	0.09	0.082	0.084	0.083	0.083	0.084	0.083	0.084
	C	25	0.101	0.086	0.089	0.086	0.084	0.083	0.082	0.084	0.084	0.085	0.083	0.085
	D	12.5	0.107	0.081	0.079	0.081	0.157	0.309	0.374	0.35	0.32	0.36	0.334	0.49
	E	6.25	0.073	0.084	0.085	0.086	0.205	0.485	0.599	0.574	0.636	0.615	0.621	0.653
	F	3.13	0.062	0.08	0.054	0.069	0.266	0.532	0.561	0.614	0.609	0.664	0.706	0.702
	G	1.56	0.06	0.064	0.07	0.071	0.363	0.484	0.602	0.527	0.625	0.6	0.583	0.71
	H	0.78	0.058	0.05	0.047	0.052	0.31	0.377	0.561	0.537	0.641	0.643	0.676	0.044

Table 12. shows serial dilutions of both of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract and their synergistic effect on *Klebsiella pneumoniae* ATCC 700603 at the absorbance of 595nm. Negative control is marked in red, and the low value is suggesting that no contamination occurred. Amoxicillin-Clavulanic acid was added in the first column and diluted, while *Vaccinium oxycoccos* extract was added in the first row and diluted.

Calculation:
A /MIC A + B/MIC B = FIC A + FIC B= FIC Index

6.25/25 + 50/200 = 0.25 + 0.25 = 0.5

FIC index is interpreted as synergistic.

4. Discussion

Table 1 presents optical density values at 595 nm for *Escherichia coli ATCC 14169*, illustrating the impact of different concentrations of Amoxicillin-Clavulanic Acid on planktonic growth. The concentrations range from 800 µg/ml to 1.56 µg/ml, with the minimum inhibitory concentration (MIC) at 6.25 µg/ml, where the average OD value is 0.049. Compared to the positive control (average OD value of 1.084), the treatment at MIC nearly inhibits microbial growth, contrasting with the positive control where growth is unaffected. The table includes positive and negative controls to ensure result accuracy and prevent contamination.

Table 2 displays the effects of Amoxicillin-Clavulanic Acid on *Escherichia coli ATCC 25922* planktonic growth. The MIC is at 6.25 µg/ml, with an average OD value of 0.053. At this concentration, microbial growth is nearly completely inhibited compared to the positive control (average OD value of 0.802). Concentration-dependent reductions in planktonic growth were observed with increasing antibiotic doses, indicating strong inhibitory effects.

Table 3 demonstrates Amoxicillin-Clavulanic Acid's impact on *Klebsiella pneumoniae ATCC BAA-1706* planktonic growth, with the MIC at 12.5 µg/ml and an average OD value of 0.053. At this concentration, growth is significantly inhibited compared to the positive control (average OD value of 0.6152). Concentration-dependent decreases in OD values suggest a dose-dependent effect on bacterial growth inhibition, even at lower concentrations.

Table 4 outlines the effect of Amoxicillin-Clavulanic Acid on *Klebsiella pneumoniae ATCC 700603* planktonic growth, with the MIC at 25 µg/ml and an average OD value of 0.0513. At this concentration, growth inhibition is notable compared to the positive control (average OD value of 0.7205). Concentration-dependent decreases in OD values indicate a strong inhibitory effect on bacterial growth, particularly at higher doses.

Table 5 examines different concentrations of cranberry extract *on Escherichia coli ATCC 14169* planktonic growth. The MIC is at 200 µg/ml, with an average OD value of 0.04075. This concentration significantly inhibits growth compared to the positive control (average OD value of 0.5687), with higher concentrations further reducing bacterial growth. Lower concentrations showed increased OD values, indicating a dose-dependent inhibitory effect of the extract.

Table 6 investigates the effect of cranberry extract on *Escherichia coli ATCC 25922* planktonic growth. The MIC is at 200 µg/ml, with an average OD value of 0.05075. At this concentration, microbial growth is significantly inhibited compared to the positive control (average OD value of 0.538). The extract exhibits inhibitory effects even at lower concentrations, with higher concentrations showing more pronounced reductions in OD values.

Table 7 presents the results of *Vaccinium oxycoccos* extract on *Klebsiella pneumoniae ATCC BAA-1706* planktonic growth, with the MIC at 200 µg/ml and an average OD value of 0.041. Growth inhibition is observed at this concentration compared to the positive control (average OD value of 0.31675). The extract's inhibitory effect is dose-dependent, with lower concentrations showing higher OD values similar to the positive control.

Table 8 evaluates the effect of *Vaccinium oxycoccos* extract on *Klebsiella pneumoniae ATCC 700603* planktonic growth, with the MIC at 200 µg/ml and an average OD value of 0.0612. At this concentration, growth inhibition is evident compared to the positive control (average OD value of 0.1007). The inhibitory effect diminishes at lower concentrations, demonstrating a dose-dependent response of the extract.

When we look at the results for Checkerboard method, we see very different results.

For *Escherichia coli ATCC 14169*, the FIC index was calculated to be 1.125. This result suggest an indifferent interaction between two antibiotic and extract. This combination did not enhance or diminish the antibacterial effect compared to the results of individual tests. This combination does not worsen the efficacy, it also does not provide a synergistic effect for this bacteria.

For *Escherichia coli ATCC 25922*, the FIC index was found to be 1.25. Results were similar to the EC1 strain. This FIC index also indicates an indifferent or additive interaction. The combined effect of the drugs is suggesting that no significant advantage is occurring when using them together for this bacteria.

For *Klebsiella pneumoniae ATCC BAA-1706*, the FIC index was 0.75. An FIC index of 0.75 results in additive interaction. This suggests some level of enhanced activity when those are used together.

Klebsiella pneumoniae ATCC 700603 showed best result. FIC index was exactly 0.5, it is indicating a synergistic interaction. This suggests that the combination of Amoxicillin-Clavulanic acid and *Vaccinium oxycoccos* extract enhances antibacterial efficacy against this strain. Such a synergistic effect can be highly beneficial, potentially allowing for lower doses of each agent and reducing the risk of side effects of antibiotics and resistance development.

5. Conclusion

In conclusion, this study demonstrates the potential of combining *Vaccinium oxycoccos* extract with Amoxicillin-Clavulanic acid to enhance antibacterial effectiveness against specific bacterial strains. The MIC and FIC values indicate that while the combination shows an indifferent effect on *Escherichia coli* strains, it exhibits additive and synergistic effects on *Klebsiella pneumoniae* strains. The synergy observed, particularly against *Klebsiella pneumoniae ATCC 700603,* suggests that such combinations could lower required doses of antibiotics, thus minimizing side effects and the development of resistance. This highlights the importance of exploring plant-derived antimicrobials in conjunction with conventional antibiotics in future for more effective and personalized treatments against resistant bacterial infections.

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