Scientific Journal of King Faisal University:
Basic and Applied Sciences

The Effect of Alcoholic Extracts of Strawberries and Green Tea on *Enterococcus faecalis* Isolated from Urinary Tract Infections

Aws Ibrahim Sulaiman [1] **and Saja Anwr Fadhil** [2]

[1] Department of Biology, College of Science, University of Mosul, Mosul, Iraq
[2] Independent Researcher, Mosul, Iraq

LINK	RECEIVED	ACCEPTED	PUBLISHED ONLINE	ASSIGNED TO AN ISSUE
https://doi.org/10.37575/b/sci/240007	30/01/2024	01/06/2024	01/06/2024	01/06/2024

NO. OF WORDS	NO. OF PAGES	YEAR	VOLUME	ISSUE
3680	5	2024	25	1

ABSTRACT

Plant extracts contain active substances that exhibit antibacterial effects. Our study aimed to evaluate the antibacterial activity of alcoholic extracts (acetone, methanol, and ethanol) of strawberries and green tea against *Enterococcus spp.* We collected 30 samples from individuals suffering from urinary tract infections. Initially, we identified 8 (26%) isolates outwardly; subsequent confirmatory molecular diagnostics resulted in one isolate, registered at the National Center for Biotechnology Information as AsAw1, marking a global first. The strawberry extract exhibited a significant inhibitory effect on *Enterococcus spp.*, with a zone of inhibition measuring 23 and 26 mm. Conversely, the green tea extract demonstrated a weaker inhibitory effect, with a zone of inhibition measuring 19 mm. These findings underscore the potential of ethanolic strawberry extract as a natural antibacterial agent against *Enterococcus spp.*, offering insights for further research and potential therapeutic applications in the treatment of urinary tract infections caused by these bacteria.

KEYWORDS
acetone, catechin, flavonoids, gelatinase, hemolytic, vancomycin

CITATION
Sulaiman, A.I. and Fadhil, S.A. (2024). The effect of alcoholic extract of strawberries and green tea on *Enterococcus faecalis* isolated from urinary tract infections. *Scientific Journal of King Faisal University: Basic and Applied Sciences*, **25**(1), 69–73. DOI: 10.37575/b/sci/240007

1. Introduction

Enterococci are part of the normal gut microbiota and can act as opportunistic pathogens, causing various infections in both community and hospital settings, particularly in individuals with compromised immune systems. These infections include bacteremia, endocarditis, and urinary tract infections (UTIs). Additionally, Enterococci can colonize mucous membranes and skin through the mouth, especially in hospital environments. These organisms are highly resistant to environmental and chemical factors and can persist on fomites (Murray *et al.*, 2015; Cornelissen and Hobbs, 2020; Sulaiman and Saad, 2024). They are the causative agents for the majority of these infections and exhibit virulence factors associated with adherence, such as the aggregation substance (Agg), which facilitates binding to renal epithelial cells, the adhesin to collagen (Ace), surface proteins (Esp), gelatinase enzymes (GelE), hyaluronidase enzymes (Hyl), and cytolysin (CylA) (Hashem *et al.*, 2021). Treatment against Enterococci presents a clinical challenge due to their increasing resistance to various antimicrobial agents, including macrolides, glycopeptides, β-lactams, aminoglycosides, and fluoroquinolones (Heidari *et al.*, 2017). Tea, available in different varieties such as green, black, and oolong, is rich in polyphenols, which vary in type and concentration depending on the preparation method. For instance, green tea contains monomeric polyphenols such as epicatechin, epicatechin gallate, and epigallocatechin, known for their antibacterial effects against various aerobic and anaerobic bacteria, viruses, fungi, and parasites (Reygaert, 2017). Strawberries, a delicious and widely consumed fruit, are rich in vitamins and possess antibacterial, antiviral, and antitumor properties, playing a significant role in reducing the formation of biofilms (Widyarman *et al.*, 2017). The purpose of our research is to identify the virulence genes of *Enterococcus spp.* and to study the effects of strawberry and green tea extracts on these pathogens.

2. Materials and Methods

2.1. Clinical Bacterial Samples:

We collected 30 clinical bacterial samples from patients with UTIs who sought treatment at AL-Salam General Teaching Hospital in Mosul, Iraq, between October 1, 2022, and January 1, 2023. These patients were diagnosed by physicians, and their samples were obtained using the mid-stream method with a clean, sterile container. These samples were subsequently transferred to the Biology Department at the College of Science, University of Mosul, for diagnostic and identification purposes.

2.2. Diagnostic and Identification:

We used blood agar (Himedia, India), m-Enterococcus agar (Himedia, India), and Azide agar (Atlas, 2010) to cultivate the bacterial samples. The composition of azide agar includes pancreatic digest of casein (15 g), yeast extract (5 g), glucose (2 g), peptic digest of soybean meal (5 g), triphenyl-tetrazolium chloride (0.1 g), KH_2PO_4 (4 g), NaN_3 (4 g), and agar (10 g) at a pH of 7.2 ± 0.2, incubated at 25°C in a 1 L medium. Various tests were performed, including Gram staining, assessing growth, and tolerance to 6.5% NaCl. Positive results for NaCl tolerance were indicated by a color change to a less intense shade, whereas negative results appeared as purple. Additionally, colonies on bile-esculin agar (HiMedia, India) displayed a black color (Tille, 2017).

2.3. Gelatinase Activity:

We followed the procedure described by Tille (2017) with a slight modification. The medium for gelatinase consisted of brain heart infusion (BHI) agar (Oxoid, UK) and 3% gelatin powder (HiMedia, India). The isolates were streaked on gelatin plates and incubated at 37°C overnight. After incubation, a Frazier solution was added to the plates. A positive result is indicated by a transparent halo around the colonies, indicating the effectiveness of the enzyme gelatinase (Hashem *et al.*, 2017).

Corresponding Author: Aws Ibrahim Sulaiman 009647503478899, awssbio61@uomosul.edu.iq

2.4. Molecular Characterization:

DNA was extracted from purified and identified colonies using the PrestoTM Mini gDNA Bacteria Kit Quick Protocol (Geneaid, Taiwan).

Table 1: Primers for this research

Primer name	Gene	Primer sequence	Product size(bp)
Ddl	(D-Ala:D-Ala)	F/ATCAAGTACAGTTAGTCTTTATTAG	941
E. faecalis	ligases	R/ACGATTCAAAGCTAACTGAATCAGT	
Esp	Enterococcal	F/ AGATTTCATCTTTGATTCTTGG	510
	surface protein	R/ AATTGATTCTTTAGCATCTGG	

We constructed the PCR program based on Creti *et al.* (2004), which includes an initial heating for 5 min at 95°C, followed by 30 cycles of 95°C for 60 sec, 60 sec at 52°C for gelE and 63°C for esp, and 72°C for 60 sec, with a final step of 72°C for 10 min.

2.5. Molecular Detection of *Enterococcus faecalis:*

Based on Khalid (2016), we used a universal primer (D-Ala: D-Ala) to detect the isolates and enterococcal surface protein. The PCR amplification products (Thermal Cycler, Eppendorf, Master-cycler Personal, Eppendorf AG, 2233, Germany) were visualized by electrophoresis (Power Supply, MS Major Science, MINIS-300VS, Taiwan) on a 1.5% agarose gel for 60 min at 80 V. The size of the amplicon was determined by comparison with the molecular marker B085 100 bp Plus DNA Ladder (TRANS Jena Bioscience GmbH, Germany).

2.6. Antimicrobial Susceptibility Test:

We performed this test according to Humphries *et al.* (2021) using the disk diffusion method. The susceptibility testing included the following antimicrobial agents (Bioanalyzer Company, Turkey) and their respective concentrations: Amoxicillin (AMC, 30 µg), Ceftriaxone (CRO, 10 µg), Daptomycin (DAP, 10 µg), Gentamicin (CN, 10 µg), Levofloxacin (LEV, 5 µg), Piperacillin (PIT, 30/6 µg), Tetracycline (TET, 10 µg), Ticarcillin (TCC, 75/10 µg), and Vancomycin (VA, 30 µg).

2.7. Hemolytic Activity:

To evaluate the hemolytic activity, the isolates were cultured on a blood agar base (Oxoid, UK) supplemented with 5% defibrinated blood. After incubating the plates at 37°C overnight, the presence of a clear zone around the colonies indicated hemolytic activity (Jasni *et al.*, 2010).

2.8. Green Tea and Strawberry Extract Preparation:

A total of 40 g of dried green tea leaf granules (*Camellia sinensis*) obtained from the DANIA Market were taken and divided into four sections, each containing 10 g. Each section was soaked separately in 100 ml of boiling water, 100 ml of ethanol, 100 ml of methanol, and 100 ml of acetone, respectively, for 24 h. The solutions were then filtered using Whatman filter paper (No.1). The filtered extracts were centrifuged at 3000 rpm for 15 min (Centrifuge REMI, India) to obtain the purified extracts, which were subsequently stored for further use (Basam *et al.*, 2016).

Similarly, 40 g of strawberry fruits were obtained from the local market in Mosul city. The strawberries were crushed using a ceramic mortar. The experiment was carried out in a manner similar to the one described for the green tea extract preparation.

2.9. Antibacterial Activity of Green Tea and Strawberry Extracts:

Recent colonies aged 18–24 h were transferred to physiological saline (2 mL) to make a bacterial suspension with a concentration of 0.5×10^8 CFU/ml. Muller-Hinton agar plates were prepared and supplemented with different concentrations of vancomycin (12.5, 25, 50, and 100 µg/ml). The Petri dishes were left at room temperature to dry for 15 min. Wells were then made in the Muller-Hinton agar plates, and the extracts of green tea and strawberry were separately added to the wells. Distilled water (D.W.) was used as a negative control. After overnight incubation, the inhibition zone was measured using an electronic calibrator (Santosh and Satya, 2010).

2.10. Statistical Analysis:

Statistical analysis was performed using the t-test to compare the average inhibition zones produced by green tea and strawberry extracts at a significance level of $p \leq 0.05$, utilizing SPSS version 21.

2.11. Sequences and Record New Strain:

To investigate the *16S rRNA* gene, we conducted PCR amplification of the target genes using specific primers (see Table 1). The PCR products were sequenced using a Genetic Analyzer 3130 from Hitachi. Subsequently, the obtained gene sequences were compared with those in the National Center for Biotechnology Information (NCBI) database using BLAST software for the result analysis.

3. Results and Discussion

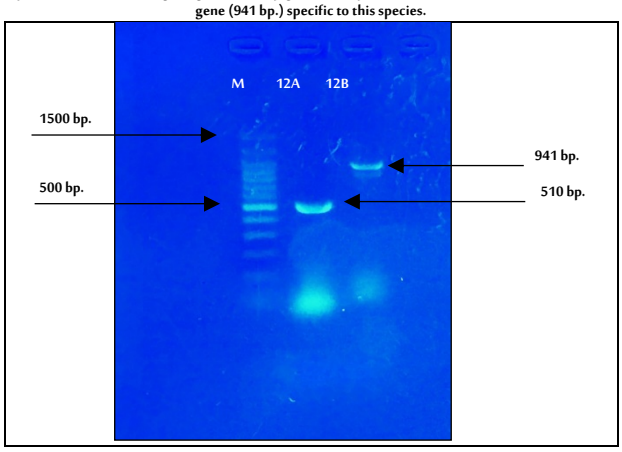
Thirty clinical samples were collected from outpatients with UTIs at AL-Salam General Teaching Hospital in Mosul, Iraq, between November 2022 and January 2023, from both sexes. All samples underwent several tests, including Gram stain, tolerance to 6.5% NaCl, gelatinase activity, and hemolytic activity, and were cultured on m-Enterococcus agar and bile esculin agar at 37°C overnight. We obtained 8 (26%) isolates belonging to *Enterococcus spp.*

Enterococci are considered to be commensal parts of the intestinal microbiota, but they are also dangerous pathogens that can cause infections in humans. Traditionally, infections caused by these bacteria ranged from 5%–10% but have recently increased and can now infect various areas of the human body, such as the bloodstream, urinary tract, surgical sites, burns, and bile ducts. Currently, the most common infections are those of the urinary tract, especially in users of urinary catheters. The ability of *Enterococcus faecalis* to cause disease is attributed to several virulence factors that promote attachment and invasion (Murray *et al.*, 2015). Our results show a convergence with the findings of Saifi *et al.* (2008), who reported 22% *Enterococcus spp.* isolates over a year, compared to our 26%. Conversely, our results differed from those of Khaled (2016), who reported a 3.2% incidence over 6 months. We believe this difference is due to the sample size and the duration of sample collection. The discrepancy with Neeva *et al.* (2024), who reported 17 isolates of *Enterococcus spp.* from 165 bacterial samples, further supports this theory. Members of the enterococci are commensals in the human intestine along with other microbes, but they are also considered one of the most significant opportunistic pathogens, leading to UTIs in both sexes and vaginal infections in females. They also have the ability to survive in harsh environments (Mahon & Lehman, 2022; Murray *et al.*, 2015).

3.1. Molecular Identification:

Based on the phenotypic diagnosis of the samples, a molecular diagnosis was performed on the eight suspected isolates, yielding one isolate that possesses the *Enterococcus faecalis Ddl* and *Esp* genes. Figure 1 illustrates this finding.

Figure 1: Molecular Identification of *Enterococcus spp.* M: 1500 ladder, 12 The isolate that showed a positive result for having the genes *12A Esp* gene (510 bp) and the *12B Ddl Enterococcus faecalis* gene (941 bp.) specific to this species.



Our findings reveal that the isolate we examined produced a 1500 bp product instead of the expected 941 bp product for the *12B Ddl Enterococcus faecalis* gene. Wang *et al.* (2024) indicated that the *Ddl* gene is specialized in the diagnosis of *Enterococcus spp.*, and they confirmed that 26 isolates belonging to these bacteria possess this gene. Our molecular results are consistent with theirs and with Khalid (2016). For the Esp result, we observed a band at 510 bp, similar to the findings of Ghazvinian *et al.* (2024), who reported 12 isolates of *Enterococcus spp.* possessing this gene, which is one of the virulence genes enabling them to adhere to the epithelial cells of the human urinary tract.

3.2. Sequences and Records in the National Center for Biotechnology Information:

The sequence of nitrogenous bases was analyzed, and this isolate was recorded in the NCBI database and named SaAw1, marking the first such recording globally from Iraq. It obtained the serial number OQ660495.1 (99.60%), as depicted in Figures 2 and 3.

Figure 2: Record of this bacterial isolate in the NCBI with the code: OQ660495.1

GenBank ▾ Send to: ▾

Enterococcus sp. strain SaAw1 16S ribosomal RNA gene, partial sequence

GenBank: OQ660495.1

FASTA Graphics

Go to: ⊻

```
LOCUS       OQ660495               1000 bp    DNA     linear   BCT 26-MAR-2023
DEFINITION  Enterococcus sp. strain SaAw1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   OQ660495
VERSION     OQ660495.1
KEYWORDS    .
SOURCE      Enterococcus sp.
  ORGANISM  Enterococcus sp.
            Bacteria; Bacillota; Bacilli; Lactobacillales; Enterococcaceae;
            Enterococcus.
REFERENCE   1  (bases 1 to 1000)
  AUTHORS   Fadhil,S.A. and Sulaiman,A.I.
  TITLE     Anti bacterial effect of green tea and strawberry extracts against
            Enterococcus spp. isolated from urinary tract infections
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1000)
  AUTHORS   Fadhil,S.A. and Sulaiman,A.I.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-MAR-2023) Biology, University of Mosul, Al-majmoaa
            street, Mosul 41002, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..1000
                     /organism="Enterococcus sp."
                     /mol_type="genomic DNA"
                     /strain="SaAw1"
                     /db_xref="taxon:35783"
                     /country="Iraq"
     rRNA            <1..>1000
                     /product="16S ribosomal RNA"
ORIGIN
        1 ttgctccatc gaaagaaaaa gagtggcgaa cgggtgagta acacgtgggt aacctgccca
       61 tcagaagggg ataacacatg gaaacaggtg ctaataccgt ataacactat tttccgcatg
      121 gaagaaagtt gaaaggcgct tttgcgtcac tgatggatgg acccgcggtg cattagctag
      181 ttggtgaggt aacggctcac caaggcaacg atgcatagcc gacctgagag ggtgatcggc
      241 cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtagg gaatcttcgg
      301 caatggacga aagtctgacc gagcaacgcc gcgtgagtga agaaggtttt cggatcgtaa
      361 aactctgttg ttagagaaga acaaggatga gagtaaaatg ttcatccctt gacggtatct
      421 aaccagaaag ccacggctaa ctacgtgcca gcagccgcgg taatacgtag gtggctagcg
      481 ttgtccggat ttattgggcg taaagcgagc gcaggcggtt tcttaagtct gatgtgatag
      541 ccccccggct aacggggagg ggtgattgga aactgggaga cttgagtgca gaagaggaga
      601 gtggaattcc atgtgtagcg gtgaaatgcg tagatatatg gaggaacacc agtggcgaag
      661 gcggctctct ggtctgtaac tgacgctgag gctcgaaagc gtggggagcg aacaggatta
      721 gataccctgg tagtccacgc cgtaaacgat gagtgctaag tgttggaggg tttccgccct
      781 tcagtgctgc agcaaacgca ttaagcactc cgcctgggga gtacgaccgc aaggttgaaa
      841 ctcaaaggaa ttgacggggg cccgcacaag cggtggagca tgtggtttaa ttcgaagcaa
      901 cgcgaagaac cttaccaggt cttgacatcc tttgacacct ctagagatag agcttcccct
      961 tcgggggcaa agtgacaggt ggtgcatggt tgtcgtcagc
//
```

Figure 3: *Enterococcus spp.* sequencing based on 16S rRNA. Record of this bacterial isolate in the NCBI with the code: OQ660495.1

Enterococcus sp. strain SaAw1 16S ribosomal RNA gene, partial sequence

GenBank: OQ660495.1

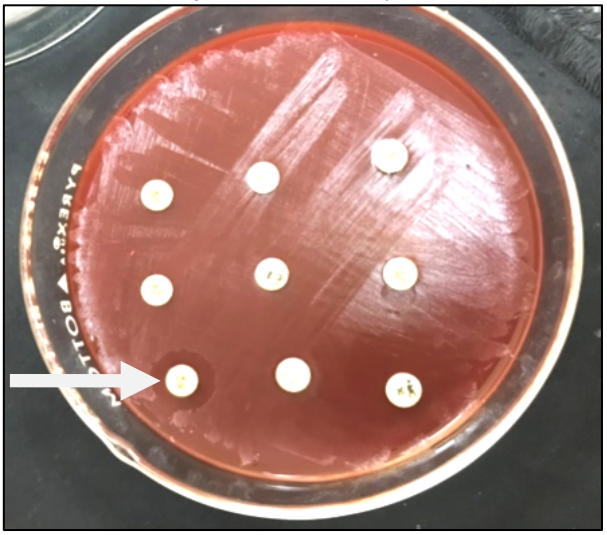
GenBank Graphics

```
>OQ660495.1 Enterococcus sp. strain SaAw1 16S ribosomal RNA gene, partial sequence
TTGCTCCATCGAAAGAAAAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGG
ATAACACATGGAAACAGGTGCTAATACCGTATAACACTATTTTCCGCATGGAAGAAAGTTGAAAGGCGCT
TTTGCGTCACTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACG
ATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTT
CGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGATGAGAGTAAAATGTTCATCCCTTGACGGTATCT
AACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCTAGCGTTGTCCGGAT
TTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAG
GGTGATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCG
TAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGC
GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGG
TTTCCGCCCTTCAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAA
CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGT
GGTGCATGGTTGTCGTCAGC
```

3.3. Antimicrobial Susceptibility:

The bacteria under study showed high resistance to the antibiotics used, except for vancomycin, which exhibited a minimal inhibitory effect at 12 mm, indicating resistance. This is shown in Figure 4.

Figure 4: The Antibiotic Sensitivity Test



Our results align with those of Khalid (2016), who reported that vancomycin-resistant enterococci (VRE) pose a significant challenge among various antibiotic-resistant microbes causing human infections. The pathogenesis of Enterococcus-caused infections is complex, involving processes such as adherence to and colonization of host tissues and resistance to host defense mechanisms (Abriouel *et al.*, 2008). There are indications that these bacteria possess specialized mechanisms of resistance (Zhou *et al.*, 2013).

Table 2: The antibacterial combination between Vancomycin and green tea and strawberry extractions on *Enterococcus spp.* (a) Green tea and (b) Strawberry

(a) Green tea

Type of extraction	The inhibition zone (mm) of green tea extract synergism with vancomycin concentrations (µg/ml)				*p*-value p ≤ 0.05
	12.5	25	50	100	
Acetone	17	16	17	19	0
Ethanol	R	R	R	R	-
Methanol	17	20	15	15	0
Hot boiling D.W.	R	R	R	R	-

(b) Strawberry

Type of extraction	The inhibition zone (mm) of strawberry extract with vancomycin concentrations (µg/ml)				*p*-value p ≤ 0.05
	12.5	25	50	100	
Acetone	R	R	R	R	-
Ethanol	15	18	23	26	0
Methanol	R	R	R	R	-
Hot boiling D.W.	R	R	R	R	-

R = resistance

3.4. Antibacterial Activity of Green Tea and Strawberry Extracts:

The synergistic effects observed between the green tea extract and the antibiotic vancomycin at different concentrations demonstrated that the antibiotic alone did not yield the same results as when combined with the green tea extract. Statistical analysis confirmed a significant difference between the use of the extract with the antibiotic and the antibiotic alone. For instance, an inhibition zone of 20 mm was observed when vancomycin (25 µg/ml) was combined with the methanol-based green tea extract, compared to the use of green tea extract prepared from acetone, which showed significant effects at an antibiotic concentration of 100 µg/ml. In contrast, the ethanolic extract of strawberries exhibited high inhibitory values with vancomycin (50 and 100 µg/ml), resulting in inhibition zones of 23 mm and 26 mm, respectively. Statistical analysis indicated that the combination of the antibiotic with the strawberry extract was significantly more effective than the antibiotic used alone, as illustrated in Table 2 and Figures 5 and 6.

Figure 5: Effect of Green Tea Extracts against *Enterococcus spp.*

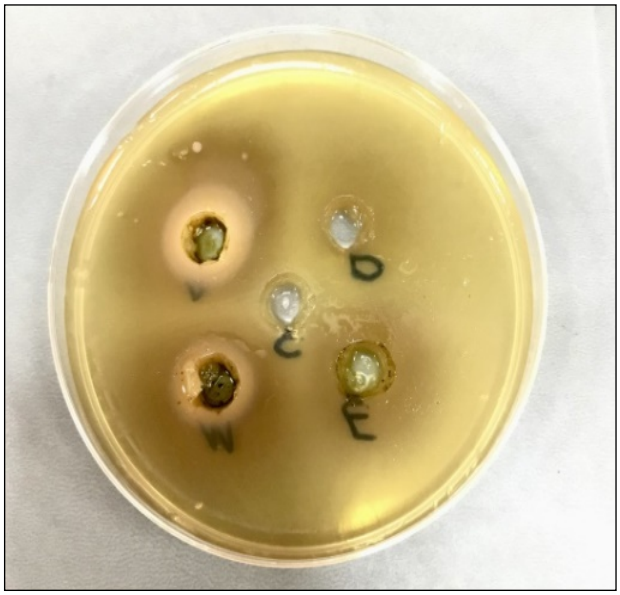
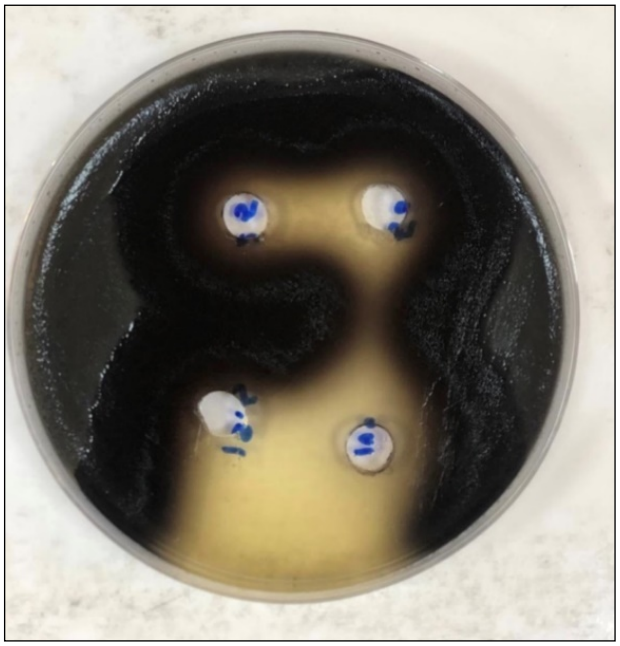


Figure 6: Effect of Strawberry Extracts against *Enterococcus spp.*



Our results align closely with those of Asmah *et al.* (2023), who found that a green tea extract concentration of 25 µg/ml had an inhibitory effect of 16.74 mm, and at 50 µg/ml, it reached 19.98 mm. Flavonoids, present in green tea, play crucial roles as antioxidants, antitumor agents, anti-inflammatory agents, and antimicrobial agents against both bacterial and viral pathogens. Similarly, a study by Liya and Siddique (2018) noted that strawberries, rich in vitamins and phenolic compounds, have ethanolic extracts characterized by significant antibacterial properties, particularly effective against *Enterococcus faecalis*. This supports our observations regarding the potent effect of strawberry ethanol extract on *Enterococcus faecalis*.

4. Conclusion

Urinary tract infections can lead to severe complications, and the use of antibiotics to treat bacterial infections has become widespread. However, the misuse of antimicrobials eventually results in the development of resistant bacterial strains. Therefore, we turned to natural plant extracts to reduce reliance on antibiotics. Both green tea and strawberries have demonstrated antibacterial effects, making this method of treatment worth adopting.

Biographies

Aws Ibrahim Sulaiman

Biology Department of Biology, College of Science, University of Mosul, Mosul, Iraq, 009647503478899, awssbio61@uomosul.edu.iq

Aws is an assistant professor at Mosul University in Iraq. He has published 16 research papers in Scopus-indexed journals and won first place in Iraq for medical and health sciences in the 2014 Iraqi Science Day Award. He has participated in 20 scientific conferences and seminars and 36 workshops and has taught students at all levels. Additionally, he has registered ten bacterial strains. Aws has received 43 letters of thanks and appreciation from the president of the Republic of Iraq.

ORCID: 0000-0002-1739-2763

Saja Anwr Fadhil

Independent Researcher, Mosul, Iraq, 009647508951653, saja.sc47@student.uomosul.edu.iq

Saja, an independent researcher from Mosul, holds a Bachelor of Science in Biology/Microbiology from the Faculty of Science, University of Mosul. She is one of the top-ranking women in her college, holding fifth rank. Saja has received thanks and appreciation from the president of the University of Mosul for registering the isolation of bacterial species for the first time in the NCBI. She has a promising future in research and postgraduate studies.

ORCID: 0009-0004-6893-2004

Acknowledgment

The researchers extend their thanks and gratitude to the Department of Biology, College of Science, University of Mosul, Iraq.

References

Abriouel, H., Omar, N. B., Molinos, A. C., López, R. L., Grande, M. J., Martínez-Viedma, P., Ortega, E., Cañamero, M. M., and Galvez, A. (2008). Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. *International Journal of Food Microbiology*, **123**(1-2), 38–49. DOI: 10.1016/j.ijfoodmicro.2007.11.067

Asmah, N., Mattulada, I. K., and Dodo, A. Z. A. (2023). Antibacterial assay of green tea (Camellia Sinensis L) against the growth of *Enterococcus faecalis*. *Journal of Syiah Kuala Dentistry Society*, **8**(2), 211–216. DOI: 10.24815/jds.v8i2.36627

Atlas, R. (2010). *Handbook of Microbiological Media* (4th ed.). Talyor and Francis Group, an Informa Business, USA.

Basam, B. M., Enas, I. J., Enaam, H. B., Zainab, H. S., and Khitam, A. O. (2016). Antimicrobial effects of Rubus idaeus, Origanum vulgare, Petroselinum crispum, and bacteria infecting urinary tract. *IJOPILS*, **2**(n/a), 1–17.

Cornelissen, C., and Hobbs, M. (2020). *Lippincott Illustrated Reviews: Microbiology* (14th ed.). India: Wolters Kluwer.

Creti, R., Imperi, M., Bertuccini, L., Fabretti, F., Orefici, G., Di Rosa, R., and Baldassarri, L. (2004). Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. *Journal of Medical Microbiology*, **53**(1), 13–20. DOI: 10.1099/jmm.0.05353-0

Ghazvinian, M., Asgharzadeh, M. S., Gholami, M., Amir, G. S., Amiri, E., and Goli, H. R. (2024). Antimicrobial resistance patterns, virulence genes, and biofilm formation in enterococci strains collected from different sources. *BMC Infectious Diseases*, **24**(1), 274. DOI: 10.1186/s12879-024-09117-2

Hashem, Y. A., Abdelrahman, K. A., and Aziz, R. K. (2021). Phenotype–genotype correlations and distribution of key virulence factors in *Enterococcus faecalis* isolated from patients with urinary tract infections. *Infection and Drug Resistance*, **14**, 1713–1723. DOI: 10.2147/IDR.S305167

Hashem, Y. A., Amin, H. M., Essam, T. M., Yassin, A. S., and Aziz, R. K. (2017). Biofilm formation in enterococci: Genotype-phenotype correlations and inhibition by vancomycin. *Scientific Reports*, **7**(1), 1–12. DOI: 10.1038/s41598-017-05901-0

Heidari, H., Hasanpour, S., Ebrahim-Saraie, H., and Motamedifar, M. (2017). High incidence of virulence factors among clinical *Enterococcus faecalis* isolates in Southwestern Iran. *Infection & Chemotherapy*, **49**(1), 51. DOI: 10.3947/ic.2017.49.1.51

Humphries, R., Bobenchik, A. M., Hindler, J. A., and Schuetz, A. N. (2021). Overview of changes to the Clinical and Laboratory Standards Institute performance standards for antimicrobial susceptibility testing, M100. *Journal of Clinical Microbiology*, **59**(12), e00213-21. DOI: 10.1128/JCM.00213-21

Jasni, A. S., Mullany, P., Hussain, H., and Roberts, A. P. (2010). Demonstration of conjugative transposon (Tn 5397)-mediated horizontal gene transfer between Clostridium difficile and *Enterococcus faecalis*. *Antimicrobial Agents and Chemotherapy*, **54**(11), 4924–4926. DOI: 10.1128/AAC.00496-10

Khalid, H. M. (2016). Molecular detection of virulence factors of *Enterococcus faecalis* isolated from urine samples in Duhok City, Kurdistan Region/Iraq. *Science Journal of University of Zakho*, **4**(1), 63–72.

Liya, S. J., and Siddique, R. (2018). Determination of antimicrobial activity of some commercial fruit (apple, papaya, lemon, and strawberry) against bacteria causing urinary tract infection. *European Journal of Microbiology and Immunology*, **8**(3), 95–99. DOI: 10.1556/1886.2018.00014

Mahon, C. R., and Lehman, D. C. (2022). *Textbook of Diagnostic Microbiology-E-Book* (7th ed.). USA: Elsevier Health Sciences.

Murray, P. R., Rosenthal, K. S., and Pfaller, M. A. (2015). *Medical Microbiology*. USA: Elsevier Health Sciences.

Neeva, N. I., Zafrin, N., Jhuma, A. A., Chowdhury, S. K., Fatema, K., and Rifat, T. A. (2024). Antimicrobial susceptibility patterns of Enterococcus species and molecular detection of *Enterococcus faecalis* isolated from patients with urinary tract infection in a tertiary care hospital in Bangladesh. *Indian Journal of Microbiology*, **n/a**(n/a), 1–10. DOI: 10.1007/s12088-024-01216-7

Reygaert, W. C. (2017). An update on the health benefits of green tea. *Beverages*, **3**(1), 6. DOI: 10.3390/beverages3010006

Saifi, M., Pourshafie, M., Eshraghian, M., and Dallal, M. (2008). Antimicrobial resistance of Enterococci isolated from urinary tract infection in Iran. *Iranian Biomedical Journal*, **12**(3), 185–190.

Santosh, K., and Satya, N. (2010). Herbal remedies of wetlands macrophytes in India. *International Journal of Pharma and Bio Sciences*, **1**(2), n/a.

Sulaiman, A. I., and Saad, A. (2024). Detection of Bacteroides fragilis LuxR gene, involved in quorum sensing, among colitis patients in Mosul, Iraq. *Journal of Applied and Natural Science*, **16**(1), 118–122. DOI: 10.31018/jans.v16i1.4716

Tille, M. P. (2017). *Bailey & Scott's Diagnostic Microbiology* (14th ed.). USA: Elsevier.

Wang, L., Zhang, T., Huo, J., Wang, Y., Lu, Y., and Zhu, X. (2024). Rapid and specific detection of Enterococcus faecium with an isothermal amplification and lateral flow strip combined method. *Archives of Microbiology*, **206**(1), 28. DOI: 10.1007/s00203-023-03758-8

Widyarman, A. S., Widjaja, S. B., and Idrus, E. (2017). Strawberry extract's effects on *Enterococcus faecalis* and Porphyromonas gingivalis biofilms in vitro. *Scientific Dental Journal*, **1**(1), 1–5. DOI: 10.26912/sdj.v1i1.1911

Zhou, X., Wang, X., Guo, B., and Wang, X. (2013). Isolation and identification of *Enterococcus faecalis* and detection of its virulence factor genes in lambs presenting with encephalitis in Xinjiang Province, China. *African Journal of Microbiology Research*, **7**(20), 2238–2244. DOI: 10.5897/AJMR12.912