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Natural Antimicrobial and Wound Healing Properties of Grape and Raisin Extracts: Insights from In Vitro Assays

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ABSTRACT

Background & Objective: This study examines the in vitro antimicrobial properties of black and yellow grape juice and raisin extracts against 12 Gram-positive and Gram-negative bacterial strains, as well as five *Candida* species.

Materials & Methods: Cell viability was assessed by MTT assay, antibacterial activity by disk diffusion, MIC and MBC tests, biofilm inhibition by a microtiter plate method, and wound healing potential by a scratch assay.

Results: MTT assay showed that black and yellow grape juices and yellow raisins were non-toxic up to 30 µg/mL, while black raisins were safe only up to 15 µg/mL. Disk diffusion demonstrated antibacterial activity of all extracts against most tested bacterial except *A. baumannii* and Methicillin-resistant *S. aureus*. The strongest inhibition zones were observed for *P. aeruginosa*, *Enterococcus*, *S. epidermidis*, *L. monocytogenes*, and *S. maltophilia*, varying by extract type, while *E. coli* and *E. coli* O157 showed the lowest sensitivity. None of the extracts showed antifungal activity against *Candida* species. The lowest MIC observed for the yellow and black raisin extracts was 15 µg/mL against *Enterococcus* and *S. maltophilia*. In the yellow and black grape juice, the lowest MIC against *S. epidermidis, Enterococcus*, and *S. maltophilia* was 30 µg/mL. Black grape juice showed the greatest inhibition of *P. aeruginosa* biofilm formation. Both yellow and black grape juices promoted wound healing.

Conclusion: Grape extracts may serve as promising natural sources for the development of novel antibacterial agents against the tested Gram-positive and Gram-negative bacteria.

Keywords: *Antibacterial activity, Biofilm inhibition, Grape extracts, Natural compounds, Wound healing*

How to Cite This Article:

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1. Introduction

To date, multiple antimicrobials have been developed, which are essential drugs used to treat mild to severe infections (1). While these antimicrobials have played a pivotal role in medical development, a growing and alarming concern in 21st-century medicine is the increasing resistance to antibiotics, which has emerged as one of the most significant problems in public health (2). This poses a threat to the community and hospitals in the treatment of common infections (3). Annual deaths worldwide due to antimicrobial resistance (AMR) reveal at least 1.27 million deaths per year and could rise even higher, to 10 million deaths a year by 2050 (4). Additionally, multi-drug resistance (MDR) of pathogenic bacterial strains to modern antibiotics has developed over the past decades (5), accounting for hundreds of thousands of infections and significant mortality (6). Therefore, the search for alternative treatments, including herbal drugs, has become a focus of research (7).

Today, in many parts of the developing world, between 70% and 95% of people continue to rely on traditional medicine (TM), and many countries have integrated herbal medicine into their mainstream healthcare systems through regulations (8). Several studies have investigated the effectiveness of plant extracts and secondary metabolites that are endowed with antimicrobial properties, either alone or in combination with existing drugs (9-12). The low cost, biocompatibility, and effectiveness of herbal drugs make them an attractive source for the development of antimicrobial agents that enhance the biological activity of existing drugs (13, 14). Examples of medicinal plants with broad-spectrum antimicrobial activities include *Allium sativum* (garlic), *Azadirachta indica* (neem), *Curcuma longa* (15).

Grapes (*Vitis vinifera* L.) and their derivatives, including juice, pomace, and raisins, are rich sources of polyphenols such as proanthocyanidins, flavonoids, and resveratrol. These compounds exhibit antioxidant, anti-inflammatory, anticancer, antibacterial, and antifungal activities. Previous studies have shown that grape polyphenols can interact with bacterial cell walls, alter membrane permeability, inhibit microbial enzymes, and reduce biofilm formation (16). Therefore, evaluating the antimicrobial effects of grape and raisin extracts provides insights into their potential as natural alternatives or adjunctive therapies for treating infectious diseases. Furthermore, in addition to their antimicrobial activity, grape extracts have been reported to promote wound healing by enhancing fibroblast proliferation, stimulating angiogenesis, and modulating inflammatory responses. This dual functionality makes grape and raisin extracts promising candidates for applications in both infection control and tissue repair (17).

This study aimed to evaluate the effects of black and yellow grape juices, as well as raisin extracts, on wound healing and their antibacterial and antifungal activities. Additionally, the cytotoxicity of these extracts in fibroblast cells and their effects at safe concentrations on standard bacterial strains, clinical isolates, biofilm formation, and wound healing were also assessed.

2. Materials and Methods

2.1 Extracts

Black and yellow raisins from Ilam Gardens were purchased from a local grocery store. Subsequently, the raisins were shade-dried and then then dried in a hot-air oven. Fifty grams of dried powdered raisins were added in 320 mL of hydroalcoholic solution (70/30) and then shaken (140 rpm, room temperature) for 72 hours. Afterwards, the extract was filtered through Whatman No. 1 filter paper (S&S, Germany).

Subsequently, the solvent was removed using a rotary evaporator and the remaining extract was dried in forced-air drying oven for 48 hours. The concentrated extract was preserved in a sterile 50 mL Falcon tube at 4°C.

Fresh black and yellow grapes were manually crushed and filtered, and aliquots of the juice were stored at -20°C. Before use, juice samples were thawed and filtered through a 0.22 µm membrane filter.

The extracts were adjusted to pH 7.4 and diluted in the culture medium to achieve the desired concentrations for all cell culture experiments.

This standardized procedure ensured reproducibility and suitability for in vitro assays.

2.2 MTT assay

Vero cells, a fibroblast-like cell line, were used for the cytotoxicity assay. These cells were obtained from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran).

The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Gibco, USA), penicillin, and streptomycin (60 µg/mL).

Cells were incubated at 37°C with 5% carbon dioxide (CO_2).

After 24–48 hours, the cells formed a monolayer and were subsequently passage into 96-well plates. Then, nine concentrations of the extracts, 60, 30, 15, 7.5, 3.75, 1.8, 0.93, 0.46, and 0.23 µg/mL, were added to the culture medium. The cytotoxicity was evaluated after 24, 48, and 72 hours using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay **(18)**.

2.3 Bacterial strains

Gram-positive bacterial strains including Methicillin-resistant *Staphylococcus aureus* (ATCC700698), Methicillin-sensitive *Staphylococcus aureus* (ATCC 29247), *Listeria monocytogenes* (ATCC 35152), Group D non-enterococcal streptococcus (ATCC 33317), *Staphylococcus epidermidis* (ATCC 14990) and Gram-negative bacterial strains: *Pseudomonas aeruginosa* (ATCC 15442) , *Stenotrophomonas maltophilia* (ATCC 13637), *Acinetobacter baumannii* (ATCC 17978), *Salmonella* Typhimurium (ATCC 13311), *Escherichia coli* (ATCC 25922), *Escherichia coli* O157 (ATCC 43894) and clinical isolates of *Helicobacter pylori* isolated from biopsies at the department of Medical Microbiology, Ilam University of Medical Sciences, were included to the survey. In addition, five fungal species were included in the study: *Candida glabrata* (ATCC 90030), *Candida parapsilosis* (ATCC 7330), *Candida albicans* (ATCC 14053), *Candida tropicalis* (ATCC MYA-3404), and *Candida krusei* (ATCC 6258).

2.4 Isolation of *H. pylori*

A homogenized suspension of antral biopsy specimens in sterile normal saline was prepared for the isolation of *H. pylori*. This suspension was then inoculated onto Brucella medium (MIRMEDIA, Iran) plates with 7% sheep blood, supplemented with the antibiotics including Vancomycin (10 mg/L), Trimethoprim (5 mg/L), and Polymyxin B (2.5 IU) to inhibit the growth of contaminating bacteria, under microaerophilic conditions using Anaerocult C (Merck, Germany) at 37°C. The plates were incubated for 48 hours; subsequently, oxidase, urease, and catalase tests, as well as Giemsa and Gram staining, confirmed the identification of the bacteria as *H. pylori* (19).

2.5 Bacterial strains and growth conditions

Stock cultures of the available bacterial strains in the microbiology collection were used to prepare 24-hour cultures on Mueller-Hinton agar (MIRMEDIA, Iran). Cultures were then incubated at 37°C. Due to the specific growth requirements of *H. pylori*, Brucella agar (MIRMEDIA, Iran) supplemented with sheep blood and appropriate antibiotics, along with GasPak Type C, was used to generate microaerophilic conditions. After 48 hours of incubation, and upon confirming adequate bacterial growth and the absence of transition to the coccoid form, the bacteria were prepared for subsequent experiments.

Disks for the disk diffusion assay (Padtan Teb, Iran) were prepared from 7.5, 15, and 30 µg/mL of black and yellow grape juices and raisins. Suspensions of all microorganisms were prepared to a turbidity equivalent to the 0.5 McFarland standard. Then, all bacterial strains were cultured on Mueller-Hinton agar, and *H. pylori* was cultured in Brucella agar with 7% sheep blood. For each bacterial strain, three disks containing the different grape extracts, along with one blank disk serving as the negative control, were placed on each agar plate. Control disks containing the appropriate reference antimicrobial agents were included for each bacterial strain and fungal species to compare the inhibition zones produced by the extracts with those of the corresponding standard antimicrobial agents .Tetracycline was used for *H. pylori,* Erythromycin for *A. baumannii,* Oxamycin for *S. epidermidis,* Methicillin-sensitive and methicillin-resistant *S. aureus,* Gentamicin for *L. monocytogenes, Enterococcus, P. aeruginosa,* Chloramphenicol for *E. coli, E. coli* O157*, S.* Typhimurium*, S. maltophilia* and finally for all five fungi amphotericin B. After 24 hours of incubation for the bacterial and fungal strains and 48 hours for *H. pylori*, the diameters of the inhibition zones for control disks and disks with different concentrations of extracts were measured (20). Results were evaluated and analyzed using two-way ANOVA, and the susceptibility of the bacterial and fungal strains and fungal species to extract disks was reported.

2.6 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The antimicrobial activity of juice and raisin extracts was examined against bacterial strains that were susceptible to the extracts in the disk diffusion test, using the broth macrodilution method to determine the MIC and MBC, as recommended by the according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20).

2.7 Biofilm

To study the inhibitory effect of the extracts on *P. aeruginosa* biofilm formation, a microtiter plate-based method was employed. In this method, 96-well flat-bottom microplates (SPL, Korea) were used. An 18-hour culture of *P. aeruginosa* culture was used to prepare a microbial suspension in Luria-Bertani (LB) broth (MIRMEDIA, Iran). The turbidity of the suspension was adjusted to an absorbance (OD600) of 0.4–0.6 by measuring its absorbance via a spectrophotometer at 600 nm. A total volume of 190 µL, consisting of the extract and LB broth, was dispensed into each well, followed by the addition of 10 µL of the bacterial suspension. The final concentrations of the extracts in the wells were 120, 60, 30, 15, 7.5, 3.75, 1.8, 0.93, and 0.45 mg/mL. Each extract dilution was tested in duplicate. LB broth was used as a negative control. The microplate was incubated at 37°C with shaking at 30 rpm for 18-20 hours. After incubation, the contents of the wells were gently discarded and rinsed

with distilled water. The wells were stained with 0.1% crystal violet (Merck, Germany) for 10 minutes at room temperature and then washed three times with distilled water. In the final stage, 200 µL of 95% ethanol (Merck, Germany) was added to each well. The absorbance was measured at 492 nm using an ELISA reader (21).

2.8 Wound Healing activity

Three final concentrations of the extracts (7.5, 15, and 30 µg/mL) were prepared in DMEM. Fibroblast cells were cultured in a six-well plate (SPL, Korea) at a density of 5 × 10^4 cells per well. The monolayer was gently scratched with a sterile 1 mL pipette tip. After scratching, the medium was removed, and the wells were washed twice with PBS. Fresh medium containing 5% FBS (Gibco, USA) and corresponding treatments was added to each well. Plates were incubated at 37°C with 5% CO_2. After 6, 8, 12, 14, 16, 18, 24, 36, and 48 hours of incubation, scratch closure was evaluated. In this test, the width of the scratches on the plate was measured and considered as a positive control and untreated scratched cells represented the control (22).

3. Result

3.1 MTT assay

The results of the cytotoxicity test are presented in Table 1. The cytotoxicity dose-response in 24 to 72 hours indicated that black and yellow juice, as well as yellow raisins, share the same non-toxic dose at 30 µg/mL, followed by black raisins at 15 µg/mL. Cell viability showed no significant difference between black and yellow juice at non-toxic doses ($P > 0.05$).

3.2 Disk diffusion

The grape juice and raisin extracts were tested against Gram-positive and Gram-negative bacterial strains, as well as five fungi, for their antimicrobial activities.

Table 1: Non-toxic concentrations of grape and raisin extracts in MTT assay

Extract	Non-toxic dose (µg/mL)	Mean Cell Viability (%) ± SD
Black Juice	30	2.3 ±92.5
Yellow Juice	30	2.0 ±93.2
Black Raisins	15	2.8 ±91.1
Yellow Raisins	30	2.1 ±92.8

Disk diffusion tests were performed for all four types of extracts at concentrations of 7.5, 15, and 30 µg/mL. All bacteria, except *A. baumannii* and Methicillin-resistant *S. aureus,* showed zones of inhibition. The maximum diameter growth of inhibition zone in millimeters (mm) for black raisin extracts in the concentration of 30 µg/mL belonged to (*P. aeruginosa*~25 mm), (*L. monocytogenes*~24 mm), (*Enterococcus*~22 mm), (*S. maltophilia*~21 mm), (*S. epidermidis*~20 mm), for yellow raisin extracts maximum growth was observed in (*Enterococcus*~30mm), (*P. aeruginosa*~28 mm), (*S. epidermidis*~24 mm), (*L. monocytogenes*~23 mm), regarding the yellow juice the maximum rate was visible in (*P. aeruginosa* and *Enterococcus*~29 mm), (*L. monocytogenes*~25 mm), (*S. maltophilia*~23 mm), and finally for black grape extract the maximum rate was dominant in (*P. aeruginosa*~26 mm) and (*Enterococcus* and *S. epidermidis*~20 mm).

The minimum growth rate, as indicated by the inhibition zone diameter, was observed for all four extracts at different concentrations *in E. coli* and *E. coli* O157. At a concentration of 7.5 µg/mL, the three extracts (yellow raisin, black raisin, and yellow raisin juice) showed no inhibitory effect against *E. coli* O157. The highest average sensitivity among the bacterial strains was observed in *Enterococcus* toward the yellow raisin extracts, and the lowest sensitivity belonged to *E. coli* O157 in yellow grape juice (Figure 1). As shown in Figure 2, among the tested extracts, black raisin and yellow grape juice exhibited the highest overall inhibitory effects. Among the bacterial strains, *P. aeruginosa* and *Enterococcus* were the most susceptible to all extracts. The zone of inhibition produced by two bacterial strains, *S. maltophilia*, and *E. coli*, are shown in Figure 3.

The results of Figure 4 show the relationship between increased concentration and the growth inhibition zone.

The anti-fungal activity of the extracts against C.glabrata, C.parapsilosis, C.albicans, C. tropicalis, and C.krusei was not detected; therefore, the tests were continued only with the sensitive bacterial strains.

3.3 MIC and MBC

The MIC values of all the plant extracts against the microorganisms are shown in Table 2. The concentrations of the plant extracts considered for the MIC assay were 7.5, 15, 30, 60, 120, and 240 µg/mL. These extracts exhibited antibacterial activity against the tested microorganisms, although the degree of activity varied among the bacterial species. The lowest MIC (highest antibacterial activity) for yellow and black raisin extracts

was 15 µg/mL against *Enterococcus*, and *S. maltophilia*. In the yellow and black grape juice, the lowest MIC for *S. epidermidis*, *S. maltophilia,* and *Enterococcus* was observed at a concentration of 30 µg/mL. The highest MIC value (240 µg/mL), indicating the lowest antibacterial activity, was observed for both yellow and black raisin extracts against *E. coli* O157.

Results from the MBC assay supported the data obtained from the disk diffusion assay and MIC assay (Table 3).

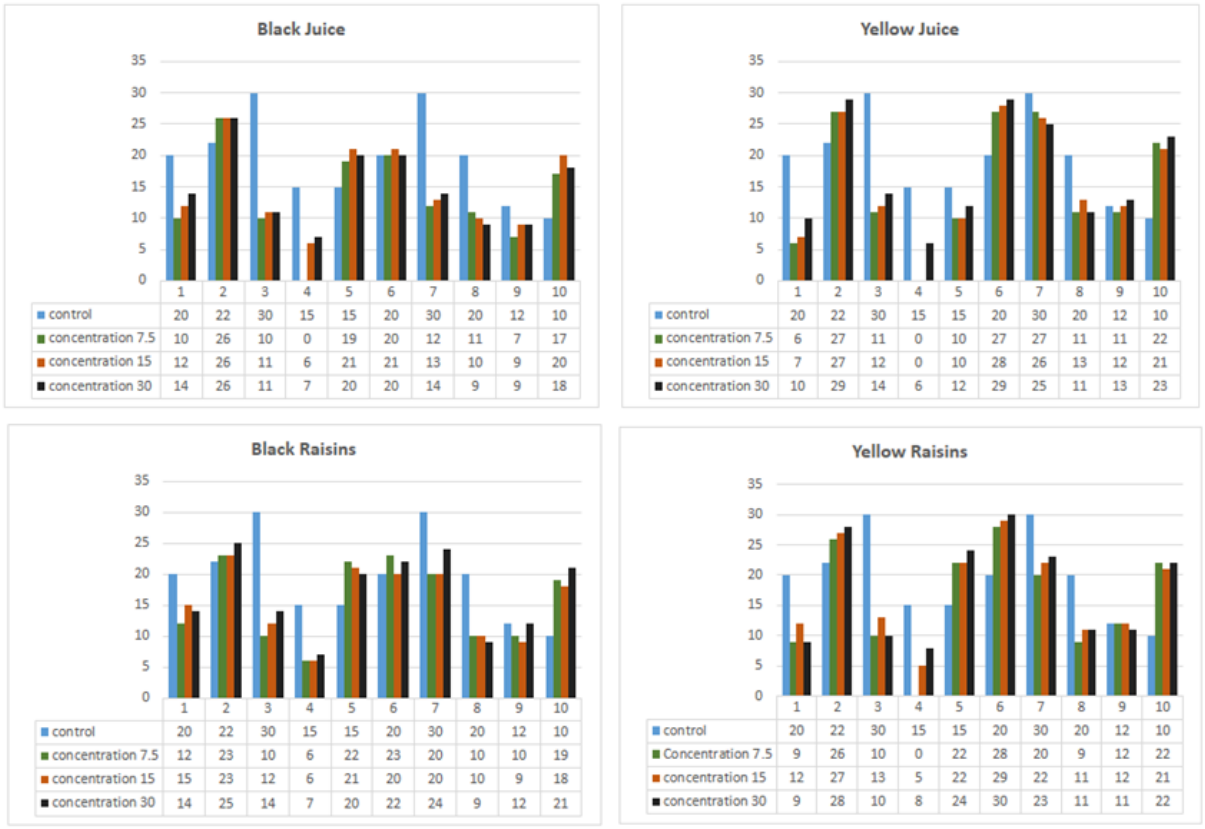

Figure 1. Growth rate of inhibition zone diameter. Bacteria name: 1. Helicobacter pylori, 2. Pseudomonas aeruginosa, 3. Escherichia coli, 4. Escherichia coli O157, 5. Staph epidermidis, 6. Enterococcus, 7. Listeria monocytogenes, 8. Methicillin sensitive staph aureus, 9. Salmonella typhimurium, 10. Stenotrophomonas maltophilia. **(Prepared by Authors, 2026)**.

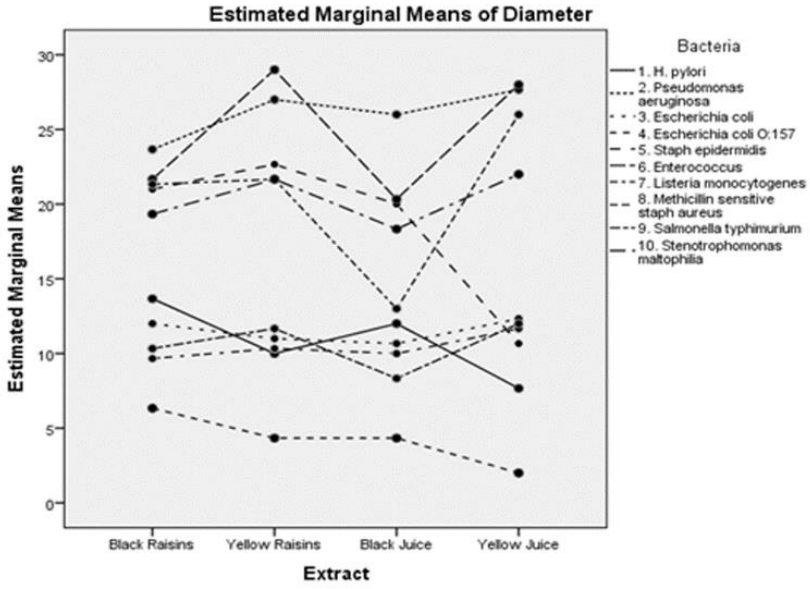

Figure 2. Estimated marginal means of inhibition zone diameters for 10 bacterial strains exposed to four grape-derived extracts (Black Raisins, Yellow Raisins, Black Juice, and Yellow Juice. **(Prepared by Authors, 2026)**.

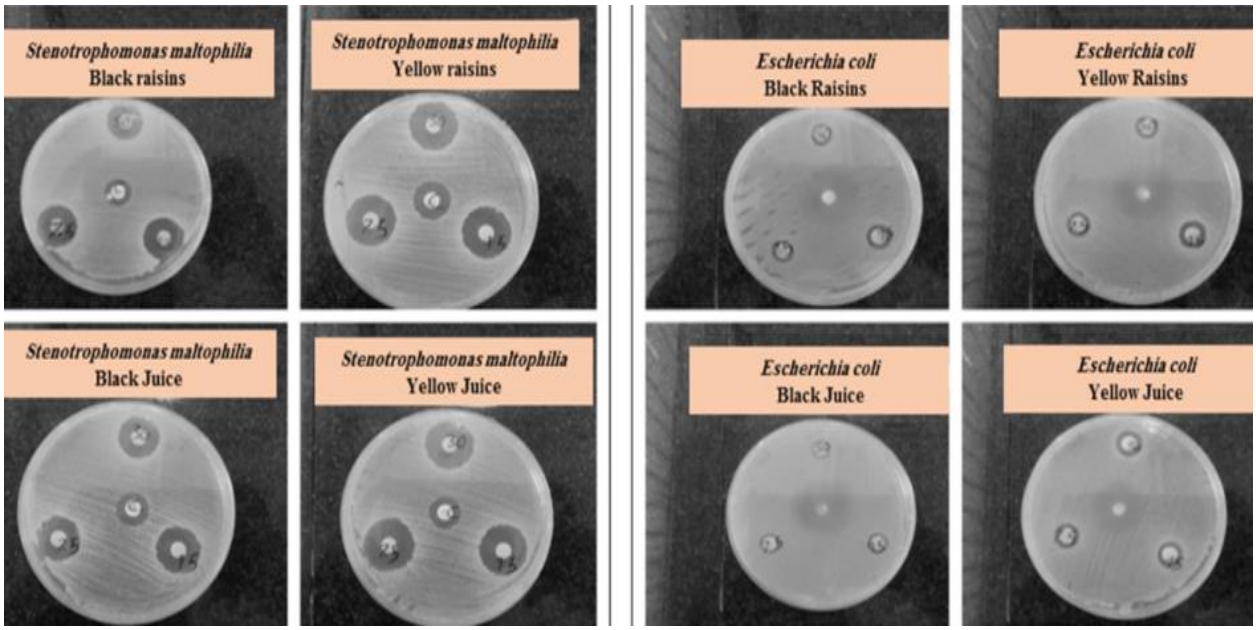

Figure 3. The diameter of inhibition zone in Stenotrophomonas maltophilia and E.coli. Left box bacteria: Stenotrophomonas maltophilia, Right box bacteria: Escherichia coli. First row in each box from left to right: Black raisins, raisins; Second row in each box from left to right: Black juice, yellow juice. **(Prepared by Authors, 2026)**.

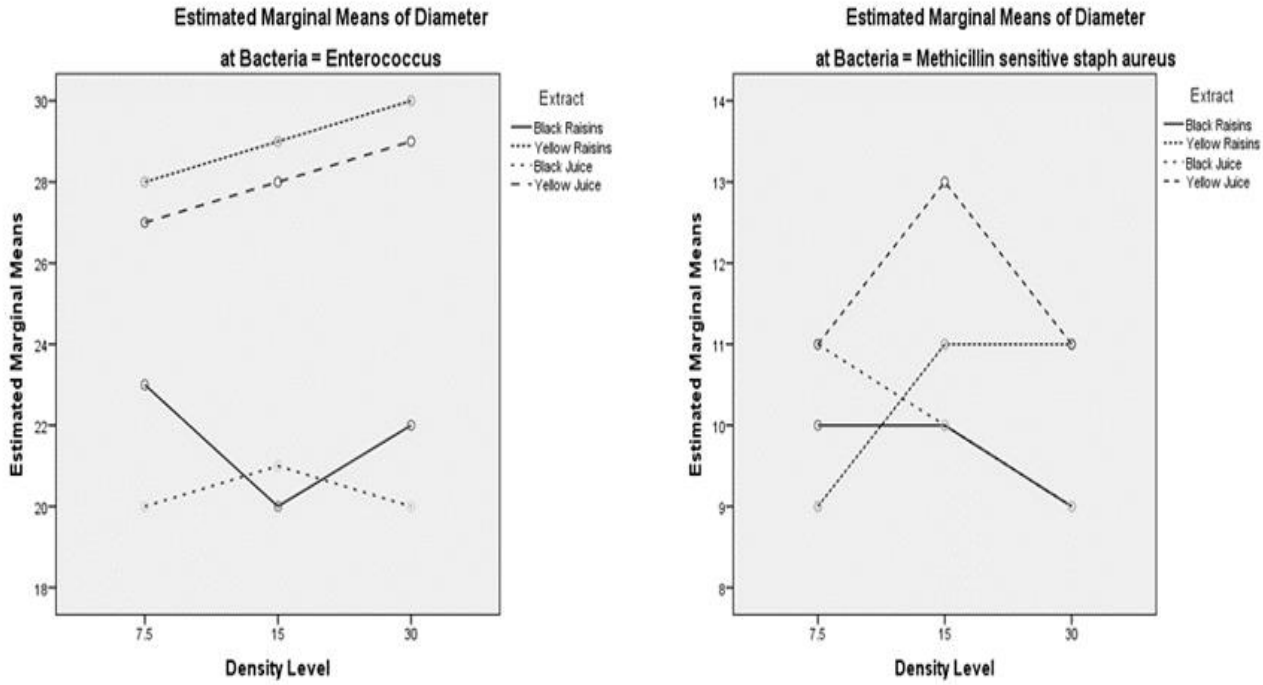

Figure 4. Relationship between increased concentration and growth inhibition zone. **(Prepared by Authors, 2026)**.

Table 2. Minimum inhibitory concentrations of the plant extracts against the pathogenic organisms

Plant extracts	Bacteria species / Concentration of Plant extracts (µg/mL)	*Helicobacter pylori*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Escherichia coli O157*	*Staph epidermidis*	*Enterococcus (sterp D Non Enterococcus)*	*Listeria monocytogenes*	Methicillin sensitive staph aureus	*Salmonella typhimurium*	*Stenotrophomonas maltophilia*
Black Juice	240	+	+	+	+	+	+	+	+	+	+
	120	+	+	+	+	+	+	+	+	+	+
	60	+	+	+	-	+	+	-	+	+	+
	30	-	-	+	-	+	+	-	-	-	+
	15	-	-	-	-	-	-	-	-	-	-
	7.5	-	-	-	-	-	-	-	-	-	-
Black Raisins	240	+	+	+	+	+	+	+	+	+	+
	120	+	+	+	-	+	+	+	+	+	+
	60	+	+	-	-	+	+	+	+	-	+
	30	-	-	-	-	+	+	-	-	-	+
	15	-	-	-	-	-	+	-	-	-	+
	7.5	-	-	-	-	-	-	-	-	-	-
Yellow Juice	240	+	+	+	+	+	+	+	+	+	+
	120	+	+	+	+	+	+	+	+	+	+
	60	-	-	-	-	+	+	-	-	+	+
	30	-	-	-	-	+	+	-	-	-	+
	15	-	-	-	-	-	-	-	-	-	-
	7.5	-	-	-	-	-	-	-	-	-	-
Yellow Raisins	240	+	+	+	+	+	+	+	+	+	+
	120	+	+	+	-	+	+	+	+	+	+
	60	+	+	+	-	+	+	+	-	-	+
	30	-	-	+	-	+	+	-	-	-	+
	15	-	-	-	-	-	+	-	-	-	+
	7.5	-	-	-	-	-	-	-	-	-	-
MHB alone		NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
MHB + plant extract		NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
MHB + Test organisms		GO	GO	GO	GO	GO	GO	GO	GO	GO	GO

Note: Negative control (MHB alone or MHB + plant extract), NG: No growth

Positive control (MHB + test organisms), GO: Growth observed

MHB: Mueller Hinton Broth

Table 3. The result of MBC assay

	Bacteria species	Plant extracts	MBC of plant extracts (µg/mL)		Bacteria species	Plant extracts	MBC of plant extracts (µg/mL)
1	*Helicobacter pylori*	Black Juice	30	6	*Enterococcus*	Black Juice	30
		Yellow Juice	60			Yellow Juice	60
		Black Raisins	30			Black Raisins	15
		Yellow Raisins	30			Yellow Raisins	7.5
2	*Pseudomonas aeruginosa*	Black Juice	30	7	*Listeria monocytogenes*	Black Juice	60
		Yellow Juice	60			Yellow Juice	60
		Black Raisins	30			Black Raisins	30
		Yellow Raisins	30			Yellow Raisins	30
3	*Escherichia coli*	Black Juice	15	8	*Methicillin sensitive staph aureus*	Black Juice	30
		Yellow Juice	60			Yellow Juice	60
		Black Raisins	60			Black Raisins	30
		Yellow Raisins	15			Yellow Raisins	60
4	*Escherichia coli O157*	Black Juice	60	9	*Salmonella typhimurium*	Black Juice	30
		Yellow Juice	60			Yellow Juice	30
		Black Raisins	120			Black Raisins	60
		Yellow Raisins	120			Yellow Raisins	60
5	*Staph epidermidis*	Black Juice	15	10	*Stenotrophomonas maltophilia*	Black Juice	15
		Yellow Juice	15			Yellow Juice	15
		Black Raisins	15			Black Raisins	7.5
		Yellow Raisins	15			Yellow Raisins	60

3.4 Inhibitory effect on biofilm

The inhibitory effects of extracts were evaluated for *P.aeruginosa* (Table 4).

At a concentration of 120 µg/mL (which is higher than the concentrations obtained from the MBC assay), black juice displayed the foremost activity in suppressing biofilm formation at an OD of 0.005. However, cytotoxic concentrations of the other two extracts, including yellow juice and black raisin extracts, were not significantly effective in inhibiting the biofilm formation. In this test, *P. aeruginosa* in LB broth was used as the control, which showed in 0.014 OD. At concentrations of 30 µg/mL and above, black juice showed the lowest OD, followed by yellow raisin extract, with an OD below 0.013. The lowest OD was at 0.005 in 120 µg/mL, indicating the maximum biofilm inhibition for black juice.

Table 4. The Pseudomonas aeruginosa biofilm inhibition presented for each extract corresponding to different concentrations.

Biofilm inhibition				
Concentrations	**Black Juice**	**Yellow Juice**	**Black Raisin**	**Yellow Raisin**
120	0.005	0.017	0.019	0.010
60	0.010	0.020	0.017	0.012
30	0.011	0.022	0.016	0.013
15	0.014	0.025	0.021	0.018
7.5	0.015	0.027	0.023	0.022
3.75	0.018	0.028	0.024	0.025
1.8	0.023	0.031	0.026	0.027
0.9	0.026	0.033	0.029	0.029
0.045	0.027	0.035*	0.031	0.030

*Represents the maximum inhibition in biofilm growth for *Pseudomonas aeruginosa*.
Control: LB Broth+*Pseudomonas aeruginosa*.

3.5 Wound Healing activity

After evaluating the cytotoxic and antimicrobial activities of the extracts, a scratch assay was carried out on fibroblast cells. None of the extracts at a concentration of 30 µg/mL had no effect on increasing cell proliferation. At lower concentrations of the black and yellow grape juice, an increase in wound-healing activity was observed. Interestingly, the black and yellow raisin extracts did not promote cell proliferation and even appeared to inhibit it; therefore, no scratch closure was observed. Nevertheless, no cell death was observed after seven days. Yellow juice extract, at concentrations of 7.5 and 15 µg/mL, showed greater wound-healing activity than black grape juice and the control on fibroblast cells. It should be noted that scratch closure by yellow juice, black juice, and control samples was evaluated after 12, 16, and 18 hours, respectively. Furthermore, Fibroblast cells exhibited increased proliferation following treatment with these extracts.

4. Discussion

The antibiotic resistance represents an existential threat to the continued control of infections. Unfortunately, it has increased due to overpopulation, the increased use of antibiotics in clinics, and poor sewage disposal systems, among other factors (23).

To overcome the emergence of AMR, various researchers have focused on the antimicrobial activity of plant extracts against different bacteria and resistant strains (24).

Grape (*Vitis spp*.) is one of the most widely grown fruit crops in the world. Its products, such as grape juice, jams, and raisins, are also essential commodities in the global market.

Grape pomace, as the richest source of PCs among fruits, is a potential source of natural antioxidant and antimicrobial agents effective against various bacterial strains (25, 26).

Gram-negative bacteria have an outer membrane of the cell wall made up of structural lipopolysaccharides, so it is impermeable to lipophilic solutions, unlike Gram-positive bacteria, which do not have this outer membrane (27). However, Cueva et al. reported that Gram-negative bacteria were more susceptible than Gram-positive bacteria to grape extracts, suggesting that the antimicrobial activity of grape-derived phytochemicals may depend on their chemical composition and the bacterial species tested rather than solely on the Gram-staining characteristics (28). The study performed by Filocamo et al. also showed that among the Gram-negative bacteria, *E. coli* was the only susceptible strain (MIC and MBC of 2000 µg/mL) to grape juice (29). Silván et al. demonstrated that the grape seed extract had a strong capacity to inhibit *Campylobacter spp* growth (30). In contrast, another study revealed that *S.* Typhimurium and *E. coli* were resistant at all concentrations of grape polyphenols (31). Additionally, a study by Dias et al. found that Gram-positive intestinal pathogens (*L. monocytogenes, S. aureus*, and *Enterococcus faecalis*) are more sensitive to extracts of grape stems than Gram-negative pathogens (*P. aeruginosa, E. coli*, *and Klebsiella pneumoniae*) (32). A recent study shows that using recommended amounts of grape waste extracts can significantly reduce the presence of *S. aureus* and *E. coli* bacterial strains (33).

In this study, the MIC and MBC findings demonstrated that the antibacterial activity of the grape-derived extracts was species-dependent. Lower MIC values observed for *Enterococcus* and *S. maltophilia*, particularly with the raisin extracts, indicate a higher susceptibility of these organisms to the bioactive compounds present in the extracts. In contrast, *E. coli* O157 exhibited the highest MIC values. Among the tested extracts, black raisin and yellow grape juice exhibited the greatest overall inhibitory activity against the tested bacterial isolates. Among the bacterial strains, *P. aeruginosa* and *Enterococcus* were the most susceptible to all extracts. Based on Figure 2, similarities between the average effects of different extract concentrations on bacteria, including *E.coli*, *S.* Typhimurium, methicillin-sensitive *S. aureus,* and *H. pylori*, can be observed. Additionally, the maximum range of the average growth of the inhibition zone was observed for *Enterococcus* (~30 mm) in yellow raisins, and the minimum was for *E. coli* O157 (~3 mm) in yellow juice. In this research, the results of the two-way ANOVA showed, in most cases, no direct link between increasing the rate of each extract concentration and the zone of inhibition produced by the different bacterial strains investigated. In Figure 4, yellow raisins and yellow juice extracts displayed a linear relationship between increased concentration and inhibition zone diameter for *Enterococcus*; however, for the other two extracts, a direct connection was not observed. Methicillin-sensitive *S. aureus* showed no explicit suppression among the increased concentration and inhibition zone. In most countries, nosocomial infections (NIs) are a significant medical problem (34). *P. aeruginosa* was the fourth most common nosocomial infection, following *E. coli*, *K. pneumoniae*, and *S. aureus*, accounting for 7.96% (35). *P. aeruginosa* is an opportunistic pathogen that infects patients with cystic fibrosis, burns wounds, immunodeficiency, chronic obstructive pulmonary disorder (COPD), cancer, and severe infection requiring ventilation, such as coronavirus disease 2019 (COVID-19) (35). Eradication of *P. aeruginosa* has become increasingly complex due to its remarkable capacity to resist antibiotics (36). Phenolic compounds, such as those found in grape extracts, are considered critical natural molecules due to their ability to damage bacterial cell walls and influence biofilm formation in different bacterial strains (37, 38). These results showed that black grape juice reduces biofilm formation, and in addition to black grape juice, extracts from yellow raisins can also effectively prevent biofilm structures formed by bacterial strains. These findings are consistent with other reports on antimicrobial herbal drugs. For example, Thymus vulgaris and Origanum vulgare extracts inhibit bacterial growth by damaging cell walls and interfering with biofilm formation (39). Similarly, Curcuma longa (turmeric) and Allium sativum (garlic) show broad-spectrum antibacterial effects, comparable to the activity observed with grape and raisin extracts (40). The healing of skin wounds, particularly chronic wounds, remains a clinical emergency (22). Grape seed extract displayed remarkable wound-healing activity via accelerated wound closure rate, enhancing Transforming growth factor β1 (TGF-β1), vascular endothelial growth factor (VEGF), collagen expression, and suppressing inflammatory markers such as Tumor necrosis factor α (TNF-α) and Interleukin-1β (IL-1β) (41-43). Hemmati et al. showed that proanthocyanidins, a class of polyphenols, in grape seed (GS) oil trigger the release of VEGF and could be an effective wound healing agent (44). It is interesting to know that the antioxidant potential of proanthocyanidins from grape seeds is 20 times greater than that of vitamin C

and 50 times greater than that of vitamin E (45). The study demonstrated that the yellow and black juice extracts were effective in increasing the proliferation of fibroblast cells. Surprisingly, black and yellow raisins did not affect cell proliferation, and even cell growth was suspended. As mentioned before, after seven days of testing, no cell death was observed. The cause of this phenomenon is obscure. These results, together with our results, indicate the positive role of grape extracts in promoting wound healing. Collectively, our results underscore the potential of grape and raisin extracts, predominantly yellow raisins and black grape juice, as natural agents with antimicrobial and wound healing properties. However, variability in efficacy across extract types and target bacterial strains highlights the need for further phytochemical analysis and in vivo validation. One limitation of the present study is that all experiments were performed in vitro; thus, the observed antimicrobial and wound healing effects may not fully translate to in vivo conditions. Additionally, only a limited number of bacterial and fungal strains were examined, and only selected concentrations of grape and raisin extracts were tested. Further studies are required to investigate dose-dependent effects, different extraction methods, and in vivo efficacy to validate these findings.

5. Conclusion

Grape and raisin extracts, particularly those from yellow raisins and black grapes, exhibited notable antibacterial activities against different bacterial strains and demonstrated wound healing properties with minimal cytotoxicity. These findings support their potential as natural therapeutic agents for combating bacterial infections and promoting tissue repair.

6. Declarations

6.1 Acknowledgments

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6.2 Ethical Considerations

This study did not involve human participants or animals. Therefore, ethical approval was not required.

6.3 Authors' Contributions

S.Aghaei: methodology, writing (original draft), sample collection; K. Heidarzadi: conceptualization; S. Shokri: writing, editing; R. Amini: sample collection, methodology; I. Pakzad, A. Keshavarzi and M. Taherikalani: conceptualization; Sh. Mahmoudvand: review and editing; N. Ansari: methodology; Z. Ramezannia: review and editing; Gh.Kalvandi and F.Azizi Jalilian: materials provision, project administration, methodology; Final approval: All authors.

6.4 Conflict of Interest

The authors declare that they have no conflict of interest.

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6.6 Using Artificial Intelligence Tools (AI Tools)

The authors did not use AI tools

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