Impact of UV-C Exposure on the Chemical Profile and Functional Properties of Grape Seed Oil

Mohammed Ibrahim Alghonaim [a,*] Sulaiman A. Alsalamah [a] Abdulmajeed Y. Alfaifi,[b] Mashael Hakami,[b] Mohammed Khalid Alhazmi,[c] and Hassan I. El Shimi [d]

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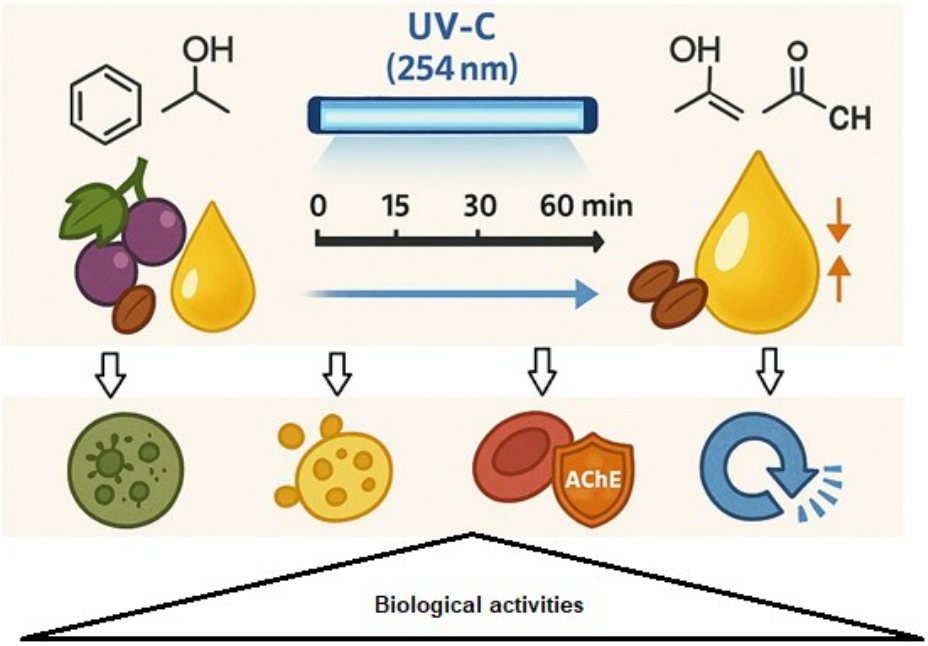
GRAPHICAL ABSTRACT



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The impact of UV-C radiation (25.0 mJ/cm², 253 nm) on grape seed oil was studied relative to varying exposure times (0, 30, and 60 min) to assess structural and biological changes. Gas chromatography-mass spectrometric analysis identified major chemical constituents such as n-hexadecenoic acid, oleic acid, and 2,6-bis(3,4-methylene dioxyphenyl)-3,7-dioxa bicyclo (3.3.0) octane, with trace amounts of other compounds that notably increasing after 60 min of exposure. Antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli,* and *Candida albicans* increased progressively with exposure time, with the 60-min treated oil showing the strongest inhibitory zones, comparable to standard drugs. However, no significant effects were observed against *Penicillium glabrum* and only weak inhibition toward *Salmonella typhi* (10±0.4 mm). Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were markedly reduced after UV-C treatment, highlighting enhanced antimicrobial potency. Furthermore, applying 75% of the MIC from 60-min treated oil markedly elevated its anti-hemolytic activity. Extended UV-C exposure also enhanced anti-inflammatory and anti-Alzheimer effects, suggesting improved therapeutic potential. Overall, results indicated that controlled UV-C irradiation substantially modifies the chemical profile and boosts the biological activities of grape seed oil. Further investigation is required to optimize exposure duration and elucidate the underlying mechanisms.

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Keywords: Grape seed oil; UV-C radiation; Antimicrobial; Antihemolytic; Anti-inflammatory; Anti-Alzheimer

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INTRODUCTION

The grapevine, or *Vitis vinifera* (L.), is an agricultural product of considerable commercial importance. It is a member of the Vitaceae family, which includes 16 genera and around 950 species distributed across the world in temperate circumstances (Ma *et al*. 2021; Ganci *et al*. 2025). Grapes (*V. vinifera* L.) have also been shown to have a variety of nutritious components. Proteins, fats, carbs, minerals, and vitamins are among the nutrients found in different portions of grapes. Grape seeds (*V. vinifera* L.) include diverse nutritional

elements comprising lipids, proteins, carbs, and fiber (Migicovsky *et al*. 2017; Di Pietro *et al*. 2023). Vegetable oil is extracted from grape seeds. Because of their long-known medicinal and cosmetic advantages for skin health, plant-based oils continue to be employed effectively in the pharmacological and cosmetic sectors worldwide (Kapcsándi *et al*. 2021; Di Stefano *et al*. 2022). Important plant components can be preserved and oxidative damage can be avoided with the usage of grape seed oil (Goufo *et al*. 2020).

A significant issue in public health is antimicrobial resistance, which can endanger humans and animals, and result in mortality (Thompson 2022). It results from overuse of antibiotics without seeking advice from experts (Rahman and Sarker 2020). Oils are an important supplier of natural antimicrobials, which enables their use as natural antimicrobial agents in food (Al-Rajhi and Abdelghany 2023; Al-Rajhi *et al*. 2025). There are innovative tools to enhance the antimicrobial capacity of natural oils (Al-Rajhi *et al*. 2024; Alsalamah *et al*. 2025a). Grape seed oil contains many constituents leading to health-promoting qualities that are primarily shown in several research, include anti-inflammatory (Garavaglia *et al*. 2016) and anti-Alzheimer effects (Berahmand *et al*. 2020), and that can collaborate with molecular and cellular pathways.

There are three categories that together constitute the ultraviolet (UV) portion of the electromagnetic spectrum: UV-C (199.0 to 280.0 nm), UV-B (281.0 to 315.0 nm), and UV-A (316.0 to 400.0 nm). Only UV-B and UV-A are able to reach the earth's surface. According to Georgieva and Vassileva (2023) this radiation induces a number of physiological changes in plants across their life cycle and may affect their capacity to react to recognized stressors. However, in the industrial domains of pharmaceutical products and food conservation technologies, UV-C irradiation can be regarded as a safe substitute for thermal processes (Tchonkouang *et al*. 2023). When used alone or in combination with a gentle heating technique, short-wave ultra violet rays (UV-C) provide a promising technical alternative for food preservation (Gürsu 2024).

Although grape berries are naturally exposed to sunlight throughout their development, the outer sheets of the fruit—particularly the skin and pulp—serve as strong protective barriers that absorb and scatter most UV radiation (Kwon *et al*. 2025). Consequently, the penetration of UV rays, particularly in the regions including UV-B and UV-C, into the inner tissues is minimal, leaving the seeds largely shielded from direct exposure. So, the chemical composition of grape seed oil is not meaningly affected by natural UV light under field conditions (Sun *et al*. 2023). In this context, the application of controlled UV-C irradiation in the current investigation provides a deliberate and reproducible means to induce structural and functional alterations in the oil, beyond those that occur naturally throughout fruit development.

The use of UV-C in food processing has several benefits over UV-A or UV-B radiation and other non-thermal methods, including minimal energy consumption (Yemmireddy *et al*. 2022), low operating expenses, and the absence of harmful residues (Salazar *et al*. 2022). Current technological advancements have made UV-C sources, including low-pressure mercury lamps and LED-based systems, commonly available at relatively low cost. Their affordability, energy efficiency, and ease of utilize make them appropriate for both industrial and research utilizations, supporting the practical application of controlled UV-C irradiation to improve the functional characteristics of natural oils (Monteiro *et al*. 2022; Alsalamah *et al*. 2025b).

Currently UV-C light is used to treat only a small number of products. Char *et al*. (2025) state that a number of factors, such as the UV-C source of illumination, reactor

conditions, and the target product—fruit juice or oil—are thought to be obstacles to realizing the technology's enormous potential and making it applicable to a wider range of different items on a commercial basis.

Little research has been done on the usage of UV light throughout various time frames. The goal of the present study was to evaluate how UV-C radiation affected the chemical composition and pharmacological properties of grape seed oil throughout a range of time durations.

EXPERIMENTAL

Oil Source, Media, and Chemicals

Grape seed oil was purchased from a certified Egyptian oil retailer *via* the code 003256, which buys verified items. The microbiological culture media were obtained from BioLab Supply Co. (Giza, Egypt)]. Sigma-Aldrich in Egypt provided the other chemical substances used in this study.

Application of Various Doses of UV-C Radiation

Three containers holding equal amounts (50.0 mL) of the grape seed oil were subjected to a UV-C irradiation dosage of 25.0 mJ/cm^2 and UV-C wavelength of 253.0 nm (commercially available, cost-effective, and widely used in laboratory and industrial applications) at room temperature (35 °C). UV-C irradiation was applied to the first, second, and third portions for varying time durations of 0, 30.0, and 60.0 min, consequently. The device was washed with water and disinfected with a 200.0 ppm hypochlorite solution after each exposure point. A UVC-28 device (UVC, MA, UK) was utilized to modify the radiation level. The applied irradiance was calculated by averaging the light obtained from various sensors in the devise (Alsalamah *et al*. 2025b).

GC-MS Analysis of Different Prepared Forms of Grape Seed Oil

About 0.5 mL of prepared grape seed oil forms were collected for GC-MS analysis. The material was mixed with 5.0 mL of methanol before being put into vials. To evaluate the specimens, a Waters (USA) equipment was utilized. With an injection volume of 1.8 µL, premium typical helium with an approximate level of 99.97% cleanliness was used at a constant rate of flow of 1.3 mL/min. An HP-6 MS column (32.0 m × 0.27 mm × 0.28 µm) was connected to a split-spitless inlet with an MS detector for the investigation. The injector and ion supplier temperature settings were set at 265 and 310 °C, respectively. As a result, the oven procedure was maintained at 97 °C for 1.0 min, increased to 160 °C at an average rate of 37 °C/min, and then maintained for 1.5 min. Then, at an effective rate of 12 °C/min, the core temperature was increased to 200 °C and held there for 2.0 min. At a rate of 12.0 °C/min, the temperature was eventually raised to 310 °C and held there for 5.0 min. The GC analysis took 90.0 min in total. All peak regions were identified and aligned with the Wiley 10 library after the mass spectra EM voltage was set at 200 Rel with an indicated frequency range of 43 to 550 *m*/*z*. The relative percentage of every component was calculated by dividing the mean area of peak by the overall peak region. Mass spectra and the chromatograms were analyzed to obtain the outcomes (Alawlaqi *et al*. 2023).

Antimicrobial Action of Various Forms of Grape Seed Oil

The *in vitro* antimicrobial capacities of grape seed oil samples' were evaluated using a range of test pathogenic microbes, such as bacteria (*Staphylococcus aureus* ATCC29213, *Enterococcus faecalis* ATCC51299, *Escherichia coli* ATCC25922, and *Salmonella typhi* ATCC6539) and fungi (*Penicillium glabrum* ATCC48440 and *Candida albicans* ATCC90028), employing the agar well diffusion method. On Mueller Hinton agar wells, the bacteria were distributed first, while molds were added on malt extract medium. Specimens were then placed over each medium-made well, using a cork borer with radius 0.6 cm. As positive controls, gentamicin (0.07 mg/mL) and fluconazole (0.28 µg/mL) were both administered, with DMSO acting as the bank. Following the 72-h growth phase, the inhibition areas was assessed at 36 °C for bacterial cells, and 27 °C after 7 days for the fungal species under investigation (Alghonaim *et al*. 2025).

Detection of MIC and MBC

The micro-dilution liquid instances and the nutrient-rich solution for bacteria were used to determine each specimen's minimum inhibition concentration (MIC). After diluting samples of grape seed oil, the amounts, which ranged from 0.95 to 1000.0 µg/mL, were determined. One hundred microliters of the constituent-diluted grape seed oil samples in a medium of broth were prepared for each place in the 96-well plate. The inoculations were made using fresh microbe cells that met the visual criteria of the 1.0 McFarland standard. To obtain a limit of 1.6×10^6 CFU/mL, 4.0 µL of sterilized 0.67 of NaCl was added to each hole. Following that, the microbes were cultivated for 72 h for pathogenic bacteria and 7 days for fungi at 36 °C. Additionally, 100 mL of the ultimate positive items, the substrate holding a 100% inhibitory substance and the setting up control microbial population were sub-cultured onto dishes in every hole before minimum bacterial concentration (MBC) was assessed. It was discovered that the MBC had the smallest instances that were unable to sustain microbial development at the appropriate temperature for the duration of the incubation period (Al-Rajhi and Abdelghany 2023).

Detection of Antihemolytic Action of Grape Seed Oil in the Presence of Microbes

Estimates were made of the hemolysin activity of testing samples with the bacteria under investigation at sub-MICs of 25%, 50%, and 75%. For this, the examined bacteria were spun up (22,000× g for 25.0 min) using different sub-MIC dosages and settled to an OD_{600} of 0.4. After mixing 500 µL of supernatants with new erythrocyte solution (2.0%) in 0.9 mL of saline, the mixture was allowed to stand for 2.0 h at 38 °C before being centrifuged for 12 min at 6 °C and 12,000× g. By addition of 0.1% sodium dodecyl sulphate to the erythrocyte suspension, a positive control (PC) of full hemolysis was created.

The erythrocytes were then incubated in Luria-Bertani (LB) broth under equivalent settings to generate a negative control (NC) of un-hemolyzed erythrocytes. The hemoglobin discharge was estimated using the absorbing capacity at 545 nm. For investigated bacteria that were sub-MIC treated, the percentage shift in sample-induced hemolysis from untreated placebo cultures was reported (Selim *et al*. 2025). After comparing the released hemoglobin with the controls that were positive and negative using Eq. 1, the hemolysis % was determined:

$$\text{Hemolysis (\%)} = \frac{\text{Samples with bacteria} - \text{NC}}{\text{PC} - \text{NC}} \times 100 \quad (1)$$

Assessment of Anti-inflammatory Impact of Different Prepared Forms of Grape Seed Oil

The beneficial effects of COX were assessed using a COX (ovine) colorimetric inhibitor screening kit that contained both ovine COX-1 and human recombinant COX-2 enzymes. To identify isozyme-specific inhibitors, the study focused on prostaglandin F2α, a compound formed from cyclooxygenase-derived prostaglandin H2, whose synthesis is inhibited by tin(II) chloride. The amount that was produced was evaluated using an enzyme immunoassay. Utilizing a microplate reader, the amount of absorption at 585 nm was determined. The test grape seed oil forms were dissolved in DMSO (1.0%) to obtain a final amount of 1.0 mL, and concentrations ranging from 1000 to 0.5 µg/mL that were examined. Celecoxib served as the COX-1 and COX-2 inhibition assay's positive control. The samples underwent triple testing at twelve distinct levels. The data were calculated in three phases. First, each grape seed oil sample's average absorption was determined. Secondly, the absorption of the background wells had been subtracted from the absorbing capability of 100% of the original COX-1 and COX-2 activities as well as the examined sample wells. Deducting every specimen with 100% initial COX-1 or COX-2 actions, reducing the result by 100% initial action, and then multiplying the outcome by 100 yielded the percentage of inhibition. The calculated outcome was referred to as the IC_{50}, or the 50% inhibition of COX-1 and COX-2 enzyme functions (Belahcene *et al*. 2024).

Testing Anti-Alzheimer Roles of Different Forms of Grape Seed Oil

The inhibition of butyrylcholinesterase (BTchI) was measured using the methodology described by Al-Rajhi *et al*. (2023). The BChE buffer and solutions were created from scratch. This required making 0.45 U/mL of BChE mixture (2.9763 mg of BChE enzyme were dissolved in 6.747 mL of buffer at a pH of 8.1) and 0.023M S-butyrylthiocholine iodide (BTchI) solution (7.1 mg of BTchI was dispersed in 1.0 mL of water). Each liquid-liquid extraction (LLE) was dispersed in DMSO first, and then in filtered water to get a level of 44.0 mg/mL followed by a final concentration of 1000.0 µg/mL. In the BChE inhibition test, a microplate reader was used to measure absorbence. About 200.0 µL of the buffer, 5.0 µL of BChE enzyme, 5.0 µL of Ellman's reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), and 5.0 µL of the LLE at a value of 40.0 mg/mL were mixed together and left in a solution for 15.0 min at 30 °C in a temperature-regulated water bath. A 5.0 µL of the BTchI solution with the substrate was then added to the mixture to initiate the enzymatic reaction. At a regulated temperature of 36° C, absorbances were determined 13.0 times at 420 nm using the microplate reader at 45-second intervals. The enzymatic inhibition was calculated from the observed absorbance values employing the subsequent calculation:

$$\text{BTchI inhibition (\%)} = 100 - \frac{R_{oil}}{R_{max}} \times 100 \quad (2)$$

where R_{max} denotes the greatest fluctuation rate in the absorption of the blank test without any inhibitor, and R_{oil} is the variation frequency in the absorption of the sample being tested comprising the LLE (Δabs/Δtime).

Statistical Analysis

The findings were calculated as a means of three repetitions and are shown as mean ± SD (standard deviation). To conduct the statistical analysis, Graph Pad Prism V8 (CA, USA) was utilized. Tukey's post hoc test and one-way assessment of variability (ANOVA) were used to assess quantitative data with a normative dispersion among all of the interventions at a 0.05 likelihood threshold.

RESULTS AND DISCUSSION

The impact of zero exposure to UV-C on chemical composition of grape seed oil was evaluated using gras chromatography. The GC-MS analysis for the three forms of grape seed oil can be seen in Figs. 1-3 and Tables 1-3. The number of compounds and their classes was slightly changed upon exposure to various time points of radiation. It is apparent that the raw grape seed oil (exposed to 0 min of UV-C) had 19 various molecules from 12 chemical classes. The grape seed oil exposed to 30 min of UV-C had a group of 19 molecules from 13 classes. Lastly, the grape seed oil (60 exposed to UV-C) contained a mixture of 20 compounds from 12 chemical classes. Many compounds (3 major and 5 minor) could be seen in all forms of oil. Three compounds in major levels could be seen in all prepared types of grape seed oil including: n-hexadecanoic acid; oleic acid; and 2,6-bis (3,4-methylene dioxyphenyl)-3,7-dioxa bicyclo(3.3.0) octane. The levels of these compounds were notably increased ($P \leq 0.05$) upon increasing of the time of exposure to radiation. Five compounds with minor levels could be seen in all forms of oil including: caryophyllene oxide; 7-isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one; 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione; 9,12-octadecadienoic acid (Z, Z)-; and *β*-sitosterol. The concentrations of these common compounds were significantly increased ($P \leq 0.05$) upon raising the time of exposure to UV-C radiation. Additionally, three compounds could only be seen in grape seed oil at 0- and 30-min exposure of UV which were: eugenol; [1,1'-bicyclopropyl]-2 octanoic acid, 2'-hexyl-, methyl ester, and 9-octadecadienoic acid (Z).

Oil exposed to UV-C light undergoes an intricate interaction of chemical modifications that affect the production of new molecules as well as the concentration of preexisting ones. While the levels of some molecules, such as certain phenolic acids, may decrease, the levels of other compounds, such as specific derivatives, may increase or even appear only in trace amounts. This is because UV-C may alter already-existing molecules and destroy chemical bonds (Araújo *et al*. 2022; Alsalamah *et al*. 2025b). There are new trends to improve the quality and activity of raw oil using various tools including ozonation and exposure to UV radiation (Di Filippo *et al*. 2020; Almuhayawi *et al*. 2025).

The process that enabled the effect of UV-C radiation was attributed to the absorption of UV light by aromatic rings and double bonds, which produced free radicals. These radicals alter the structure and characteristics of the produced films by forming intermolecular covalent connections. Furthermore, according to a recent study, UV-C radiation enhanced the effective properties of starch-based films (Uyarcan and Güngör 2024). Different categories of chemicals were seen in different types of produced oil in this study. According to the source, grape seed oil is a product with several potential uses in the nutritional, medicinal, nutrient-rich, and cosmetics sectors due to its wide range of

biologically active substances with well-known health-promoting qualities (Lucarini *et al*. 2022).

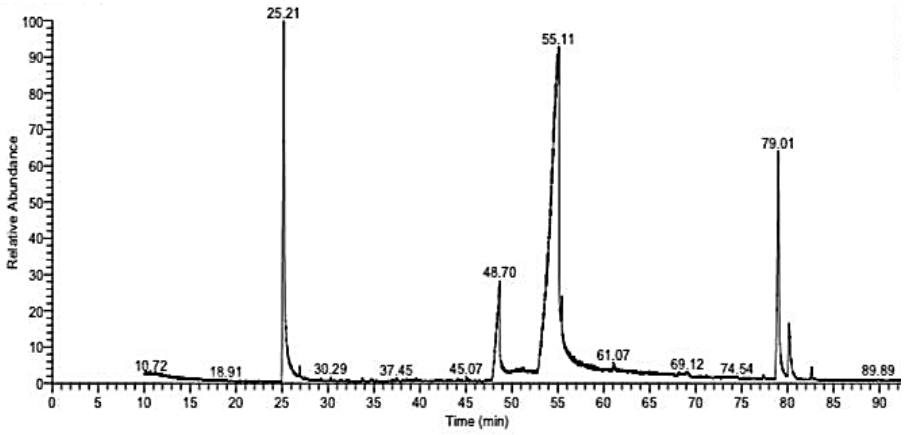

Fig. 1. GC-MS pattern for raw grape seed oil (0 min exposure to UV-C)

Table 1. Different Compounds and their Corresponding Details from GC-MS Analysis of Raw Grape Seed Oil

Class	Area (%)	Molecular Weight	Molecular Formula	Compound Name	RT (min)
Allylbenzene	21.21	164	$C_{10}H_{12}O_2$	Eugenol	25.21
Sesquiterpene	0.54	204	$C_{15}H_{24}$	3H-3a,7-Methanoazule ne, 2,4,5,6,7,8-hexahydro 1,4,9,9-tetramethyl-, [3aR-(3aα,4α,7α)]	26.92
Epoxide	0.33	220	$C_{15}H_{24}O$	Caryophyllene oxide	33.72
Sesquiterpenoids	0.13	220	$C_{15}H_{24}O$	Humulenol-II	34.69
Allylic alcohol	0.17	218	$C_{15}H_{22}O$	2,2,7,7-Tetramethyltri cyclo[6.2.1.0(1,6)]unde c-4-en-3-one	37.46
Hydrocarbon	0.28	218	$C_{15}H_{22}O$	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexa hydro-3H-naphthalen-2-one	39.57
Spirocyclic diketone	0.20	276	$C_{17}H_{24}O_3$	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-die ne-2,8-dione	45.07
Fatty acid	5.40	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	48.70
Fatty acid	4.51	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z, Z)-	54.00
Fatty acid	48.19	282	$C_{18}H_{34}O_2$	9-Octadecadienoic acid (Z)	55.11
Fatty acid	1.61	282	$C_{18}H_{34}O_2$	Oleic Acid	55.44
Fatty acid ester	0.4	266	$C_{17}H_{30}O_2$	Hexadecadienoic acid methyl ester	61.08
Fatty acid ester	0.11	322	$C_{21}H_{38}O_2$	[1,1'-Bicyclopropyl]-2 octanoic acid, 2'-hexyl-, methyl ester	67.57
Fatty acid ester	0.17	356	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxy methyl) ethyl ester	69.12
Phenol	0.14	416	$C_{28}H_{48}O_2$	ç-Tocopherol	77.39
Alkane	12.07	354	$C_{20}H_{18}O_6$	2,6-Bis (3,4-methylene dioxyphenyl)-3,7-dioxa bicycle(3.3.0) octane	79.01
Oxazoles	3.71	233	$C_{12}H_{11}NO_4$	3-Methyl-4-piperonyl-5-isoxazolone	80.17
Phytosterol	0.12	400	$C_{28}H_{48}O$	Campesterol	80.72
Phytosterol	0.71	414	$C_{29}H_{50}O$	ç-Sitosterol	82.63

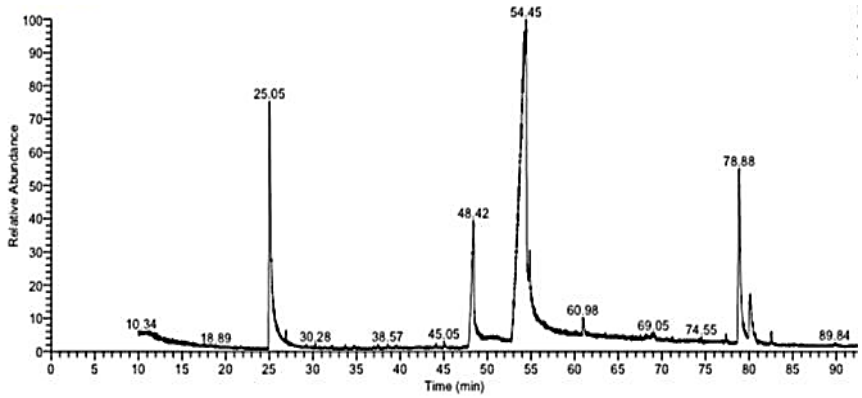

Fig. 2. GC-MS pattern for raw grape seed oil (30 min exposure to UV-C)

Table 2. Different Compounds and their Corresponding Details from GC-MS Analysis of Grape Seed Oil Exposed to 30 min of UV-C

Class	Area (%)	Molecular Weight	Molecular Formula	Compound Name	RT (min)
Alkane	0.11	170	$C_{12}H_{26}$	Dodecane	18.90
Allylbenzene	21.48	164	$C_{10}H_{12}O_2$	Eugenol	25.05
Sesquiterpene	0.67	204	$C_{15}H_{24}$	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro 1,4,9,9-tetramethyl-, [3aR-(3aα,4α,7α)]	26.90
Alcohol	0.18	164	$C_{11}H_{16}O$	Bicyclo[3.3.0] octan-2-one, 7-isopropylidene	29.23
Epoxide	0.62	220	$C_{15}H_{24}O$	Caryophyllene oxide	33.70
Alkane	0.13	450	$C_{32}H_{66}$	Dotriacontane	35.24
Hydrocarbon	0.98	218	$C_{15}H_{22}O$	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexa hydro-3H-naphthalen-2-one	37.44
Spirocyclic diketone	0.43	276	$C_{17}H_{24}O_3$	7,9-Di-tert-butyl-1-oxa spiro (4,5) deca-6,9-diene-2,8-dione	45.05
Fatty acid	8.70	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	48.42
Fatty acid	3.38	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z, Z)	51.03
Fatty acid	37.88	282	$C_{18}H_{34}O_2$	Oleic Acid	54.45
Fatty acid	3.59	282	$C_{18}H_{34}O_2$	9-Octadecadienoic acid (Z)	58.90
Fatty acid ester	0.62	322	$C_{21}H_{38}O_2$	[1,1'-Bicyclopropyl]-2 octanoic acid, 2'-hexyl-, methyl ester	71.21
Phenol	0.63	416	$C_{28}H_{48}O_2$	ç-Tocopherol	77.36
Fatty acid ester	0.46	884	$C_{57}H_{104}O_6$	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E, E, E)	77.60
Alkane	14.14	354	$C_{20}H_{18}O_6$	2,6-Bis (3,4-methylene dioxyphenyl)-3,7-dioxa bicyclo(3.3.0) octane	78.88
Cyclic ketone	4.46	150	$C_{10}H_{14}O$	Cyclohexanone, 2-(2-butynyl)-	80.10
Sterol	0.47	436	C26H44O5	Ethyl iso-allocholate	80.66
Phytosterol	1.07	414	$C_{29}H_{50}O$	ç-Sitosterol	82.55

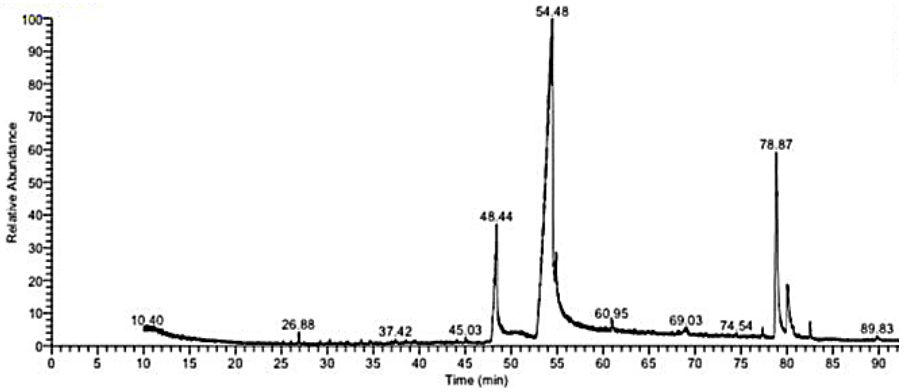

Fig. 3. GC-MS pattern for grape seed oil (60 min exposure to UV-C)

Table 3. Different Compounds and their Corresponding Details from GC-MS Analysis of Grape Seed Oil Exposed to 60 min of UV-C

Class	Area (%)	Molecular Weight	Molecular Formula	Compound Name	RT (min)
Hydrocarbon	0.32	204	$C_{15}H_{24}$	Copaene	26.02
Hydrocarbon	1.93	204	$C_{15}H_{24}$	1H-Cycloprop[e]azule ne, 1a,2,3,4,4a,5,6,7b-octa hydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4α,4aβ,7bα)1-	26.88
Sesquiterpene	0.42	204	$C_{15}H_{24}$	ç-Elemene	29.22
Hydrocarbon	0.45	204	$C_{15}H_{24}$	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aα,7α,8aα)]	30.26
Alkane	0.34	310	$C_{22}H_{46}$	Docosane	31.38
Epoxide	0.75	220	$C_{15}H_{24}O$	Caryophyllene oxide	33.68
Cyclic enones	0.65	206	$C_{14}H_{22}O$	2,5,9-Trimethylcyclou ndeca-4,8-dienone	34.65
Pyrans	0.71	336	$C_{22}H_{40}O_2$	2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-	36.97
Hydrocarbon	0.59	218	$C_{15}H_{22}O$	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexa hydro-3H-naphthalen-2-one	37.42
Spirocyclic diketone	0.67	276	$C_{17}H_{24}O_3$	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	45.03
Fatty acid	12.70	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	48.44
Fatty acid	16.12	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z, Z)-	54.35
Fatty acid	28.78	282	$C_{18}H_{34}O_2$	Oleic acid	54.48
Fatty acid	3.70	282	$C_{18}H_{34}O_2$	9-Octadecanoic acid	60.95
Fatty acid ester	0.60	356	$C_{21}H_{40}O_4$	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxy methyl) ethyl ester	71.20
Phenol	1.60	416	$C_{28}H_{48}O_2$	β-Tocopherol	77.34
Alkane	23.12	354	$C_{20}H_{18}O_6$	2,6-Bis (3,4-methylene dioxyphenyl)-3,7-dioxa bicyclo(3.3.0) octane	78.86
Cyclic ketones	5.13	150	$C_{10}H_{14}O$	Cyclohexanone, 2-(2-butynyl)-	80.06
Phytosterol	0.40	400	$C_{28}H_{48}O$	Campesterol	80.64
Phytosterol	1.02	414	$C_{29}H_{50}O$	β-Sitosterol	82.52

Various forms of grape seed oil were tested in comparison with a group of pathogenic microbes, as illustrated in Fig. 4 and Table 4. It could be noticed that raw grape seed oil (0 min exposure to UV-C) as well as the exposed oil to 30 min of UV-C had a weak antimicrobial impact towards *S. aureus, E. faecalis*, *E. coli*, and *C. albicans*. Moreover, both oil forms had no impact towards *S. typhi* and *P. glabrum.* However, the exposed oil to 60 min of UV-C showed a promising *in vitro* antimicrobial action *versus S. aureus, E. faecalis*, *E. coli*, and *C. albicans*, where it had a higher inhibition zone compared the inhibition zones detected for standard drug. Furthermore, the exposed oil to 60 min of radiation showed a weak action versus *S. typhi* with inhibition zone = 10 ± 0.4 mm. Also, it showed no antifungal impact towards *P. glabrum.* The absence of antifungal activity of grape seed oil against *P. glabrum*, despite its effectiveness against bacteria and *C. albicans*, is likely due to variations in cell wall composition and membrane structure among these microorganisms. The antibacterial potential of grape seed oil may be attributed to its diverse bioactive constituents, which exert several biochemical and structural influences on bacterial cells. Some lipophilic fatty acids can integrate into the bacterial cell membrane, disrupting its structure and increasing membrane permeability. Additionally, phenolic compounds and unsaturated fatty acids may promote the generation of reactive oxygen species inside bacterial cells, leading to oxidative damage of vital biomolecules such as DNA, lipids, and proteins, thereby impairing essential metabolic and replication processes. Based on numerous investigations, the oil that was derived from grape seeds really inhibited the development of *E. coli* and *S. aureus* (Krasteva *et al*. 2023; Fernández-Pérez *et al*. 2025). Oleic acid, n-hexadecanoic acid, and 2,6-bis (3,4-methylene dioxyphenyl)-3,7-dioxabicyclo (3.3.0) octane are among the major chemicals that have antibacterial action. Oleic acid is an unsaturated fatty acid that occurs naturally has antibacterial properties, especially against Gram-positive bacteria, which it can kill or hinder in growth. This action is probably caused by its capacity to damage the membrane of the bacterial cell (Casillas-Vargas *et al*. 2021). The saturated fatty acids such as hexadecanoic acid have been shown to have antibacterial properties. The biological consequences of the molecule 3,4-methylene dioxyphenyl-2,6-bis (3,7-dioxa bicycle (3.3.0) may be significantly influenced by the existence of carboxylic groups (Nabi *et al*. 2022). Additionally, simple hydrocarbons having carbon-carbon single bonds, such as octane, are alkane compounds that often have antibacterial properties. Based on other studies, some alkanes are more successful than others at combating particular fungi or bacteria (Libisch 2024). The findings of the current investigation suggest a close relationship among the chemical modifications induced by UV-C exposure and the improved antimicrobial activity of grape seed oil. The GC–MS analysis revealed noticeable increases in key constituents such as oleic acid and 2,6-bis(3,4-methylenedioxyphenyl)-3,7-dioxa-bicyclo(3.3.0)octane after 60 minutes of irradiation, demonstrating the transformation or formation of bioactive compounds that may contribute to bacterial inhibition. These ingredients are known to disrupt cell membrane integrity and promote oxidative stress within microbial cells, thereby increasing antibacterial potency. Nevertheless, establishing a direct cause–effect relationship among specific chemical alterations and antimicrobial efficacy remains complex, as the biological activity likely arises from synergistic interactions among multiple constituents. In agreement with recent study, the exposed *A. vera* oil to UV-C for 30 and 60 min incresed its antimicrobial and anti-biofilm effects against various bacteria. This might be due to the presence of large number of chemical derivatives which generated from exposure to UV-C (Alsalamah *et al*. 2025b). Also, the present findings are consistent with a recent report

(Alsalamah *et al.* 2025a), which showed that exposure of sage oil to UV radiation enhanced its chemical composition and biological activities—including antimicrobial, antidiabetic, anti-inflammatory, and anti-Alzheimer features—compared to the unexposed oil.

Table 4. Antimicrobial Action (mm) of Various Types of Grape Seed Oil Exposed to UV-C (0, 30, and 60 min)

Microorganism	0 min	30 min	60 min	Standard Drug
S. aureus	17±0.8	19±0.4	25±0.3	26±0.4
E. faecalis	16±0.2	18±0.2	23±0.1	16±0.1
E. coli	9±0.5	11±0.4	24±0.1	22±0.5
S. typhi	NA	NA	10±0.4	15±0.2
C. albicans	16±0.1	22±0.2	25±0.5	22±0.2
P. glabrum	NA	NA	NA	36±0.2

Data are measured as means ± SD.

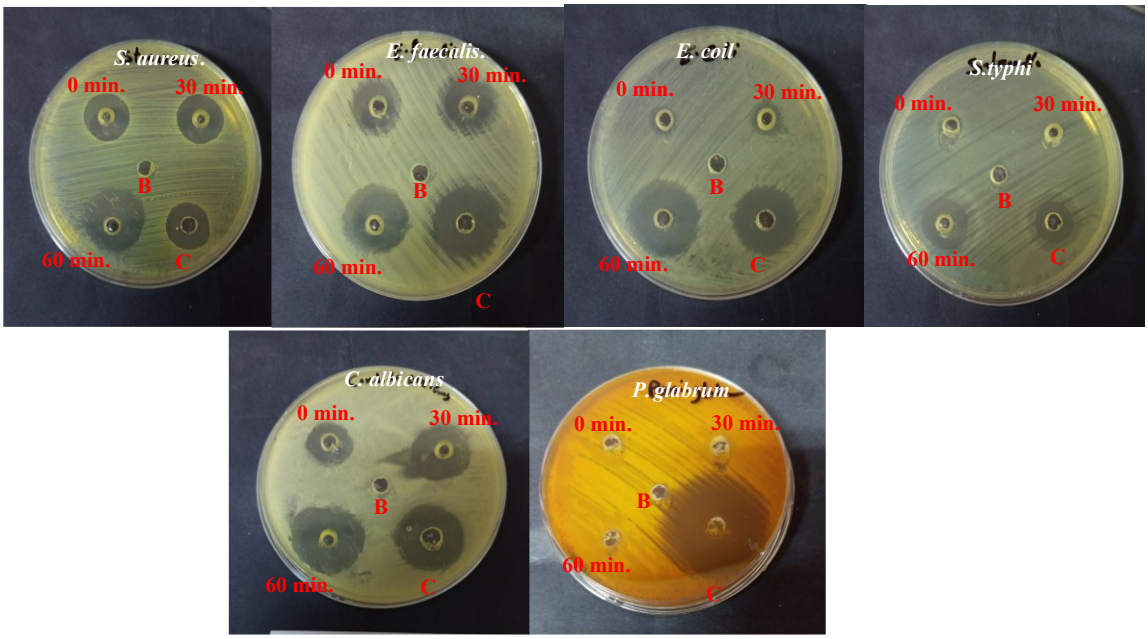

Fig. 4. Agar diffusion testing for grape seed oil exposed to 0, 30, and 60 minutes of UV-C towards various pathogenic microbes in comparison to standard drugs (C)

Application of UV-C on grape seed oil at different time intervals led to reduction of MIC and MBC towards tested microorganisms. There was an inverse relationship between exposure time and levels of MIC and MBC. Increasing the exposure time to UV-C led to lower values of MIC and MBC, as illustrated in Table 5. Clinical testing facilities frequently use MIC tests to evaluate the susceptibility to medications of infections caused by bacteria. Clinical breakpoints, which indicate the MIC values under which a medical condition is probably highly curable, have been established for authorized antibiotics by means of this approach. As a result, specialists can utilize clinical breakpoints in addition to empirical data to assist choose an appropriate antimicrobial treatment strategy (Kadeřábková *et al*. 2024). The reduction in MIC and MBC may be due the elevation of the levels of bioactive compounds.

MIC is directly impacted by the concentration of bioactive substances. A bioactive chemical's MIC value decreases with increasing concentrations, indicating that less of the molecule is required to stop microbial growth (Altemimi *et al*. 2017; Salem *et al*. 2023). This is due to the fact that there are more chemicals accessible that combine with and disturb the bacteria. Besides, there is frequently a dose-response relationship in which a compound's inhibitory impact grows with concentration until it reaches a threshold, beyond which additional increases could not yield appreciable further advantages (Cairone *et al*. 2025).

Table 5. Values of MIC and MBC (µg/mL) for Grape Seed Oil Exposed to 0, 30, and 60 min of UV-C

Microorganism	0 min		30 min		60 min	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	62.5±0.1	250±0.2	31.25±0.4	62.5±0.2	15.62±0.3	15.62±0.3
E. faecalis	62.5±0.2	125±0.3	15.62±0.2	62.5±0.2	7.8±0.1	15.62±0.3
E. coli	250±0.5	1000±0.6	250±0.4	250±0.1	15.62±0.1	15.62±0.3
S. typhi	NA	NA	NA	NA	1000±0.2	NA
C. albicans	62.5±0.1	250±0.2	15.62±0.2	250±0.2	15.62±0.3	15.62±0.3

Some bacteria are serious infectious agents that produce a variety of factors that promote virulence, such as hemolytic compounds called toxins. Grape seed oil forms reduced the hemolysis induced by the studied microorganisms to varying degrees (Fig. 5). The present results revealed that exposure of grape seed oil for 60 min of UV-C induced the highest hemolytic percentages. When the tested bacteria — *S. aureus*, *E. faecalis*, *E. coli*, and *S. typhi* — were exposed to grape seed oil treated with UV-C for 60 minutes, the lowest inhibition effects were observed at 25% of the MIC, showing values of 82.7%, 91.1%, 61.8%, and 89.6%, respectively. Meanwhile, the percentages were 90.0%, 94.6%, 83.6%, and 94.4% at 50% MIC of grape seed oil exposed to 60 min of UV-C. Lastly, the percentages were 98.0%, 96.4%, 98.0%, and 99.0% at 75% MIC of grape seed oil exposed to 60 min of UV-C (Fig. 6).

The purposed of the anti-hemolytic function in an abundance of bacteria is to ascertain whether the material can stop hemolysis even while bacteria are proactively participating. Assessing the appropriateness of antimicrobial compounds that may come into contact with the body's blood cells and bacteria is pertinent to this (Kumar *et al*. 2024). According to earlier research, it was found that several essential oils are crucial for preventing hemolysis (Lee *et al*. 2014; Vemuri *et al*. 2018). Furthermore Kim *et al*. (2018) reported that cinnamon bark oil has the ability to prevent *P. aeruginosa* from forming biofilms, which results in reduced hemolytic properties. According to a different study, herring oil lessens *S. aureus*'s hemolytic effect on red blood cells. Additionally, it has been determined that *S. aureus* produces hemolysin, a marker of virulence that promotes infection formation and is broken down by red blood cells (Lee *et al*. 2022). The current investigation's suppression/inhibition of hemolytic function would emphasize the significance of grape seed oil subjected to UV-C for possible therapeutic possibilities to regulate hemolysis, a virulence factor induced by all tested bacteria. UV-C radiation can change the concentrations of important chemicals in oils, which can result in the formation of new molecules and their derivatives (Kumar *et al*. 2024).

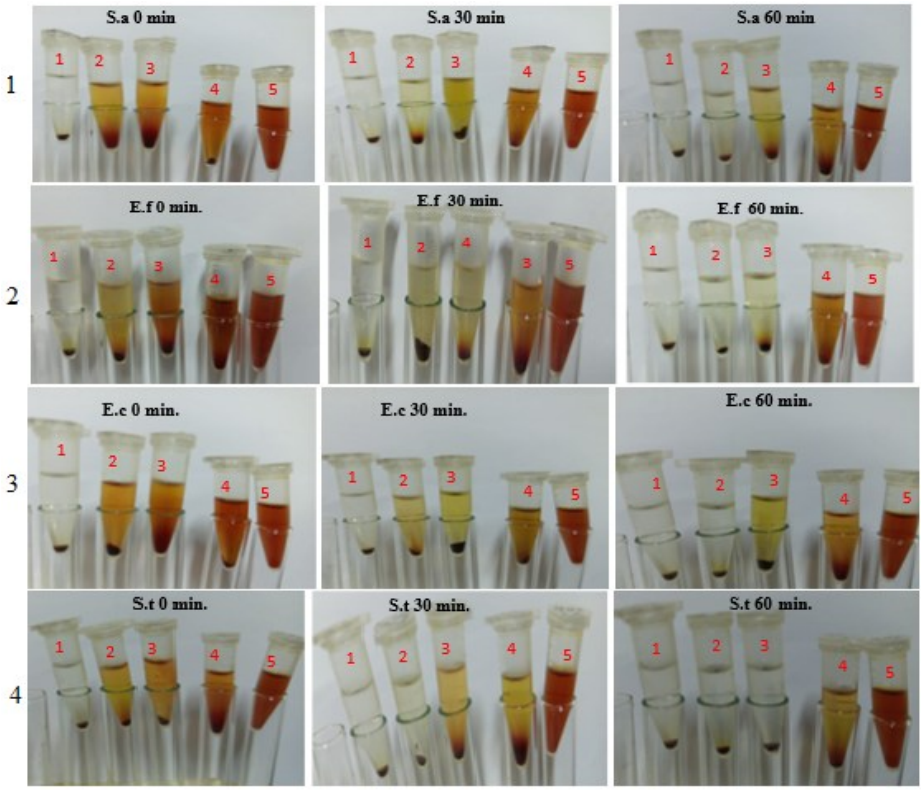

Fig. 5. Visual examination of antihemolytic action of grape seed oil upon exposure to 0, 30, and 60 min of UV-C where **I**: in presence of *S. aureus*; **II** in presence of *E. faecalis*; **III** in presence of *E. coli*; and **VI** in presence of *S. typhi*

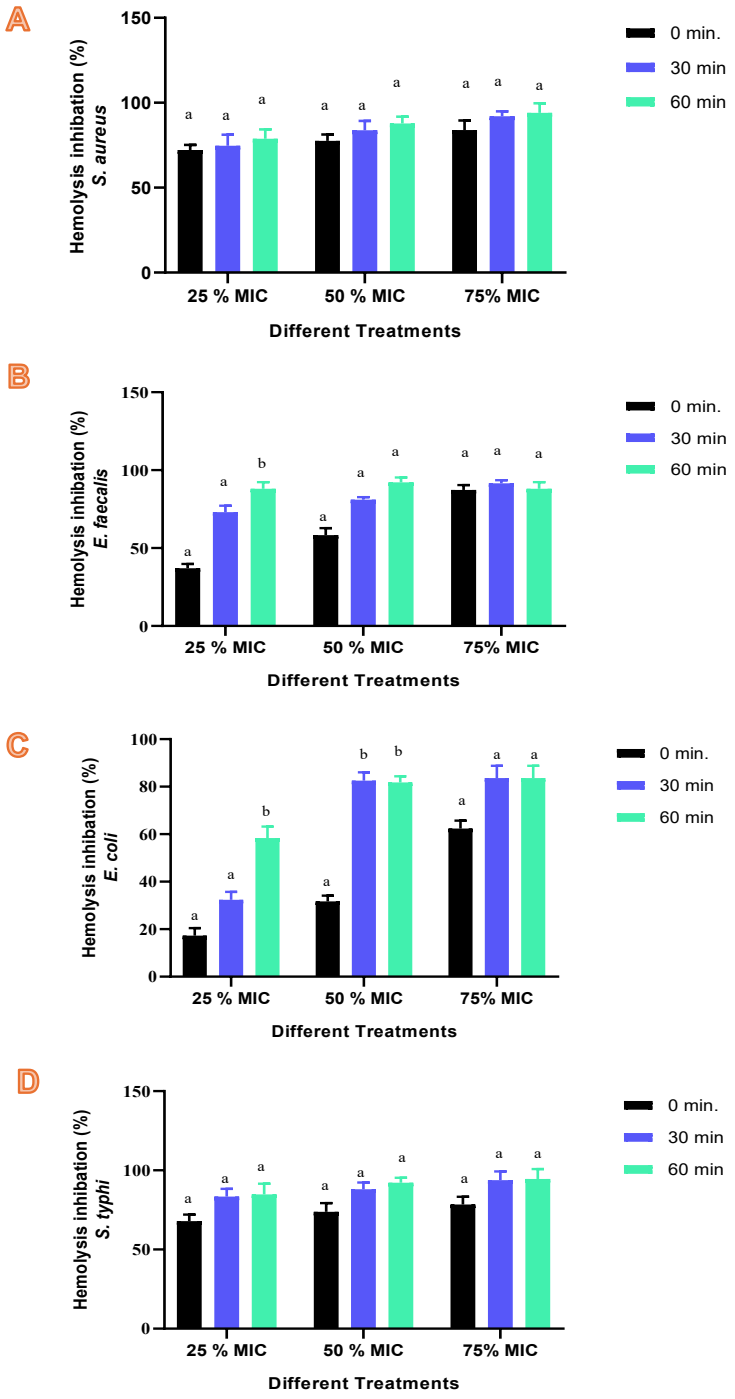

Fig. 6. Bar graph analysis for antihemolytic action of grape seed oil upon exposure to 0, 30, and 60 minutes of UV-C (using 25%, 50%, and 75% of MIC) where **A**: in presence of *S. aureus*; **B** in presence of *E. faecalis*; **C** in presence of *E. coli*; and **D** in presence of *S. typhi* (results are drawn as means ± SD; different letters refer to dramatic difference where $P \leq 0.05$)

The anti-inflammatory capacity for various examined forms of grape seed oil *via* COX-1 is depicted in (Fig. 7A), and COX-2 is illustrated in (Fig. 7B). There is a proportional rise in COX-1 impact with the increase of exposure time where the levels for raw grape seed oil had $IC_{50} = 87.31 \pm 0.5$ µg/mL, while the levels for oil exposed to 30, and

60 min were 46.84±0.1 µg/mL and 26.91±0.1 µg/mL, compared to the level of COX-1 standard was at IC_{50} = 10.33± 0.3 µg/mL. On the other hand, there was a notable improvement in COX-2 value in accordance of rise of exposing period to UV-C where the levels for raw grape seed oil had IC_{50} = 57.38 ± 0.1 µg/mL , while the levels for oil exposed to 30, and 60 min were 32.73±0.1 µg/mL and 24.99±0.4 µg/mL compared to the level of COX-1 standard, which was at IC_{50} = 6.44±0.5 µg/mL. Inflammatory processes are typically present in chronic illnesses, which may be linked to higher rates of death and morbidity globally. These processes are frequently challenging to manage with current treatments and procedures. In this regard, chronic illness therapy might benefit from the ingestion of foods that have anti-inflammatory properties. Olas *et al*. (2012) found that grape seed oil was more efficient than natural oils at reducing platelet adhesion. The main ingredients in the oil could be the cause of the anti-inflammatory effect. Hexadecanoic acid has been shown to have anti-inflammatory properties by blocking the COX-1 and COX-2 enzymes, which participate in the synthesis of inflammatory mediators (Jiménez-Nevárez *et al*. 2023), oleic acid's anti-inflammatory properties can be strengthened when coupled with other bioactive substances (Santa-María *et al*. 2023). Numerous fruits and vegetables naturally contain alkenes, which help control inflammation (McDougall and McKenna 2022).

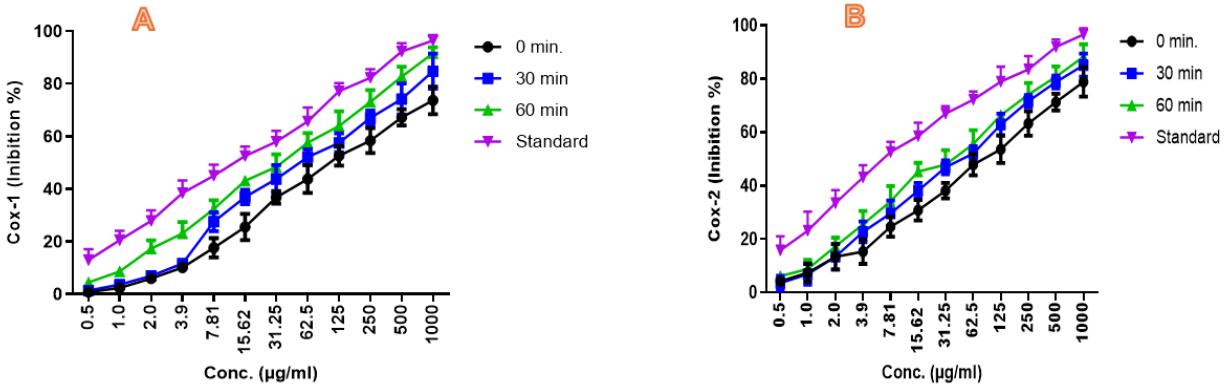

Fig. 7. Anti-inflammatory impact for grape seed oil exposed to different periods of UV-C (A) COX-1 inhibition assay; (B) COX-2 inhibition assay. Data are represented as means ± SD

Raw grape seed oil had a weak anti-butyrylcholinesterase inhibition with IC_{50} = 42.78 ±0.6 µg/mL. There was a significant improvement ($P \leq 0.05$) of anti-butylcholinesterase inhibition level upon exposing the oil for 30 min of UV-C with IC_{50} = 19.15 ± 0.4 µg/mL. The maximal inhibition level could be seen for the oil exposed to 60 min of UV-C with IC_{50} = 12.13 ± 0.1 µg/mL compared to the anti-butylcholinesterase inhibition of the standard with IC_{50} = 0.58 ± 0.1 µg/mL (Fig. 8). Natural products including grape seed oil can be made more valuable through a variety of processes, such as oxidative stress suppression, which lowers the production of reactive oxygen species and prevents oxidation of biomolecules and cellular damage, depending on their different constituents. Neurodegenerative disorders can be suppressed by this route (Berahmand *et al*. 2020). Hexadecenoic and oleic acids are suggested to be involved by binding to the butyryl-cholinesterase enzyme's active site (Wechakorn *et al*. 2025). Alkenes, which represent one of the major constituents of grape seed oil, have the ability to alter how molecules react with butyrylcholinesterase (BChE), which may have an impact on the enzyme's inhibitory

activity. Both the entire chemical composition of the inhibitor and the location and configuration of the alkene inside the molecule determine the precise consequences (Li *et al*. 2021).

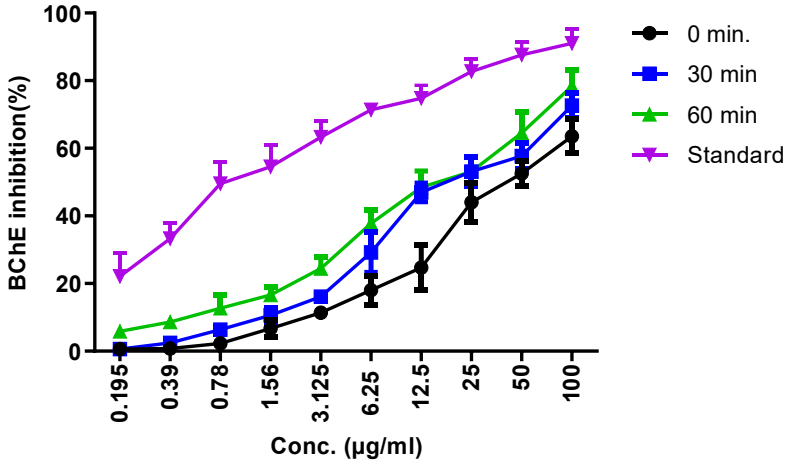

Fig. 8. Comparative analysis for butylcholinesterase inhibition activity of various types of grape seed oil relative to standard rivastigmine. Results are drawn as means ± SD.

CONCLUSIONS

1. The exposure of grape seed oil to ultraviolet light (UV-C) raised the contents of certain chemical components in the oil with a particular rise of the levels of oleic, hexadecenoic acids as well as 2,6-bis (3,4-methylene dioxyphenyl)-3,7-dioxabicyclo (3.3.0) octane
2. A direct correlation was found between UV-C exposure and the improvement of biological activities of the oil, including its antimicrobial, antihemolytic, and anti-inflammatory effects *via* inhibition of COX-1 and COX-2 enzymes; as well as anti-Alzheimer *via* inhibition of BChE actions.

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