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Effect of UV-C radiation on chemical profile and pharmaceutical application in vitro of *Aloe vera* oil

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Abstract

The uncontrolled exposure to ultra violet (UV) radiation have many adverse impacts on human health. UV could be divided into three main classes according to wave length range where UV-C (200-280) is one of these classes. Aloe vera oil is a common product in markets with many cosmetic and medicinal applications. The purpose of this investigation is to expose the oil to UV-C at a dose of 17.0 mJ/cm² (254 nm) for 0, 30, and 60 min to examine the impact of change of exposure periods on the alteration in chemical composition of Aloe vera oil as well as its various biological activities. GC-MS examination of various oil forms reveled the proportional elevation in the number of compounds in the oil with increasing time of exposure to UV-C where linoleic acid ethyl ester and ethyl oleate were the most common compounds in oil forms with emergence of trace molecules and their derivatives from various chemical classes especially in the exposed oil to radiation especially after 60 min. The antimicrobial action towards Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, and Salmonella typhi) and fungi (Aspergillus niger and Candida albicans) reflecting the successive to boost the activity versus examined microbes except for A. niger with inhibition zones ranged from 16 ± 1 to 24 ± 1 mm, 18 ± 1 to 25 ± 1 mm, and 22 ± 1 to 26±1 mm using unexposed A. vera oil, exposed A. vera oil to UV for 30, and 60 min, respectively against tested microorganisms. Moreover, exposure to radiation at various time points resulted in substantial reduction of minimal inhibitory concentration (MIC) as well as minimal bactericidal concentration (MBC) for the applied oil forms. For instance the MIC values were 62.5 ± 0.1 , 31.25 ± 0.1 , and 15.62 ± 0.1 µg/ml for *S. aureus*, 125 ± 0.2 , 62.5 ± 0.2 , and 31.25 \pm 0.2 μ g/ml for B. subtilis, moreover MBC values were 125 \pm 0.1, 125 \pm 0.2, and 31.25 \pm 0.3 μ g/ml for S. aureus, 250±0.3, 125±0.2, and 31.25±0.3 µg/ml for B. subtilis using unexposed A. vera oil, exposed A. vera oil to UV for 30, and 60 min, respectively. There is a notable improvement of anti-biofilm action of examined oil especially upon using 25% of MBC. Furthermore, antioxidant, anti-inflammatory, anticancer, and wound healing capabilities of the tested oils dramatically raised with increase time of exposure. Future studies are required to detect the maximal beneficial gain upon exposure to various natural oils UV-C.

Keywords Aloe vera, UV-C, Chemical classes, Antioxidant, Anticancer, Healing

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Introduction

Phytomedicinal chemicals are primarily found in plants (Alsolami et al. 2023; Almehayawi et al. 2024). Because they are less expensive and have a comparatively lower prevalence of adverse effects than contemporary pharmaceuticals, plant-based medications are being used as substitutes to synthetic treatments (Sharifi-Rad et al. 2020; Al-Rajhi et al. 2023). In addition to improving general health, natural substances and pharmaceutical active components (APIs) assist with managing diseases (Sun et al. 2020; Abdelghany et al. 2021; Alawlaqi et al. 2023). Aloe plants are an exclusive supplier of phytochemicals since they are able to endure hot, dry weather, which allows them for maintaining water and essential chemical substances in their swelling, succulent foliage (Wójcik et al. 2021). Several investigations carried out both in vitro and in vivo have verified the biological features of Aloe species, involving wound closure, anti-inflammatory, antimicrobial, and cancer-fighting abilities. Most of these attributes could not be attributed to a single class of molecules, but mostly to a wide range of molecules that compose the phytochemical composition of Aloe extracts (Yahya et al., 2022; Yadeta 2024).

A perpetual green shrub with vivid yellow cylindrical blossoms, A. vera (Aloe barbadensis Miller, family Xanthorrhoeaceae) is widely found in hot, arid regions. A. vera is derived from the Arabic term "Allaeh", referring to "reflected stinging materials", and the Latin title "Vera", which indicates "true". A. vera leaves' colorless mucilaginous gel has been widely employed in both cosmetic and medicinal uses. More than 75 distinct substances, such as vitamins, proteins, minerals, carbohydrates, anthraquinones, lipids, hormonal substances, as well as others, are found in A. vera (De Oliveira et al. 2018; Maan et al. 2018; Sánchez et al. 2020). According to literature review of Kumar et al. (2024), pharmacological utilization of A. vera oil and its contents are becoming attractive for several investigators due to its biological functions without any occurring side effects. A. vera possesses several functional properties were attributed to A. vera such as antioxidant and antimicrobial activities, as well as it was utilized for management of diabetes, wound healing, and skin related problems (Olán-Jiménez et al. 2024). Additionally, recent study by Mensah et al. (2025) reported the utilization of A. vera and its ingredients in the industry of foods as safe preservatives, nutraceuticals, and functional components. Biofilms containing A. vera oil were found to possess antibacterial activity versus Aspergillus niger, Staphylococcus aureus, Candida albicans, and Escherichia coli as well as antioxidant properties which increased with percentage A. vera oil in the biofilm (Isık et al. 2023).

Eight to nine percent of the total solar radiation is UV irradiation (Cockell and Knowland 1999;

Del-Castillo-Alonso et al. 2015). Throughout its life cycle, this radiation causes a variety of adaptive changes and may have an impact on plants' ability to respond to known stressors (Kovács et al. 2014). Typically, UV radiation is separated into three categories according to its wavelength: UV-A (320.0-400.0 nm), UV-B (280.0-320.0 nm), and UV-C (200.0-280.0 nm). All of the bands can have a very diverse biochemical impact in biological systems (Maharaj 2015). UV-C irradiation can be considered safe alternative to thermal process in industrial fields of pharmacological materials and food preservation technology (Gopisetty et al. 2018). Short-wave ultraviolet radiation (UV-C) is a desirable technological option for maintaining food, either by itself or in conjunction with a mild heating process (Rodríguez-Rodríguez et al. 2019). Cell structures rapidly sustain significant damage from UV-C (Verdaguer et al. 2017). The applied UV-C in food technology compared to UV-A or UV-B radiation as well as other non-thermal approaches possess numerous advantages such as lack of toxic remains, low operational costs (Gayán et al. 2014)., and little energy usage (Rodríguez-Rodríguez et al. 2020). According to Meléndez-Pizarro et al. (2020) the continuous flow of UV-C stabilized the betalains content in the blends of Aloe vera-pitaya and therefore its chemical properties were enhanced. Nonetheless, information about the use of UV light over various time periods is not well researched. The present investigation aimed to assess the impact of UV-C radiation across different time periods on the chemical structure and biological characteristics of *A. vera* oil.

Materials and methods

The used oil & reagents

A. vera oil was procured a specified megastore in Saudi Arabia with a code of (289467) which purchase authenticated products. The oil was split into 3 different containers. Media of cell culture and antibiotics were purchased from Biocom Biotech Ltd. (Pretoria, South Africa). Other chemicals in the present paper were obtained from (Sigma, USA). The cell lines used alongside were kindly provided from VACSERA, Egypt.

Exposure of oil to UV-C for various time points

Three equal parts of the *A. vera* oil were exposed to a UV-C irradiation dose of 17.0 mJ/cm² UV-C (254 nm) in three similar chambers at ambient temperature (28 °C). The 1st, 2nd, and 3rd part were exposed to UV-C irradiation for different periods including 0, 30, and 60 min, respectively. Following every session, the unit was cleaned with water and sanitized with a 200 ppm hypochlorite solution. We used a UVX-25 sensor (UVP, CA, USA) to adjust the radiation level. The average of the illumination measured by each sensor was used to get the actual irradiance (Koutchma et al. 2016).

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Characterization in the change in chemical composition using GC–MS examination

Oil treatments (1.0 ml) were gathered for GC-MS measurement and diluted with 10 ml of methanol prior to being placed in vials. A device made by (Thermo 800 Evo®, USA) was used to analyze the specimens. At a steady flow rate of 1.4 ml/min, high-quality natural helium with a minimum of 99.98% cleanliness was employed with an injection volume of 2.0 µl. A split-splitless intake with an MS detector was coupled to an HP-5 MS column $(30.0 \text{ m} \times 0.26 \text{ mm} \times 0.26 \text{ } \mu\text{m})$ for the study. The operating temperatures of the injector and ion supply were adjusted to 270 °C and 305 °C, consequently Oven program: kept at 95 °C for 1 min, raised to 155 °C at a rate of 35 °C/min, and then kept for 1 min. The temperature was then raised to 195 °C at a rate of 10 °C per minute and maintained for two minutes. The temperature was then raised to 290 °C at a rate of 10.0 °C per minute and maintained for 5.0 min. The entire GC operation duration was 25 min. The mass spectra EM voltage was adjusted at 200 Rel with a reading frequency of 45–560 m/z, and all peak locations were detected and matched with the WILEY 9 library. The mean peak area was divided by the total peak region to determine the relative percent of each ingredient. (Trace Finder®, USA) 3.0 was used to analyze chromatograms and mass bands (Abdelghany et al. 2020).

Testing the antimicrobial pattern for various treatments

A variety of test pathogenic organisms, including bacteria (Staphylococcus aureus ATCC12600, Bacillus subtilis ATCC9372, Klebsiella pneumonia ATCC33495, Salmonella typhi ATCC14028) and fungi (Aspergillus niger ATCC1015, Candida albicans ATCC10231), were used to assess the examined A. vera samples in vitro antimicrobial roles using the well distribution of agar testing. Molds were inoculated on malt extract media, whereas bacteria were spread on Mueller Hinton agar plates. A cork borer edge was then used to place the assessed samples onto the medium-made well. Both, gentamicin (0.09 mg/ml) and fluconazole (0.27 µg/ml) was applied as positive controls, with DMSO serving as the bank. After a 72-h growth period, the inhibitory area was evaluated at 35 °C for bacterial cells, 4-8 days, and 29 °C for the investigated fungus species (Al-Rajhi and Abdelghany 2023).

Determination the levels for minimal inhibitory concentration and minimum bactericidal roles

The least inhibiting value (MIC) of each specimen was assessed using the nutrient-rich liquid for bacteria and the micro-dilution liquid steps. The values, which varied from 0.95 to 1000.0 μ g/ml, were obtained by diluting specimens of *A. vera* oil. For every spot in the 96-well dish, 100 μ l of the component diluted specimens of *A.*

vera oil in a broth medium have been prepared. Fresh microbe colonies that satisfied the visual requirements of the 1.0 McFarland standard were used to create the inoculation, and each hole was treated with 3.0 μl of sanitized 0.6% of sodium chloride to reach a threshold of 1.8×10^6 CFU/ml. After that, the microorganisms were grown at 36 °C for 72 h for bacteria and 4–8 days for molds. Besides, MBC was evaluated after sub-culturing 100 ml of the final positive objects, the substrate containing a 100% inhibitory agent, and the setup control microbial culture onto plates in each hole. The MBC was found to have the fewest specimens that could not support microbial growth at the proper temperature throughout the incubation time species (Al-Rajihi et al. 2025).

Detection the anti-biofilm action at various MBC levels

To test anti-biofilm activity was identified using 96-well plates that contained 300.0 µl of fresh trypticase soy yeast broth in each well and were supplemented with sub-lethal dosages (75, 50, and 25% of MBC of studied A. vera oils). A level of 10⁶ CFU/ml of (S. aureus, B. subtilis, K. pneumonia, S. typhi, subsequently) was used as the inoculant on the plates. After 48 h of incubation at 37 °C, the plates were rinsed with sterile deionized water to get rid of any free-floating bacterial cells and the supernatant broth was removed. After 30 min of air drying, the plates were dyed with a 0.1% crystal violet solution dissolved in the sterile deionized water for 20 min at 27 °C to reveal the bacterial biofilm that had formed. After removing the extra staining, the plates underwent three rounds of washing. Each well received 250 µl of 96% ethanol to dissolve the attached dye in the bacterial cells. A microplate reader was used to record the absorbance at a wavelength of 580 nm following the 17.0-min incubation period (Qanash et al. 2023). The following equation was used to determine the bacteria's biofilm inhibition:

Percentages of Inhibition = 1-(Absorbance of wells treated by samples- Blank absorbance/Absorbance of control Wells-Blank absorbance) \times 100 (Where blank absorbance is for media only, while absorbance of control is the examined bacterial strains with no application of *A. vera* oils).

Elucidation the change in antioxidant role upon change time of exposure to UV-C

By applying ascorbic acid as an imitation antioxidant and the 1-diphenyl-2-picryl hydrazyl (DPPH) method, the free radical scavenger capabilities of oils were evaluated in order to determine the effectiveness of their antioxidants. First, 3 ml of various latex dosages (3.90–1000.0 $\mu g/ml$) in ethanol made using the dilution procedure were combined with 2.0 ml of DPPH (0.20 mM) solution in ethanol. After giving the mixture a good shake, it was allowed to stand at 30 °C for 35.0 min. A

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spectrum analyzer (UV–VIS Thermo, USA) was used to detect the reaction mixture's absorbance at 522 nm. Using a log dosage inhibitory curve, the inhibitory amount 50% (IC $_{50}$) of latex needed for inhibiting 50% of the free radical formed by DPPH was determined (Al-Rajhi et al. 2022).

Evaluation the anti-inflammatory using protein denaturation testing

The basis for this assay is the compounds' capacity to prevent protein denaturation, as explained by (Kamal et al. 2022). The reaction mixture was made up of 1.0 ml of a fresh egg-albumin solution, 1.0 ml of standard diclofenac sodium, and serial concentrations of *A. vera* oil samples (1.56–200 µg/ml). It was then incubated for 20 min at 29 °C. The reaction mixture is kept at 80 °C in a water bath for 15 min to produce denaturation. Following cooling, the sample's turbidity was assessed using spectrophotometry at 670 nm. The blank, to which no samples were introduced, was used to measure the suppression ratio of denaturation. Three duplicates of each test were conducted, and the mean was taken. Formulation was utilized to assess the protein denaturation activity.

Protein denaturation Percentages (%) = [Absorbance (blank) - Absorbance (sample) /Absorbance (blank)] × 100

Application of MTT procedure for detection of antineoplastic impact in vitro

American Type Tissue Collection (ATCC) was the source of cultured cells in our study. The used cells namely A431 (epidermoid carcinoma, skin cell lines), and normal Vero cells (CCL-81). After adding heat-inactivated fetal bovine serum (11%) and streptomycin (105.0 mg/ml) to DMEM substrate, it was hydrated and maintained at 38 °C with CO₂ (5% v/v). The MTT assay was used to evaluate the specimens' effect on the cell lines' viability. 100.0 µl of cell suspensions containing 6.0×10^3 cells were added to 96-well plates, which subsequently underwent incubation for a full day. The growing cells were treated with 100.0 µl of the specimen-containing media for three days after different amounts of the specimens were added to the cell media. After fixing the cells with 150.0 µl of 10.0% TCA instead of medium, they underwent incubation for an hour at 5 °C. The cells were then rinsed five times with distilled water after the TCA solution was removed, and then they were undergoing incubation for 12 min at 28 °C in a dark environment. After four passes of washing with 1% acetic acid and a 12-h air- drying, 150.0 µl of TRIS (10.0 mM), which was required to break down the protein-dye complex, was added, and the intensity of absorption at 540 nm was measured using a reader (Apport 394, USA at this point) (Abdelghany et al. 2019).

Determination of the variation in wound healing role upon using various treatments

Scratch wounds were examined using a multi-well plate coated with an extracellular matrix substrate of 9.0 µg/ ml fibronectin, incubated for 2.0 h at 35 °C, and then the unbound extracellular matrices were eliminated and washed with phosphate-buffered saline. The cells from the dish were split with trypsin, and then developed on the scratch wound assay plate, which was then incubated to allow the cells to spread and to produce a confluent monolayer. A pipette tip was used to remove the monolayer cell, which included the confluent monolayer. To get rid of split cells, gently wash the cell monolayer after scratching. After that, swap it out for new medium that contains different samples of A. vera oil that have been evaluated. The plate has been kept for 48 h at 38 °C in the cell culture incubator. Following the time spent incubating, phosphate-buffered saline was used to clean the cell monolayer. The cells were subsequently fixed with 4.0% paraformaldehyde for 20 min, stained with crystal violet (2% in ethanol) for 15 min, and the cell culture was viewed under a microscope (Martinotti and Ranzato 2020). The data analysis that followed was computed using the subsequent formulas:

Migration Level (ML) of cells = (Ws-We/t) \times 100; (where, Ws = Mean diameter of stating wound (μ m), We = average of final wound width (μ m), t = time of the experiment in hours)

Percentage of closing for Wound = (As - Adt/As)

where, A_s = starting wound region, Adt = wound region in hours

Percentage of area variation = Starting area - Finished area

Analysis for the outcomes

To perform analysis, SPSS version 16.0 was used to examine the acquired records (SPSS Inc., USA). The standard deviation (SD) of the average values is used to express the whole data. When P0.05 was taken into consideration and results were significant, the ANNOVA testing was utilized to find meaning (Jabir et al. 2018).

Results

The variation in the chemical pattern of *A. vera* oils via GC–MS

It could be noticed that *A. vera* which exposed to 0 min (unexposed-control) had a group of nineteen (19) different molecules from four different chemical classes which were: (Alkane (4), Fatty acid (5), Fatty alcohol (2), Fatty

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Table 1 Different molecules, retention times, their corresponding formulas, areas and classes in *A. vera* oil exposed to 0 min, of UV-C using GC–MS analysis

RT	Compound Name	Mo-	Мо-	Area	Class
		lecular formula	lecular weight	%	
42.85	Docosane	C ₂₂ H ₄₆	310	0.29	Alkane
46.16	Nonadecane	C ₁₉ H ₄₀	268	0.49	Alkane
47.90	9-Hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	0.34	Fatty acid
48.19	12-Methyl-E,E-2,13-oc tadecadien-1-ol	C ₁₉ H ₃₆ O	280	0.55	Fatty alcohol
48.90	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	10.15	Fatty acid ester
49.09	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.98	Fatty acid
49.36	Heneicosane	$C_{21}H_{44}$	296	0.80	Alkane
51.80	Oleic Acid	$C_{18}H_{34}O_2$	282	1.0	Fatty acid
52.36	1-Hexadecanol, 2-methyl	C ₁₇ H ₃₆ O	256	0.44	Fatty alcohol
54.10	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	16.13	Fatty acid ester
54.52	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310	41.53	Fatty acid ester
55.13	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312	11.36	Fatty acid ester
55.83	Undec-10-ynoic acid, dodecyl ester	C ₂₃ H ₄₂ O ₂	350	0.33	Fatty acid ester
56.75	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	3.68	Fatty acid
61.91	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	2.21	Fatty acid
62.23	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy- 1-(hydroxy methyl) ethyl ester	C ₂₁ H ₃₈ O ₄	354	3.11	Fatty acid ester
62.47	9-Octadecenoic acid (Z)-, 2-hydroxy- 1-(hydroxy methyl) ethyl ester	C ₂₁ H ₄₀ O ₄	356	4.00	Fatty acid ester
68.06	1,2-15,16-Diepoxyhex adecane	$C_{16}H_{30}O_2$	254	0.79	Alkane
87.67	9-Hexadecenoic acid, 9-hexadecenyl ester, (Z,Z)-	C ₃₂ H ₆₀ O ₂	476	1.82	Fatty acid ester

acid ester (8) (Supplementary 1A, Table 1). While, GC–MS examination for the oil exposed to 30 min of UV-C revealed the existence of 23 compounds which consisted of 5 various chemical classes which were: (Alkane (3), Phthalic acid esters (1), Fatty acid (7), Fatty alcohol (4), Fatty acid ester (8)) (Supplementary 1B, Table 2). Lastly, *A. vera* oil exposed to 60 min of UV-C radiation showed the maximal number of molecules which were 33 various molecules from 10 different classes which were: (Alkane (7), Cyclo-alkane (1), Phthalic acid esters (1), Fatty acyls (1), Alcohol (1), Aldehyde (1), Fatty acid (8), Fatty alcohol (5), Vinyl ester (1), Fatty acid ester (7)) (Supplementary 1C, Table 3). There were 11 compounds could be seen in all forms of oils which were: Docosane; Nonadecane;

Table 2 Different molecules, retention times, their corresponding formulas, areas and classes in *A. vera* oil exposed to 30 min, of UV-C using GC–MS analysis

RT	Compound Name	Mo- lecular formula	Mo- lecular weight	Area %	Class
42.90	Docosane	C ₂₂ H ₄₆	310	0.52	Alkane
43.83	1,2-Benzenedicar- boxylic acid, butyl 8-methylnonyl ester	C ₂₂ H ₃₄ O ₄	362	0.62	Phthalic acid esters
46.22	Nonadecane	$C_{19}H_{40}$	268	0.59	Alkane
47.55	1-Dodecanol, 2-octyl-	C ₂₀ H ₄₂ O	298	0.33	Fatty alcohol
47.94	cis-13-Eicosenoic acid	$C_{20}H_{38}O_{2}$	310	0.78	Fatty acid
48.24	11,14-Eicosadienoic acid, methyl ester	C ₂₁ H ₃₈ O ₂	322	0.73	Fatty acid ester
48.51	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	3.12	Fatty acid
48.95	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	8.95	Fatty acid ester
49.10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.62	Fatty acid
49.42	Eicosane	$C_{20}H_{42}$	282	0.89	Alkane
50.96	12-Methyl-E,E-2,13-oc tadecadien-1-ol	C ₁₉ H ₃₆ O	280	0.63	Fatty alcohol
51.86	Oleic Acid	$C_{18}H_{34}O_2$	282	1.34	Fatty acid
52.42	1-Hexadecanol, 2-methyl	C ₁₇ H ₃₆ O	256	2.46	Fatty alcohol
53.90	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308	15.21	Fatty acid ester
54.62	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310	39.69	Fatty acid ester
55.39	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	8.75	Fatty acid ester
57.17	9,12-Octadecadi- enoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	2.61	Fatty acid
62.29	17-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	3.21	Fatty acid
62.53	Oleic acid, 3-hydroxy- propyl ester	$C_{21}H_{40}O_3$	340	3.4	Fatty acid ester
63.37	cis-13-Eicosenoic acid	$C_{20}H_{38}O_{2}$	310	0.54	Fatty acid
66.83	Erucic acid	$C_{22}H_{42}O_2$	338	0.47	Fatty acid
68.69	9,12-Octadecadi- enoic acid (Z,Z)-, 2-hydroxy-1-(hydroxy methyl)ethyl ester	C ₂₁ H ₃₈ O ₄	354	0.73	Fatty acid ester
71.60	2-Methyl-Z,Z-3,13-oct adecadienol	C ₁₉ H ₃₆ O	280	1.41	Fatty alcohol
87.69	Oleic acid, 3-(octa- decyloxy)propyl ester	C ₃₉ H ₇₆ O ₃	592	2.4	Fatty acid ester

12-Methyl-E, E-2,13-oc tadecadien-1-ol; Hexadecanoic acid, ethyl ester; n-Hexadecanoic acid; Oleic Acid; 1-Hexadecanol, 2-methyl; Linoleic acid ethyl ester; Ethyl Oleate; Octadecanoic acid, ethyl ester; and 9-Octadecenoic acid (Z)-. Besides, four molecules could be seen in the control oil and could be seen in the exposed oils for 30 and 60 min, respectively. These compounds were: 9,12-Octadecadienoic acid (Z, Z)-; 9,12-Octadecadienoic

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Table 3 Different molecules, retention times, their corresponding formulas, areas and classes in *A. vera* oil exposed to 60 min, of UV-C using GC–MS analysis

RT	Compound name	Mo-	Mo-	Area	Class
		lecular	lecular	%	
		formula	weight		
39.44	Docosane	C ₂₂ H ₄₆	310	0.73	Alkane
41.01	1-Hexadecanol, 2-methyl-	C ₁₇ H ₃₆ O	256	1.57	Fatty alcohol
42.94	Eicosane	$C_{20}H_{42}$	282	1.2	Alkane
43.33	Heneicosane	$C_{21}H_{44}$	296	0.43	Alkane
43.85	1,2-Benzenedicar- boxylic acid, butyl 8-methylnonyl ester	C ₂₂ H ₃₄ O ₄	362	1.36	Phthalic acid esters
45.30	Cyclopentane, (4-octyldodecyl)-	$C_{25}H_{50}$	350	1.44	Cyclo-alkane
46.28	Nonadecane	$C_{19}H_{40}$	268	1.68	Alkane
47.60	1-Dodecanol, 2-octyl-	C ₂₀ H ₄₂ O	298	2.56	Alcohol
48.54	Dotriacontane	$C_{32}H_{66}$	450	0.79	Alkane
48.97	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	7.89	Fatty acid ester
49.11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.96	Fatty acid
49.49	Carbonic acid, eico- syl vinyl ester	$C_{23}H_{44}O_3$	368	1.89	Vinyl ester
50.35	cis-13-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310	1.0	Fatty acid
50.89	Erucic acid	C ₂₂ H ₄₂ O ₂	338	1.97	Fatty acid
51.24	17-Pentatriacontene	$C_{35}H_{70}$	490	2.48	Fatty acyls
51.64	1-Docosanol	$C_{22}H_{46}O$	326	0.67	Alkane
51.91	13-Docosenoic acid	$C_{22}H_{42}O_2$	338	4.68	Fatty acid
52.50	2,2,3,3,4,4 Hexadeutero octadecanal	C ₁₈ H- ₃₀ D ₆ O	274	1.77	Fatty alcohol
54.19	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	10.59	Fatty acid ester
54.61	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310	18.65	Fatty acid ester
55.04	Undec-10-ynoic acid, dodecyl ester	$C_{23}H_{42}O_2$	350	0.40	Fatty acid ester
55.45	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	9.30	Fatty acid ester
56.01	9-Octadecenoic acid (Z)-	C ₁₈ H ₃₄ O ₂	282	5.98	Fatty acid
57.25	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.59	Fatty acid
58.49	Behenic alcohol	$C_{22}H_{46}O$	326	2.57	Alcohol
59.46	1-Heptacosanol	$C_{27}H_{56}O$	396	1.39	Fatty alcohol
62.09	Undec-10-ynoic acid, tetradecyl ester	C ₂₅ H ₄₆ O ₂	378	1.81	Fatty acid ester
62.33	12-Methyl-E,E-2,13- oc tadecadien-1-ol	C ₁₉ H ₃₆ O	280	3.45	Fatty alcohol
62.60	Oleic Acid	$C_{18}H_{34}O_2$	282	3.97	Fatty acid
63.90	cis-11-Eicosenoic acid	C ₂₀ H ₃₈ O ₂	310	2.90	Fatty acid
64.87	Nonacosanol	$C_{29}H_{60}O$	424	1.22	Fatty alcohol
73.13	Dotriacontane	$C_{32}H_{66}$	450	0.79	Alkane
81.80	2,2-Dideutero octadecanal	C ₁₈ H- ₃₄ D ₂ O	270	0.32	Aldehyde

acid (Z, Z)- 2-hydroxy-1-(hydroxy methyl) ethyl ester {shared among 0 and 30 min}; Heneicosane; Undec-10-ynoic acid, dodecyl ester {shared among 0 and 60 min}. Furthermore, it could be noticed that there were two major compounds could be seen in all oil forms which were: Ethyl Oleate and Linoleic acid ethyl ester. There was a slight decrease in the levels of these compounds upon exposing the oil to a UV-c to 30 min. However, there is a significant decrease ($p \le 0.05$) via exposure of oil to radiation for 60 min reflecting the proportional impact of the UV-C radiation time to impact the major compounds and yield new groups of compounds and their derivatives.

The impact of change of exposure periods of UV-C on antimicrobial impact

It could be noticed that *A. vera* oil had a promising antimicrobial impact towards tested microbes including: *S. aureus, B. subtilis, K. pneumonia, S. typhi,* and *C. albicans.* Besides, all oil forms had a negative antifungal action versus *A. niger* (Fig. 1 and Table 4). There is a gradual elevation of the antimicrobial action of the tested oil in proportionally with the raise of the time of exposure to UV-C reaching maximal values upon using exposed oil to 60 min. of UV-C radiation. Moreover, the antimicrobial effects versus microbes had slightly higher levels relative to applied standard norms.

MIC& MBC for antimicrobial actions

Application of UV-C for various time points of 0, 30, and 60 min to A. vera oil led to dramatic reduction ($p \le 0.05$) in the levels of minimal inhibitory levels as minimal bactericidal impact towards test microbes including S. aureus, B. subtilis, K. pneumonia and S. typhi. However, the minimal inhibitory levels for oils versus C. albicans were similar in all treatments. While, there is a significant decrease in minimal bactericidal impact towards for oils versus C. albicans upon increase the exposure time of UV-C to A. vera oil (Table 5).

Anti-biofilm for various oil forms

The impact of various forms of A. vera oil to reduce biofilm formation of S. aureus, B. subtilis, K. pneumonia and S. typhi upon using 25%, 50 and 75% of MBC levels towards tested Gram positive and Gram negative bacteria could be seen in (Supplementary 2). It could be noticed that the exposed oil for 0, 30 and 60 min had a dramatic improvement ($p \le 0.05$) in biofilm inhibition towards tested bacteria upon using 25% of MBC. There was a sight enhancement of anti-biofilm roles upon using 50% and 75% of MBC levels of various oil forms towards S. aureus, B. subtilis, K. pneumonia and S. typhi.

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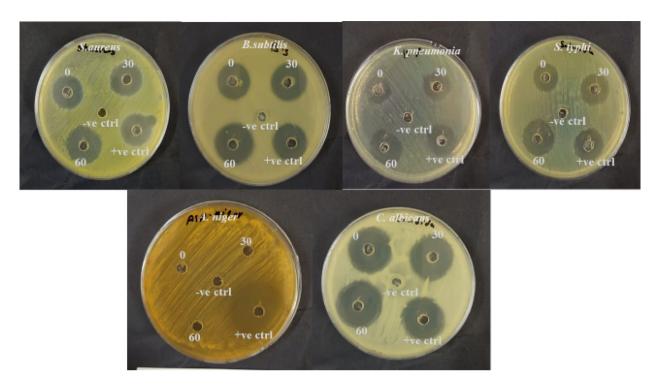


Fig. 1 Testing the impact of change in exposure time points (0, 30, and 60 min.) for the antimicrobial action of *A. vera* oil using agar diffusion technique towards test microorganisms of bacteria and fungi

Table 4 Antimicrobial (mm) action of A. vera oil upon exposure to 0, 30, and 60 min. of UV-C (Data are tabulated as means \pm SD)

Microbe	Treatmer	SD*		
	0 min	30 min	60 min	
S. aureus	18±1	20±1	23 ± 1	20±1
B. subtilis	19±1	21 ± 1	24 ± 1	20 ± 1
K. pneumonia	16 ± 1	18 ± 2	24 ± 2	19±1
S. typhi	15 ± 2	18±1	22 ± 1	21 ± 1
C. albicans	24 ± 1	25 ± 1	26 ± 1	25 ± 2
A. niger	NA [#]	NA	NA	30 ± 3

^{*}SD standard drug; *Not detected

The effect of variation of exposure periods of UV-C on antioxidant action

The antioxidant action of *A. vera* oils exposed to 0 min of UV-C (control) was determined at $IC_{50} = 38.33 \pm 0.29$ µg/ml. There is a slight improvement in the level of

antioxidant role of the tested oil exposed to 30 and 60 min of radiation reached the level of $IC_{50}s=32.87\pm0.31$ µg/ml, and 28.84 ± 0.22 µg/ml, subsequently. While, ascorbic acid standard had $IC_{50}=4.29\pm0.11$ µg/ml as depicted in (Fig. 2). There is a proportional elevation in antioxidant capacity of oil upon increasing the exposure time to UV-C.

The role of change of exposure periods of UV-C on antiinflammatory activity

The anti-inflammatory action of *A. vera* oils exposed to 0 min of UV-C (control) was determined at $IC_{50} = 13.03 \pm 0.17$ µg/ml. Exposure of oil to 30 and 60 min of radiation resulted in a notable enhancement in the concentration of anti-inflammatory action of the tested oil reached the level of $IC_{50} = 8.89 \pm 0.21$ µg/ml, and 7.32 ± 0.12 µg/ml, consecutively. On the other hand,

Table 5 MIC & MBC (μ g/ml) for *A. vera* oil upon exposure to 0, 30, and 60 min. of UV-C (Data are tabulated as means \pm SD; Different small superscript letters (for MIC) in the same raw refer to significant difference $P \le 0.05$; Different capital superscript letters (for MBC) in the same raw refer to significant difference $P \le 0.05$)

Microbe	Treatment						
	0 min		30 min		60 min		
	MIC	MBC	MIC	MBC	MIC	МВС	
S. aureus	62.5 ± 0.1 ^a	125±0.1 ^A	31.25 ± 0.1 ^b	62.5 ± 0.1 ^B	15.62 ± 0.1 ^c	31.25 ± 0.1 ^C	
B. subtilis	125 ± 0.2^{a}	250 ± 0.3^{A}	62.5 ± 0.2^{b}	125 ± 0.2^{B}	31.25 ± 0.2^{c}	$31.25 \pm 0.3^{\circ}$	
K. pneumonia	62.5 ± 0.3^{a}	250 ± 0.2^{A}	62.5 ± 0.2^{a}	125 ± 0.3^{B}	15.62 ± 0.3^{b}	$31.25 \pm 0.2^{\circ}$	
S. typhi	250 ± 0.4^{a}	500 ± 0.4^{A}	125 ± 0.3^{b}	250 ± 0.2^{B}	$62.5 \pm 0.2^{\circ}$	125 ± 0.3^{A}	
C. albicans	15.62 ± 0.1^a	62.5 ± 0.1^{A}	15.62 ± 0.1^a	31.25 ± 0.1^{B}	15.62 ± 0.1^a	$31.25 \pm 0.1^{\circ}$	

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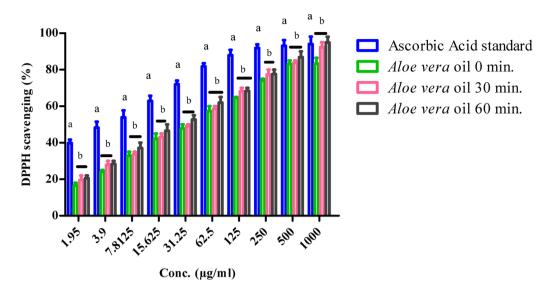


Fig. 2 Antioxidant activity using DPPH technique of *A. vera* oil exposed to (0, 30, and 60 min.) of UV-C relative to ascorbic acid standard (Data are drawn as means \pm SD; various superscript letters above columns refer to significant difference where $p \le 0.05$)

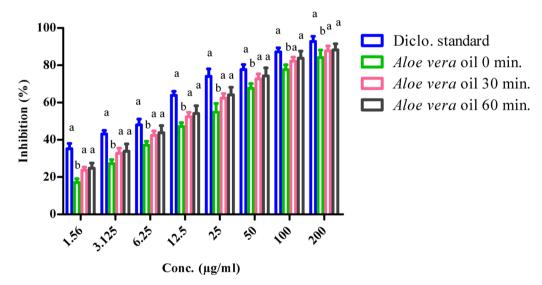


Fig. 3 Anti-inflammatory activity using protein denaturation test of *A. vera* oil exposed to (0, 30, and 60 min.) of UV-C relative to standard (Data are drawn as means \pm SD; various superscript letters above columns refer to significant difference where $p \le 0.05$)

the examined standard had $IC_{50} = 5.96 \pm 0.11$ µg/ml as depicted in (Fig. 3). There is a proportional rise in anti-inflammatory roles of oil upon increasing the exposure time to UV-C.

Anticancer activity of A. vera oil types

Various forms of *A. vera* were tested at different levels ranged from 1000 to 31.25 µg/ml towards A431 cancerous cell line using MTT test and examined under microscope as depicted (Fig. 4). It could be notice that *A. vera* oil at 0 min of exposure had a promising anticancer impact with $IC_{50} = 180.75 \pm 1.38$ µg/ml. A slight improvement for anticancer role upon exposure to 30 min of UV-C to reach $IC_{50} = 155.44 \pm 0.41$ µg/ml. A

dramatic enhancement ($p \le 0.05$) in anticancer impact for the tested oil upon exposure to 60 min of UV-C to reach $IC_{50} = 80.91 \pm 0.61$ µg/ml reflecting the positive role of exposure time o improve anticancer activity. The toxicity of unexposed *A. vera* oil to UV-C, upon exposure to 30 min of UV-C, and upon exposure to 60 min of UV-C was tested on normal vero cells which giving high IC_{50} 375.75 ± 2.38 µg/ml, 355.75 ± 2.66 µg/ml, and 346.75 ± 3.36 µg/ml (data not tabulated) indicating little toxicity on normal cells.

Wound healing outcome upon using various types of oil

The variations healing probabilities of various oil forms were examined using scratch experiment as illustrated

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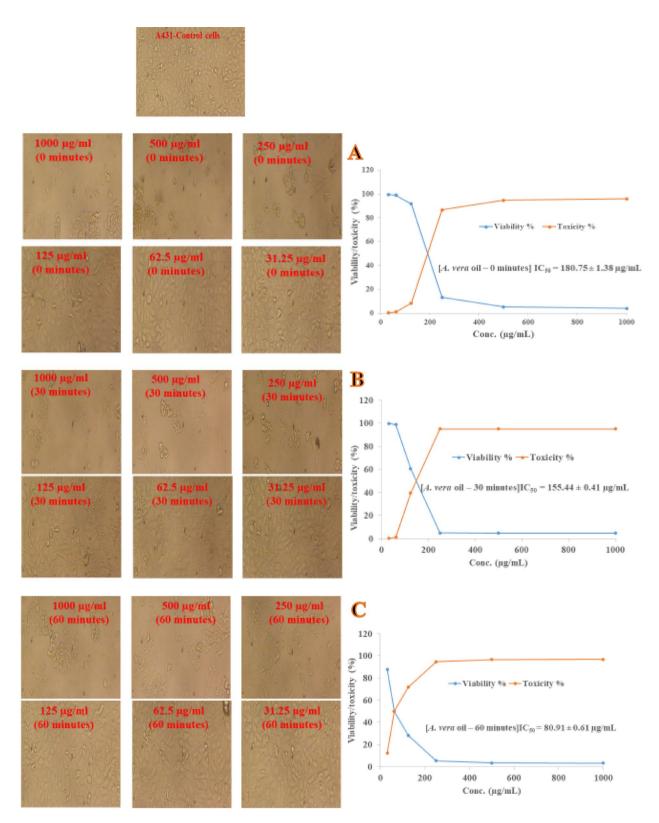


Fig. 4 Evaluation of anticancer impact versus A431- cell line of various oil forms of *A. vera* treated by UV-C for 0, 30, and 60 min. at concentrations ranging from (1000–31.25 μ g/ml) for each treatment; left pane: exmiantion using inverted microscoe (Magnification = \times 40); right panel: statistical analysis for vaibility and toxicity of various treatments(results are drawn as means \pm SD)

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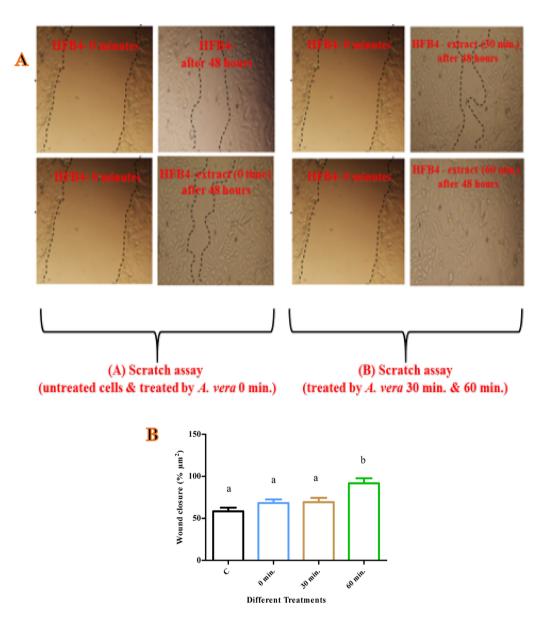


Fig. 5 Wound healing assessment upon application of *A. vera* oil forms exposed to 0, 30, and 60 min of UV-C upon using scratch assay **A** Microscopic examinations of various treatments; **B** Statistical analysis for measurement of healing improvement upon using various oil forms (results are drawn as means \pm SD; various superscript letters above columns refer to significant difference where $p \le 0.05$)

in (Fig. 5). It could be noticed that using the *A. vera* oil exposed to 0, and 30 min of UV-C has slightly improved the healing for the cells after 48 h. While, application of the exposed oil to 60 min of radiation led to maximal healing activity with a dramatic difference ($p \le 0.05$) relative to untreated cells reflecting the role of exposing of oil to UV-C for various periods to enhance healing capacity of oil.

Discussion

Through the years, individuals have used different portions of *Aloe* species and its oil either alone or in combination with other substances for a variety of purposes (Salehi et al. 2018; Leitgeb et al. 2021). These uses of Aloe

species result from the plants' chemicals, which give them biological activity (Yadeta 2022). On the other hand, one appealing technical option for preserving produce is the administration of short-wave ultraviolet radiation (UV-C), either by itself or in conjunction with thermal conditioning. The lack of harmful residues, minimal use of energy, and inexpensive operating expenses are some benefits of UV-C over alternative techniques (Gayán et al. 2014). Radiation diffusion reduces the effectiveness of UV-C in blends; consequently, for an effective treatment, the UV-C dose, substance pH, and liquid optical characteristics should be taken into account (Ferrarioet al. 2018). The purpose of the current study was to assess how being subjected to various UV-C time points (0, 30,

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and 60 min) affected the chemical makeup and biological functioning of *A. vera* oil in each condition.

In the present work, the chemical composition revealed the number of chemical compounds and their corresponding classes which exist in A. vera oil has gradually increased from nineteen compound (4 classes) in 0 min' exposure to twenty-three molecules (5 classes) via exposing oil to 30 min of UV-C and reaching maximal number of compounds of thirty-three compounds (10 classes) after 60 min of exposure to radiation. An investigation on Asparagus that received UV-C revealed an important rise in cutting potential and an apparent rise in tissue strength, even when using a lesser UV-C dose of 1.0 kJ/ m² and a time frame of 8 min (Huyskens-Keil et al. 2012). Numerous studies have demonstrated that UV-C irradiation causes the synthesis of chemical substances, contingent on the dose. One crucial consideration in therapy is the quantity of UV-C energy generated from an illumination supply per unit of time (Shama 2007). The potential biochemical mechanisms of the UV-C effect on A. vera oil may be due to the ability of UV-C photons to break the bonds between chemical constituents of oil lead to chemical construction modifying. UV-C photons can break chemical bonds, and could result in modifying compounds in the A. vera oil. Islam et al. (2016) reported that some chlorogenic acid, epicatechin, and phloridzin concentrations were decreased while concentration of catechin was increased in apple juices treated by UV-C irradiation. In recent investigation the efficient features of starch-based films were improved by UV-C radiation (Uyarcan and Güngör 2024), the mechanism of UV-C radiation was explained via absorption of UV radiation by aromatic rings and double bonds generating free radicals. These radicals create intermolecular covalent bonds which modifying the properties and structure of the created films.

In this study the antimicrobial impact of antimicrobial roles versus S. aureus, B. subtilis, K. pneumonia, S. typhi, and C. albicans has been enhanced upon exposure to UV-C for 0, 30, and 60 min this might be due to the existence of large number of derivatives which boost the antimicrobial action of the treated oil. Additionally, the detected levels MIC and MBC for the tested oil forms has been significantly reduced upon increasing time of exposure. The oxygenated terpenoids, aldehydes, fatty acids, and fatty acid esters in oils provide the majority of the antimicrobial properties. However some hydrocarbons also have antimicrobial abilities a reported in earlier investigations (Koroch et al. 2007; Gutierrez et al. 2008). Synergistic, additive, or antagonistic effects could result from interactions between these elements. While opposing and additive properties have also been noted, historical research has shown that entire oils typically exhibit a stronger antimicrobial than combinations of their primary constituents, indicating that minor constituents are essential to the synergistic effects (Hazzit et al. 2009; Bassolé and Juliani 2012).

The need to find ways to prevent biofilm development stems from the fact that this cellular state plays a major role in antibiotic resistance and infection complications (Mortazavi-Tabatabaei et al. 2019). In fact, bacteria that reside in these matrices gain from better lateral transfers of genes (Añibarro-Ortega et al. 2021), access to nutrients and water, and defense against harmful outside influences and antibiotics (Arsène et al. 2022). The present work reveled the effective application of various times of exposure of *A. vera* oil to UV-C to improve antibiofilm formation for examined bacteria especially upon using 25% of MBC levels.

Another biological characteristic of natural oils that is of considerable significance is their antioxidant capacity, which may protect food from oxidants' detrimental impacts (Hu et al. 2003). Furthermore, because essential oils can scavenge free radicals, they may be useful in preventing diseases including cardiovascular illness, cancer, and immune system deterioration. More and more data points to the possibility that free radical-induced cell destruction is the etiology of these illnesses (Hoang et al. 2021). UV-C at suitable doses reported to improve antioxidant capacity of natural products (Skowron.et al. 2024). The current investigation reveled the successive role of radiation using UV-C to improve antioxidant, anti-inflammatory as well as anticancer capacity of A. vera oil towards A4321 cells reflecting that direct role of raising time frame to enhance biological capabilities of A. vera oil especially after 60 min of exposure to radiation. To document the harmful-less of UV-C irradiation, Gopisetty et al. (2018) tested the irradiated beverage by UV-C on healthy mice liver and normal colon cells, who confirmed the absence of cytotoxic effects.

Application of UV-C radiation improve the wound healing ability of A.era oil especially after 60 min of exposure to radiation. Many studies report the effective role of A. vera products for skin care and improve wound healing capabilities (Hekmatpou et al. 2019; Mariana et al. 2023). The radiation results in raising the number of compounds even in trace levels which has many biomedical applications (Kumar et al. 2019; Nguyen et al. 2023). Generally, our investigation was similar to recent report which demonstrated that the chemical ingredients, biological activities including antimicrobial, anti-inflammatory, antidiabetic, anti-alzheimer properties of exposed Sage Oil to UV radiation were enhanced compared to un-exposed to UV radiation (Alsalamah et al. 2025). Also, earlier study found that pharmacological properties of Eclipta alba oil as a result of exposure to UV radiation Rai and Agrawal (2020). Li et al. (2016) studied the treated essential oil of white Guanxi honey pummelo by

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UV rays, who found that UV rays induced the aldehyde ingredients and its transformations, for instance, citronellal content was increased but other aldehydes contents decreased, also transformation of citral to geranic acid, neric acid, and cyclocitral was recorded as a result of exposure to UV rays. In another study, Rahman et al. (2020) observed that the level of limonene which represent the major constituent of orange essential oil was decreased (20%) after exposure the oil to UV rays. Finally, the application of UV-C irradiation dose of 17.0 mJ/ cm² UV-C to A. vera oil for 0, 30 and 60 min resulted in boosting in the number of chemical compounds in the oil to reach maximal number of molecules of 33 compounds from 10 various chemical classes. The present outcomes suggest a direct correlation with the exposure time with experimental biological activities. Various bacteria as well as C. albicans were inhibited A. vera oil with different levels of inhibition depending on the exposure time of oil to UV-C irradiation particularly for 60 min. For example, the inhibition zone of K. pneumonia was 16 ± 1 mm using un-exposed oil to UV-C, while become 24 ± 2 mm using exposed oil to UV-C for 60 min. The obtained IC50 values indicated that UV-C exposure time was effective for the anticancer properties of A. vera oil against A431 cell line where un-exposed oil to UV-C, exposed oil to UV-C to 30 min, and 60 min reflected IC₅₀ values 180.75 ± 1.38 , 155.44 ± 0.41 , and 80.91 ± 0.61 µg/ml, respectively. The same effect of UV-C exposure time was recorded in the antioxidant, anti-inflammatory, and wound healing properties of *A. vera* oil. Future research is required to screen the optimal maximal dose of radiation which might raise the biological impact of A. vera oil for various beneficial applications in vitro and in vivo for management of various health issue such as skin care, wounds particularly in diabetes. Also, future investigation is required to understand the action mechanisms of A. vera oil constituents against pathogenic microorganisms and cancer cells at ultrastructure and oxidative enzymes level.

Supplementary Information

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Supplementary Material 1.

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Author contributions

S.A.A. and M.S.A. Investigation, writing—review and editing; M.I.A., T.M.A., S.S., and M.T.A Conceptualization and methodology. All authors agreed to the published version of the manuscript.

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Data availability

The results from the current investigation are available from the coauthor (Sulaiman A. Alsalamah: SAAlsalamah@imamu.edu.sa) upon reasonable appeal.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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