Ultraviolet Radiation Effect on Chemical Profile of Sage Oil and its Inhibitor Capacity for Butyrylcholinesterase, α-Amylase, Protein Denaturation, Cancer and Pathogenic Yeasts

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Sage oil plays a vital role in various fields, including health and food. The effects of UV radiation (UVR) can increase the bioactive content of medicinal plants, but there has been little research on how this affects sage oil. Therefore, this study aimed to evaluate the impact of UVR on the sage oil phytoconstituents and its biological activity. GC-MS analysis detected 20, 23, and 25 different compounds in sage un-exposed and exposed to UVR for 30 and 60 min, respectively. *Candida albicans, C. tropicalis,* and *C. glabrata* were suppressed with inhibition zones 21.62 ± 1.22, 16.20 ± 1.23, and 8.20 ± 0.66 mm by sage oil, while the exposed sage oil to UVR for 60 min exhibited 26.50 ± 1.33, 21.43 ± 2.12, and 20.25 ± 0.50 mm inhibition zone, respectively. The required IC_{50} to inhibit butyrylcholinesterase, α-amylase, and protein denaturation was 95.3, 14.9, and 10.7 µg/mL in sage oil that was not exposed to UVR, and 35.1, 7.1, and 7.1 µg/mL in exposed sage oil to UVR for 60 min, respectively. There were negligible effects between the unexposed and exposed sage oil to UVR for 30 and 60 min against Hela cells with IC_{50} 193.19 ± 0.98, 149.71 ± 0.18, and 148.19 ± 0.66 µg/mL, respectively.

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INTRODUCTION

Plants and their products still represent the main sources for management of numerous health problems (Alsolami *et al*. 2023; Al-Rajhi *et al*. 2024). *Salvia*, a popular herbal plant identified as sage, is widespread in numerous cultures because of its cookery, psychological, and medical effects. The common sage (*Salvia officinalis* L.) plant is found all over the world, but it is especially common in tropical and temperate areas of the Mediterranean region of Europa, South-East Asia, and Central and South America (Afonso *et al*. 2019). Because of their health benefits, many species of *Salvia* are thought to be favorable for utilization in a variety of fields, such as the food, therapeutic, and cosmetic industries. Crucially, despite the number of analyses that have been operated in recent years

to screen the physiological characteristics of *Salvia* plants, many species are still not well understood (Carović-Stanko *et al*. 2016, Ozkan *et al*. 2016).

Raal *et al*. (2007) reported that essential oil of sage was applied for the management of dyspeptic signs and excessive perspiration. Longaray *et al*. (2007) explained that antibacterial and antifungal activities of sage oils can be attributed to the presence of camphor, 1,8-cineole, and thujone. Also, Pinto *et al*. (2007) documented the activity of sage oil against yeasts and filamentous fungi. It has been employed for the treatment of various types of illnesses such as ulcers, seizure, rheumatism, gout, tremor, inflammation, dizziness, hyperglycemia, diarrhea, and cancer; it also has shown efficacy as an antioxidant, anti-mutagenic, antidementia, antimicrobial, and hypolipidemic agent (Ghorbani and Esmaeilizadeh 2017).

According to Abu-Darwish *et al*. (2013), camphor and 1,8-cineole represented the main contents of sage oil. In addition, sage oil exhibits antifungal potential towards several strains of dermatophyte, and it is applied as a cosmetic for skin care and other pharmaceutical purposes. Alzheimer's disease (AD) is a chronic neurodegenerative illness that influences elderly individuals (Al-Rajhi *et al*. 2023). Gad *et al*. (2022) documented that essential oils of *Salvia* are attractive candidates for the management of AD. Prior clinical investigation reflected that utilization of sage oil enhanced cognitive and mental function in the infected individuals by AD (Babault *et al*. 2021).

The second leading reason of mortality in the world is cancers (Siegel *et al.* 2024). The 4th common cancer is cervical cancer among women worldwide (Hull *et al*. 2020). Based on the World Health Organization, the recommended managements of this cancer type are chemotherapy, surgery, and radiation. Natural sources from herbal plants have been progressively documented as an alternative treatment for cancer infections (Qanash *et al*. 2022; Selim *et al*. 2024). Three categories of UV radiation were reported, namely UV-A, UV-B, and UV-C with 315 to 400, 280 to 315 mμ, and 190 to 280 nm wavelength ranges, respectively (Pattanaik *et al.* 2007). The detrimental effects of UV-B radiation on medicinal plants and other kinds of plants have been recognized (Manukyan 2013). Prior research has revealed that UV radiation can increase the amounts of biologically active substances in medicinal plants while inducing secondary metabolism pathways (Nishimura *et al*. 2008; Lee *et al*. 2013). These trials assessed UV-B radiation during the developmental phase of each plant, which was challenging to operate and necessitated a significant financial outlay for production. Therefore, more research is required to determine the best way and dose to use UV-B radiation to increase the amount of bioactive chemicals in medicinal vegetation (Chen *et al*. 2018).

Previous studies have only focused on the chemical characterization of sage oil without considering the effects of UV exposure. There are few studies that have focused on the effects of UV on the chemical profile of sage oil and their biological activities. This study examined the effects of UV on the biological activity of sage oil, including antiyeast, anti-inflammatory, anticancer, anti-Alzheimer's, and antidiabetic activities *in vitro*. In addition, the effect of UV radiation on the chemical profile of sage oils was studied, giving insight on the biological activities of sage oil.

EXPERIMENTAL

Materials and Characterization of UV Radiation Used

Sage oil was obtained from Alhedaia Company, which is a source of natural oils and herbal cosmetics (Egypt). One liter of the oil was exposed to 0, 30, and 60 min in three separate containers with UV-B (SUN V-17/F UV lamp 235 V; 50.0 Hz; 0.16 Amplitude with an emission of 280 nm). The chemicals used alongside the work have been purchased from (Sigma, Egypt).

GC-MS Analysis of Sage Oil

The chemical components of the sage extract were ascertained by gas chromatography/mass spectral (GC-MS) analysis. The specimens of sage extract prior to exposure, and exposure to 30 and 60 min of UV were diluted with 9:1 dichloromethane: methanol and analyzed in Agilent Gas Chromatography (7880A) and mass spectrometry (5976C). After injecting 1.0 µL of the substance into the gas chromatography inlet, it was volatilized into the apparatus's column (Agilent 19091S-433: 468.56509. HP-5MS 5% Phenyl Methyl Silox 326 °C: 30.0 m × 250.0 µm x 0.25.0 µm). The temperature during the program was started at 85 °C for 1.0 min. The temperature was subsequently raised by 16 °C/min to 250.0 °C and held for 6.0 min, for a total duration of 20 min. Additionally, a split proportion of 1:1 infusion was used for the specimens, and 280.0 °C was retained. Additionally, high-purity helium gas was employed as a carrying gas at a pressure of 9.3826 pounds per square inch (psi) and an average flow rate of 1 mL/min. Ultimately, the samples' identification, structure, and molecular masses were determined through mass spectrometry analysis utilizing the National Institute of Standards and Technological repository (NIST 2014 and NIST 2011) (Al-Rajhi and Abdelghany 2023).

Antimicrobial Action, Minimum Inhibitory Concentration (MIC), and Minimum Fungicidal Concentration (MFC)

The tested examination of *in vitro* antiyeast properties were compared to a variety of yeast pathogens, *Candida albicans* (ATCC 10221), *Candida tropicalis* (66029), and *Candida glabrata* (66032), with the well dissemination of agar process using malt extract medium. About 100 µL of various suspended cells of yeast (1.5×10^4 colony producing units/mL) were utilized. After that, the inspected oil (25 µg/well) was placed into the medium-made well applying a polished cork borer tip. DMSO was used as the negative control product, and the commonly administered compound was fluconazole (0.24 µg/mL). The inhibition region was assessed at 30 °C for the investigated species of yeast following a 48-h development period (Almehayawi *et al*. 2024).

For MIC estimation: By using nutrient-rich and the micro-dilution broths technique, the samples' MIC was ascertained. The ultimate concentrations, which ranged from 0.97 to 1000 µg/mL, were calculated by diluting every sample twice. In every space of the 96-well micro-titrate dish, 100 µL of the component dilutions under evaluation in broth media have been prepared. To attain a 1.9×10^6 CFU/mL threshold, each well was treated with 2.6 µL of sterile 0.8% NaCl after the inoculum was prepared using fresh microbe cultures that satisfied the visibility requirements of the 1.0 McFarland standard. After that, the microorganisms were grown at 30 °C for 48 h. Each sample was optically measured to determine the MIC values that occurred when the standard strain's growth was entirely halted. A negative standard (estimated items without the infection) and a positive

reference (an inoculum containing the items being studied) were present in every microplate. For MFC detection: 100 mL of the control microbe culture, the final samples that were positive, and the medium with 100% growth inhibition were sub-cultured onto dishes in every hole so that MBC could be measured. The MFC was discovered to contain the fewest specimens that failed to support microbial development at the proper temperature during the time spent incubating.

Anti-inflammatory Impacts

The anti-inflammatory properties were assessed within a concentration range of 1.56 to 200 µg/mL. Diclofenac sodium, a strong non-steroidal anti-inflammatory reagent, was utilized as a norm routine medication. The resulting reaction combination, which had been suspended for 16 min at 29 ± 1 °C, contained 2 mL of the produced samples, 2.7 mL of phosphate-buffered saline (pH 6.5), and 2 mL of egg albumin (from fresh hen's egg, 1.0 mM). The denaturation process was achieved by heating the combination for 10 min at 75 °C in a water bath. Following normal room cooling, the intensity of absorption at 670 nm was obtained. Three runs of each experiment were conducted. The level of protein denaturation was determined as following: % inhibition = $(A_s/A_c\text{-}1) \times 100$ where A_s is the absorbance (Abs) of the specimen and A_c is the absorbance of the control.

Evaluation of Cytotoxic Action *versus* HeLa cells

The vitality of HeLa cells (obtained from the American Type Culture Collection, USA) was tested following the method explained by Abdelghany *et al*. (2019) employing an established colorimetric MTT technique with 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyl tetrazolium bromide reagent (Sigma, Egypt). The amount of the experimental oil in each specimen (31.25 to 1000 µg/mL) required to inhibit the proliferation of cells by 50% (IC_{50}) was calculated using the dose-response graph generated for each treatment. The MTT is converted to a violet formazan remnant by the respiratory dehydrogenase of healthy cells.

Anti-diabetic Evaluation *via* α-Amylase Inhibition

A revised description of the method used by Wickramaratne *et al*. (2016) was utilized to examine the sage oil for inhibition of α-amylase as a marker of diabetic development *via* 3,5-dinitrosalicylic acid (DNSA). Sage oil (20 to 100 µg/mL) was dissolved in DMSO (10%) and then mixed in a buffer composed from 0.006 M, 0.02M, and 0.02 M of NaCl, Na_2HPO_4, and NaH_2PO_4, respectively and adjusted to pH 6.9 to obtain final doses that ranged from 1.9 to 1000 µg/mL. Two units/mL of α-amylase (200 µL) were added to each dose of sage oil and kept for 10 min at 30 °C. This was followed by addition of 200 µL aqueous suspended starch (1%). The reaction mixture was continued for 3 min, and then finished through DNSA reagent (200 µL) with a boiling water bath adjusted to 85 °C for 12 min. Five mL of H_2O were added to the reaction mixture after cooling to 30 °C (Bakri *et al*. 2024). The absorbance was determined at 540 nm for treated and untreated α-amylase by the sage oil.

$$\alpha - \text{Amylase inhibition } (\%) = 100 - \frac{\text{Untreated } \alpha\text{–amylase } - \text{Treated } \alpha\text{–amylase}}{\text{Untreated } \alpha\text{–amylase}} \times 100 \quad (1)$$

Butyrylcholinesterase Inhibition Assay

The inhibition assay of butyrylcholinesterase (BChE) was achieved by using a modified description of the Ellman technique. The solutions and buffer for BChE were prepared as follows: 0.022 M S-butyrylthiocholine iodide (BChI) (7.01 mL/1 mL water) solution and 0.44 U/mL of BChE (2.9762 mg / 6.746 mL of buffer) solution adjusted at 8.0 pH. Buffer (200 µL), BChE enzyme (5 µL), Ellman's reagent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (5 µL), and varying doses of sage oil (0.195 to 100 µg/mL) were combined and left in a solution for 15 min at 30 °C using a water bath (Qanash *et al*. 2023). This allowed for the determination of the BChE inhibition assay by assessing absorbance *via* a microplate reader. Next, 5 µL of the BTchI substrate solution was added to the mixture to the beginning of the enzymatic reaction. The absorbance was measured at 410 nm employing a microplate reader to determine the inhibition of BTchI at 45 s, 13 times using the following equation,

$$\text{Butyrylcholinesterase inhibition } (\%) = 100 - \frac{\text{CR sage oil} - \text{R max}}{H_{\max}} \times 100 \qquad (2)$$

where *CR* sage oil means the alteration rate in the absorbance of the test including the sage oil (Δabs/Δtime), whereas $H_{\max}$ means the highest change rate in the absorbance of the sample of blank deprived of any inhibitor. Rivastigmine (inhibitor for cholinesterase to treat AD dementia) was applied as a standard drug without exposure to UV radiation.

Statistical Investigation

The findings were offered as mean ± standard deviation (SD) for three replicates. These were calculated using Microsoft Excel 365 and SPSS v.25.

RESULTS AND DISCUSSION

Results from GC-MS examination for bioactive compounds of the sage extract in regular conditions and upon exposure to UV radiation for 30 and 60 min are shown in (Figs. 1 to 3 and Tables 1 to 2). The GC profile for the unexposed sage extract showed the presence of 20 different compounds, which mainly consisted of 10 different fatty acid esters and 6 different fatty acids, as well as 4 other compounds from various classes. Furthermore, eight major molecules could be seen, which were: ethyl (9Z)-octadec-9-enoate, octadec-9-enoic acid, ethyl linoleate, methyl (9E)-9-octadecenoate, 3-[(*Z*)-octadec-9-enoyl]oxypropyl octadecanoate, ethyl palmitate, and methyl (9Z,12Z)-octadeca-9,12-dienoate. Meanwhile, the GC profile for the 30 min exposed extract of UV radiation revealed the presence of 23 various compounds, which essentially formed from 13 various fatty acid esters and 4 different fatty acids and 6 other molecules form various classes. Besides, eight major compounds could be seen, which were: methyl hexadecanoate; octadec-9-enoic acid; ethyl (9Z)-octadec-9-enoate; 9,11-octadecadienoic acid, methyl ester, (E,E)-; methyl octadecanoate; (Z)-octadec-9-enoic acid; hexadecanoic acid; (9E,12Z)-octadeca-9,12-dienoic acid; and octadec-9-enoic acid, constitutively. Lastly, the GC pattern upon exposure to 60 min of UV radiation showed the presence of 25 deferent compounds, which mainly consisted of 12 different fatty acid esters and 5 various fatty acids, and 8 different compounds from other classes. Additionally; eight major compounds could be seen, which were: octadec-9-enoic acid, methyl (9E)-9-octadecenoate; ethyl linoleate, ethyl (9Z)-octadec-9-enoate, ethyl linoleate, hexadecanoic acid, 3-[(*Z*)-octadec-

9-enoyl]oxypropyl octadecanoate, and (9E,12Z)-octadeca-9,12-dienoic acid. It could be noticed that ethyl (9Z)-octadec-9-enoate (21.87), octadec-9-enoic acid (20.38), and ethyl linoleate (17.66) were the highest three compounds in sage extract. Exposure to UV radiation led to reduction of the levels of these molecules and the yield of other bioactive derivatives, which increased upon elevation of the exposing time *i.e.*, number of bioactive compounds proportional to the increase upon raising the time of exposure to UV.

Light serves as a vital signal that controls the development, growth, and physiology in higher plants in addition to being a source of energy (Krizek and Chalker-Scott 2005). However, it has been shown that the spectrum equilibrium of UV and radiation that stimulates photosynthesis (PAR) intensity affects how plants react to ultraviolet (UV) light (Matsuura *et al*. 2013). To deal with UV stressors in the environment, plants have developed a variety of defense mechanisms. Exposure of plants to ultraviolet (UV) rays can increase the creation of antioxidant agents as a preventative measure (Dolzhenko *et al*. 2010; Raffo *et al*. 2020). Analysis of various extracts of sage using GC-MS reveal that the number of bioactive molecules of sage extract has been increased upon increasing the time of exposure to UV radiation. It has been demonstrated that UV changed the regulation of genes in peppermint that are included in the manufacture of essential oils, which in turn changed the chemical composition of essential oils (Alagupalamuthirsolai *et al*. 2019). The composition of essential oils changes when exposed to UV light, according to numerous publications. For instance, lemongrass's essential oil level rose when exposed to UV light (Kumari *et al*. 2009). Significant increases in a few medicinally significant components of *E. alba* subjected to prolonged UV irradiation were similarly found by Rai and Agrawal (2020). GC-MS for sage extract reflect the predominance of fatty acids and their ester in various test samples. Two kinds of lipids, fatty acids (chains of hydrocarbon with a reactive group of carboxylic acid) and monoglycerides (enriched additives of a fatty acid and glycerol molecule), are of particular significance because of their diverse biological activities (Yoon *et al*. 2018; Zhu *et al*. 2025).

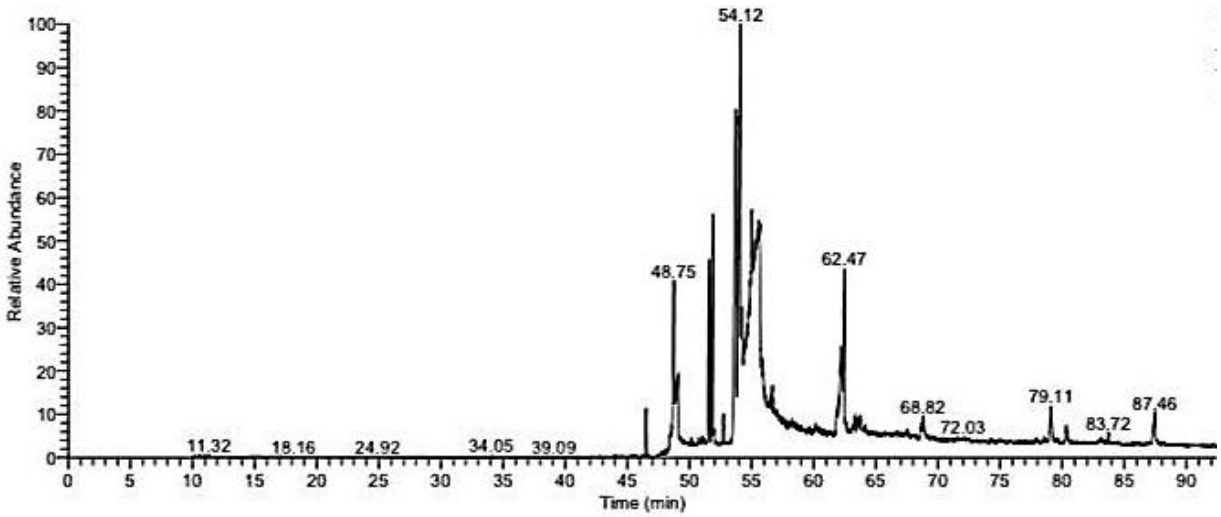

Fig. 1. GC-MS analysis of non-exposed sage oil to UV

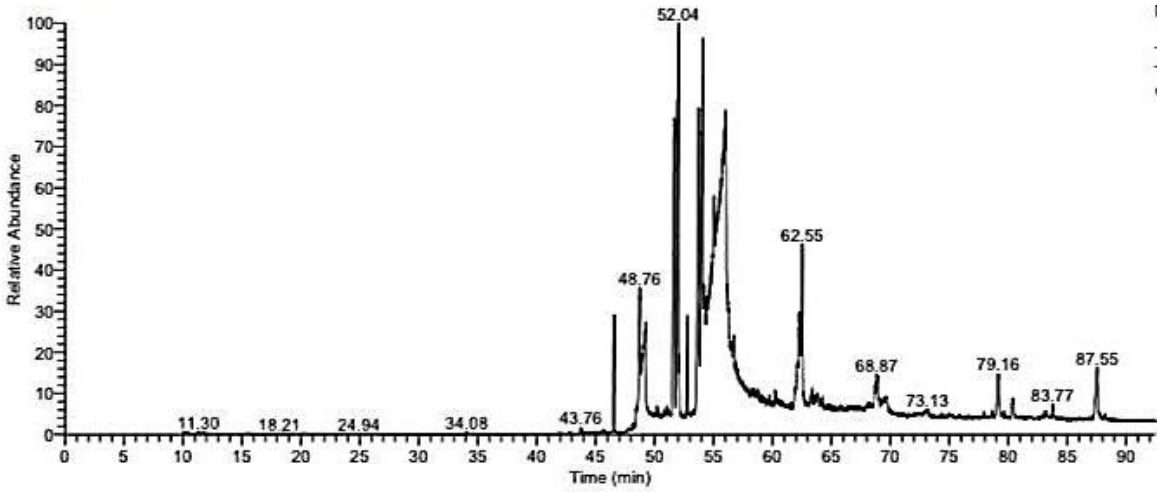

Fig. 2. GC-MS analysis of exposed sage oil to UV for 30 min

Table 1. Detected Compounds in Non-exposed Sage Oil to UV *via* GC-MS Investigation

*RT (min)	Area (%)	Constituent Name	Group	Formula	***MW (g/mol)
46.52	1.47	Methyl hexadecanoate	Fatty acid ester	$C_{17}H_{34}O_2$	270
47.84	0.16	(*Z*)-Hexadec-9-enoic acid	Fatty acid	$C_{16}H_{30}O_2$	254
48.14	0.17	(9E)-Octadeca-9,17-dienal	Fatty aldehyde	$C_{18}H_{32}O$	264
48.75	4.46	Ethyl palmitate	Fatty acid ester	$C_{18}H_{36}O_2$	284
49.09	3.03	Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256
50.19	1.08	Oleic Acid (Z)-Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
51.58	7.62	Methyl (9Z,12Z)-octadeca-9,12-dienoate	Fatty acid ester	$C_{19}H_{34}O_2$	294
51.90	8.02	Methyl (9E)-9-octadecenoate	Fatty acid ester	$C_{19}H_{36}O_2$	296
52.72	1.11	Methyl octadecanoate	Fatty acid ester	$C_{19}H_{38}O_2$	298
53.74	17.66	Ethyl linoleate	Fatty acid ester	$C_{20}H_{36}O_2$	308
54.12	21.87	Ethyl (9Z)-octadec-9-enoate	Fatty acid ester	$C_{20}H_{38}O_2$	310
55.58	20.38	Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
62.19	2.54	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxy methyl)ethyl ester	Fatty acid ester	$C_{21}H_{38}O_4$	354
62.47	4.49	3-[(*Z*)-Octadec-9-enoyl]oxypropyl octadecanoate	Fatty acid ester	$C_{21}H_{40}O_3$	340
63.50	0.83	(9E,12Z)-Octadeca-9,12-dienoic acid	Fatty acid	$C_{18}H_{32}O_2$	280
79.10	1.29	5-[(3S,3aR,6S,6aR)-3-(1,3-benzodioxol-5-yl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-1,3-benzodioxole	*Lignan*	$C_{20}H_{18}O_6$	354
79.61	0.25	Methyl (11E,14E)-icosa-11,14-dienoate	Fatty acid ester	$C_{21}H_{38}O_2$	322
80.32	0.77	(3E,13Z)-2-Methyloctadeca-3,13-dien-1-ol	Fatty acid alchol	$C_{19}H_{36}O$	280
83.72	0.84	Dotriacontane	Alkane	$C_{32}H_{66}$	450
87.47	1.96	[(E)-Hexadec-9-enyl] (E)-hexadec-9-enoate	Fatty acid	$C_{18}H_{34}O_2$	282

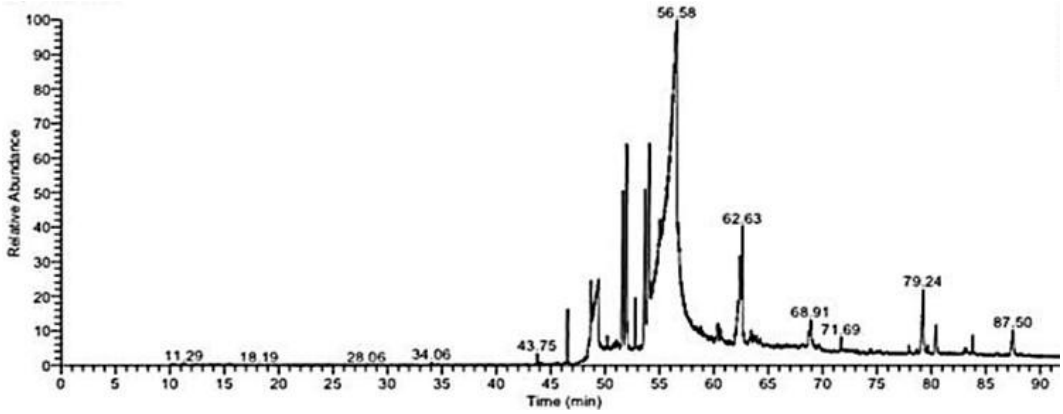

Fig. 3. GC-MS analysis of exposed sage oil to UV for 30 min

Table 2. Detected Compounds in Sage Oil Exposed to UV at 30 min *via* GC-MS Investigation

*RT (min)	Area (%)	Constituent Name	Group	Formula	***MW (g/mol)
43.75	0.16	Bis(2-methylpropyl) benzene-1,2-dicarboxylate	Carboxlate ester	$C_{16}H_{22}O_4$	278
46.57	19.03	Methyl hexadecanoate	Fatty acid ester	$C_{17}H_{34}O_2$	270
48.16	0.18	(9E)-Octadeca-9,17-dienal	Fatty aldehyde	$C_{18}H_{32}O$	264
48.75	1.84	Ethyl palmitate	Fatty acid ester	$C_{18}H_{36}O_2$	284
49.25	5.4	n-Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	256
51.69	11.22	9,11-Octadecadienoic acid, methyl ester, (E,E)-	Fatty acid ester	$C_{19}H_{34}O_2$	294
52.04	15.91	Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
52.10	0.18	Methyl octadec-11-enoate	Fatty acid ester	$C_{19}H_{36}O_2$	296
52.79	2.72	Methyl octadecanoate	Fatty acid ester	$C_{19}H_{38}O_2$	298
53.75	9.09	Ethyl linoleate	Fatty acid ester	$C_{20}H_{36}O_2$	308
54.12	12.45	Ethyl (9Z)-octadec-9-enoate	Fatty acid ester	$C_{20}H_{38}O_2$	310
55.06	5.33	(9E,12Z)-Octadeca-9,12-dienoic acid	Fatty acid ester	$C_{18}H_{32}O_2$	280
56.05	5.56	(Z)-Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
62.30	2.10	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	Fatty acid ester	$C_{21}H_{38}O_4$	354
62.55	3.01	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Fatty acid ester	$C_{21}H_{40}O_4$	356
73.14	0.16	(E)-Octadec-13-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
77.95	0.19	Methyl (8E,11E,14E)-docosa-8,11,14-trienoate	Fatty acid ester	$C_{23}H_{40}O_2$	348
78.63	0.2	[(8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-Ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl] (Z)-octadec-9-enoate	Beta-sitosterols	$C_{47}H_{82}O_2$	678
80.38	0.67	(3*E*,13*Z*)-2-Methyloctadeca-3,13-dien-1-ol	Fatty acyls	$C_{19}H_{36}O$	280
83.76	0.41	Dotriacontane	Alkane	$C_{32}H_{66}$	450
87.55	2.78	1,2,3-Propanetriyl tris[(E)-9-octadecenoate]	Fatty acid ester	$C_{57}H_{104}O_6$	884

The recorded inhibition zones indicated that sage oil exhibited activity against examined yeasts *C. albicans*, *C. tropicalis*, and *C. glabrata*, with zones of inhibition 21.62±1.22, 16.20±1.23, and 8.20±0.66 mm at 0 time of UV exposure, 22.65±2.2, 18.22.±1.3, and 14.21±1.5 mm at 30 min of UV exposure, 26.50±1.33, 21.43±2.12, and 20.25±0.50 mm, respectively (Table 4 and Fig. 4).

Table 3. Detected Compounds in Sage Oil Exposed to UV at 60 min *via* GC-MS Investigation

*RT (min)	Area (%)	Constituent Name	Group	Formula	***MW (g/mol)
43.57	0.52	Bis(2-methylpropyl) benzene-1,2-dicarboxylate	Carboxlate ester	$C_{16}H_{22}O_4$	278
46.54	2.30	Methyl hexadecanoate	Fatty acid ester	$C_{17}H_{34}O_2$	270
48.72	2.13	Ethyl palmitate	Fatty acid ester	$C_{18}H_{36}O_2$	284
49.38	8.09	Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$	265
50.21	0.67	Octadec-9-enoic acid	Fatty acid	$C_{21}H34O_2$	341
51.65	13.57	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid ester	$C_{19}H_{34}O_2$	294
51.98	11.79	Methyl octadec-9-enoate	Fatty acid ester	$C_{19}H_{36}O_2$	296
52.77	2.10	Methyl octadecanoate	Fatty acid ester	$C_{19}H_{38}O_2$	298
53.70	10.26	Ethyl linoleate	Fatty acid ester	$C_{20}H_{36}O_2$	308
54.05	10.77	Ethyl (9Z)-octadec-9-enoate	Fatty acid ester	$C_{20}H_{38}O_2$	310
55.01	2.24	(Z)-Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
56.59	14.94	Octadec-9-enoic acid	Fatty acid	$C_{18}H_{34}O_2$	282
62.17	0.39	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxy methyl)ethyl ester	Fatty acid ester	$C_{21}H_{38}O_4$	354
62.42	3.05	(3E,12Z)-Nonadeca-1,3,12-triene-5,14-diol	Fatty acyls	$C_{19}H_{34}O_2$	294
62.64	4.39	3-[(*Z*)-Octadec-9-enoyl]oxypropyl octadecanoate	Fatty acid ester	$C_{21}H_{40}O_3$	340
63.43	2.47	(9E,12Z)-Octadeca-9,12-dienoic acid	Fatty acid	$C_{18}H_{32}O_2$	280
63.94	0.17	Oxiran-2-ylmethyl octadec-9-enoate	Fatty acid ester	$C_{21}H_{38}O_3$	338
71.69	0.69	(9Z)-Octadec-9-enamide	Fatty amide	$C_{18}H_{35}NO$	281
75.31	0.49	Methyl (11E,14E)-icosa-11,14-dienoate	Fatty acid ester	$C_{21}H_{38}O_2$	322
79.24	4.05	5-[(3S,3aR,6S,6aR)-3-(1,3-benzodioxol-5-yl)-1,3,3a,4,6,6a-hexahydrofuro[3,4-c]furan-6-yl]-1,3-benzodioxole	Lignan	$C_{20}H_{18}O_6$	354
79.65	0.36	Dotriacontane	Alkane	$C_{32}H_{66}$	450
80.42	1.56	2-(2-Butynyl)cyclohexanone	Cyclic ketone	$C_{10}H_{14}O$	150
83.14	0.32	17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-Dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	Phytosterols	$C_{29}H_{50}O$	414
83.78	0.84	Heptacosane	Alkane	$C_{27}H_{56}$	380
87.50	1.84	Pentyl (9E)-9-octadecenoate Pentyl oleate	Fatty acid ester	$C_{23}H_{44}O_2$	352

These results indicated that UV exhibited an inducer effect for sage oil to inhibit yeasts. This was perhaps due to the occurrence of active ingredients in the oil exposed to UV. The outcomes of MIC and MFC indicated that the sage oil exposed to UV was more effective than non-exposed sage oil to UV against examined yeasts. The MIC and MFC values were recorded with low quantity, particularly at 60 min compared to that at 0 min (Table 5). According to Abu-Darwish *et al*. (2013) sage oil inhibited some fungi particularly dermatophyte such as *Epidermophyton floccosum* and *Trichophyton rubrum*, *Cryptococcus neoformans* besides different strains of *Aspergillus* and *Candida*. Some compounds in sage oil including linalyl acetate, α-terpineol, and camphor showed anti-inflammatory, antifungal, and anticancer effects (Itani *et al*. 2008).

Table 4. Effect of Sage Oil Exposed to UV at Different Times Against Growth of Different Yeasts

Examined Yeast	Inhibition Zone at Different Time (min)			
	0	30	60	Control
C. albicans	21.62 ± 1.22	22.65 ± 2.2	26.50 ± 1.33	29.50 ± 1.25
C. tropicalis	16.20 ± 1.23	18.22 ± 1.3	21.43 ± 2.12	20.66 ± 2.30
C. glabrata	8.20 ± 0.66	14.21 ± 1.5	20.25 ± 0.50	26.25 ± 1.11

Table 5. MIC and MFC of Sage Oil Exposed to UV at Different Times against Different Yeasts

Examined Yeast	MIC at Different Time (min)			MFC at Different Time (min)		
	0	30	60	0	30	60
C. albicans	31.25	15.62	15.62	125	62.5	31.25
C. tropicalis	125	62.5	31.25	250	125	62.50
C. *glabrata*	250	62.5	31.25	500	125	31.25

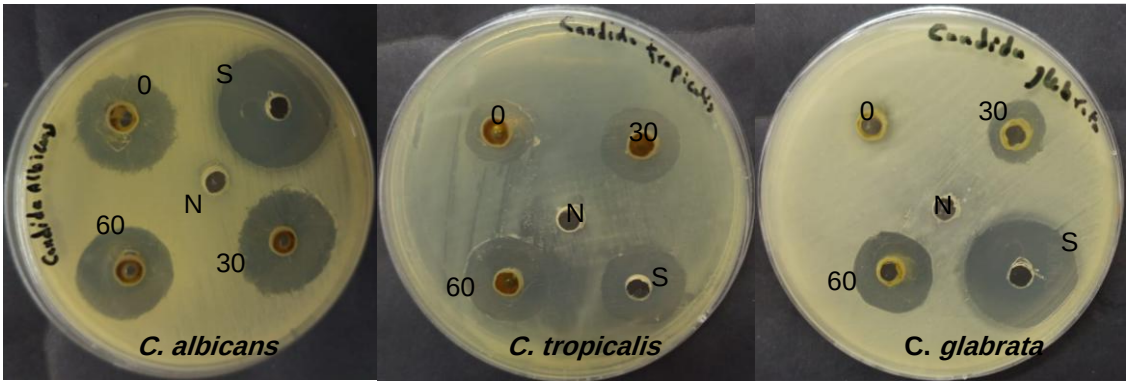

Fig. 4. Growth inhibition of examined yeasts by unexposed sage oil to UV (0), UV-exposed sage oil at 30 min (30), and UV-exposed sage oil at 60 min (60) compared to standard (S) and negative control (N)

Inhibition of BChE is one of the main management strategies to minimize AD. The percentage of BChE inhibition increased with increasing the dose of sage oil either exposed to UV or not, but the exposed oil to UV increased the BChE inhibition %, particularly when exposed to UV for 60 min. Compared to standard rivastigmine, the sage oil possess moderate anti-Alzheimer activity; however the UV increased its activity against BChE action (Fig. 5), where the IC_{50} values were 0.587, 95.29, 46.28, and 35.06 µg/mL, for

rivastigmine, unexposed sage oil to UV, UV-exposed sage oil at 30 min, UV-exposed sage oil at 60 min, respectively. Another enzyme acetyl cholinesterase (AChE) as biomarker of AD development was inhibited at the level 46% by 500 µg/mL of sage oil (Ferreira *et al*. 2006). The cited article reported enhancing the memory *via* reducing the acetylcholine shortage and improving the brain content of acetylcholine. Also, a previous study by Eidi *et al*. (2006) recorded that sage oil enhance the immediate word recall. Additionally, the essential oil of sage (*S. officinalis*) displayed inhibitions of 63.8% and 66.3% of AChE and BChE, respectively (Orhan *et al*. 2008). The essential oils (EO) activity of *Salvia* species namely *S. sclarea*, *S. virgata*, and *S. officinalis*, were estimated against AChE (Gad *et al*. 2022), where EO of *S. virgata* was more effective than *S. officinalis*, but EO of *S. virgata* was non-effective.

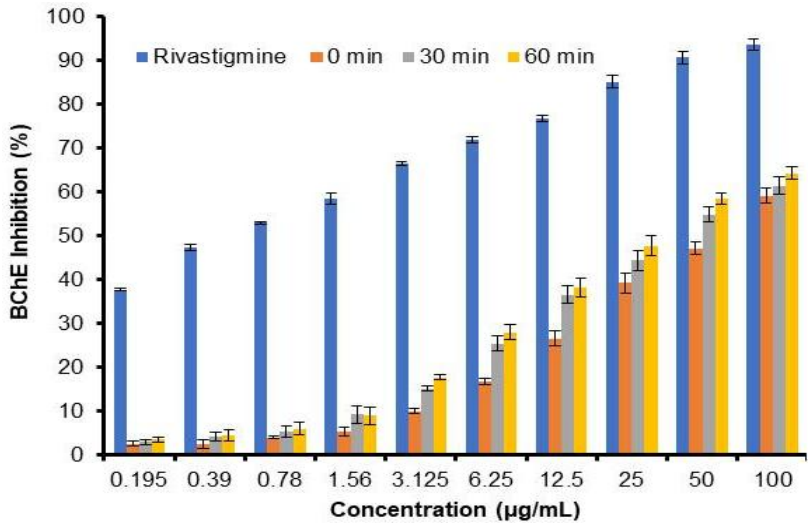

Fig. 5. Inhibition of BChE by unexposed sage oil to UV, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min compared to standard Rivastigmine

Sage oil reflected anti-diabetic effect *in vitro* through prevention of α-amylase activity, as illustrated in Fig. (6).

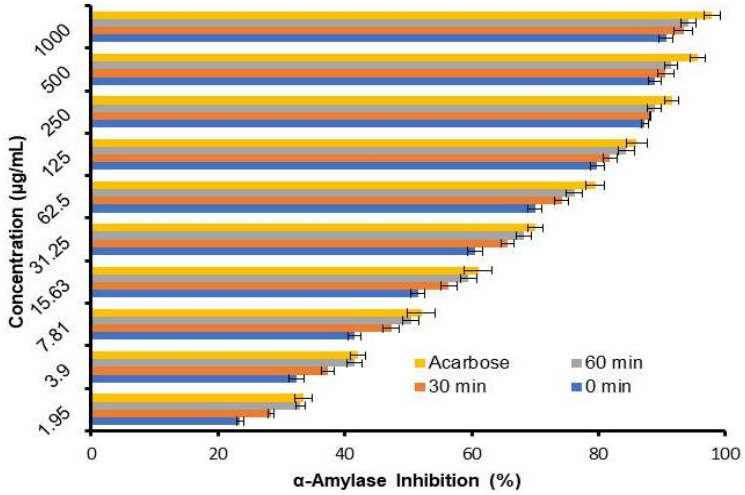

Fig. 6. Inhibition of α-amylase by unexposed sage oil to UV, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min compared to standard acarbose

The α-amylase activity declined as the dose of oil increased, where the inhibition reached to 90.7, 93.4, and 94.2% when using 1000 µg/mL of unexposed sage oil to UV, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min, respectively. It is clear that maximum inhibition of α-amylase activity was recorded when employing UV-exposed sage oil at 60 min, followed by UV-exposed sage oil at 30 min, and unexposed sage oil to UV with IC_{50} values of 7.1, 9.85, and 14.89 µg/mL, respectively. The obtained finding was comparable to the IC_{50} value (6.37 µg/mL) of acarbose, which supported the outcome of anti-diabetic potential of our sage oil. The hypoglycemic properties of sage oil were documented in earlier studies *in vivo* and *in vitro* (Eidi *et al.* 2005).

Protein denaturation is a well-recorded explanation of inflammation. As a part of the examination on the anti-inflammatory mechanism, testing of the capability of sage oil to inhibit denaturation of protein was planned. Protein denaturation inhibition as a marker of anti-inflammatory effect was ducumented bysage oil , indicating this oil play an important role in the minimize inflammation. Based on results shown in Fig. 7, sage oil played a critical role for preventing protein denaturation with excellent IC_{50} 10.74 µg/mL. However, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min reflected the highest activity with IC_{50} values 7.07 and 9.12 µg/mL, respectively than the effect of unexposed sage oil to UV. Our experiment used standard diclofenac sodium, which reflected IC_{50} value 6.46 µg/mL. Sage oil showed anti-inflammatory action in an earlier study (Abu-Darwish *et al.* 2013). Vaishali *et al.* (2018) *via* protein denaturation inhibition documented the anti-inflammatory of sage oil.

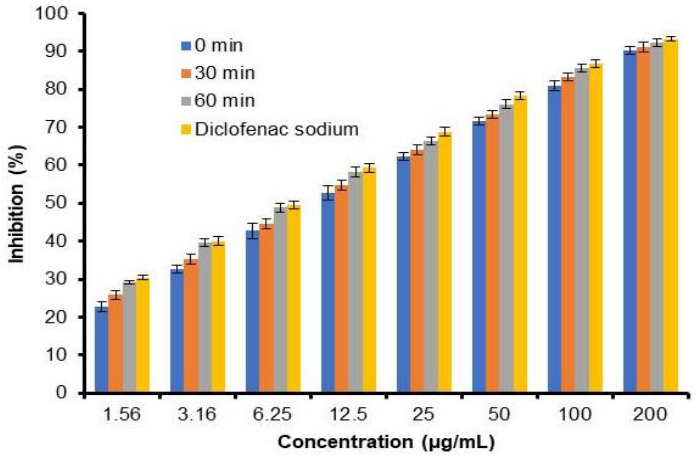

Fig. 7. Inhibition of protein denaturation by unexposed sage oil to UV, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min compared to standard diclofenac sodium

From the experiments, it is clear that low concentrations (31.25, 62.5, and 125 µg/mL) of sage oil did not exhibit anticancer activity, while there were clear effects at high concentrations (250, 500, and 1000 µg/mL) (Fig. 8). There was a negligible difference among the effect of UV-unexposed sage oil, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min on proliferation of HeLa cells, which indicated 96.3%, 96.5%, and 97.1% toxicity level, respectively. Also, the calculation of IC_{50} quantities of UV-unexposed sage oil, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min were 193.19 ± 0.98, 149.71 ± 0.18, and 148.19 ± 0.66 µg/mL, respectively. In a recent study, *Salvia officinalis* L essential oil was shown to have potent anticancer activities

(Babaei-Ghaghelestany *et al*. 2023). The sage oil exhibited anti-cancer effect with IC_{50} 17.32µg/mL toward hepatocellular carcinoma (Falih and Al-Ali 2024).

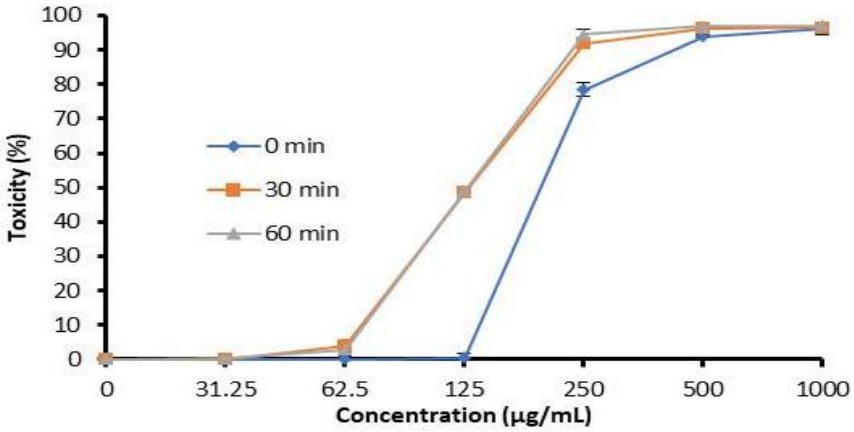

Fig. 8. Toxicity potential of UV-unexposed sage oil, UV-exposed sage oil at 30 min, and UV-exposed sage oil at 60 min against HeLa cells

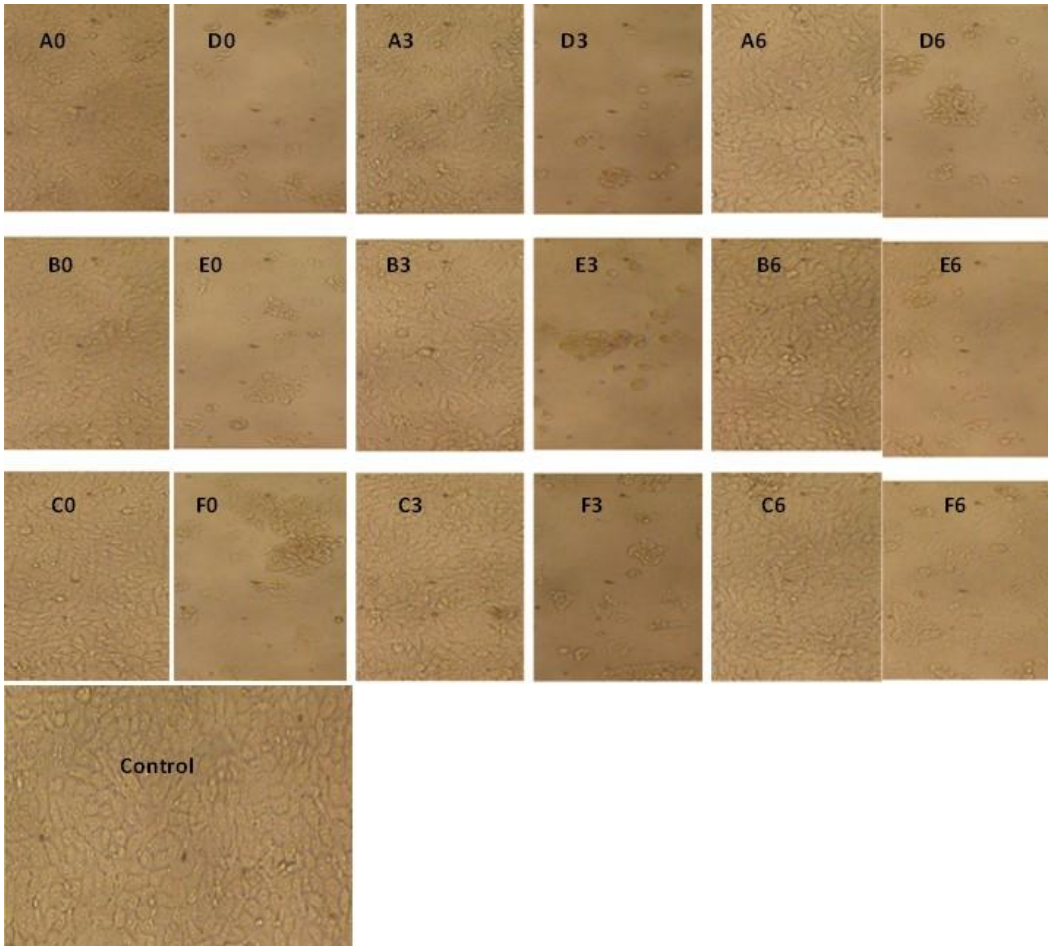

Fig. 9. Morphological features of PC3 at various doses of UV-unexposed sage oil (0), exposed sage oil to UV for 30 min (3), and exposed sage oil to UV for 60 min (6) (31.25 µg/mL (A), 62.5 µg/mL (B), 125 µg/mL (C), 250 µg/mL (D), 500 µg/mL (E), 1000 µg/mL (F) and untreated cells (Control)

Microscopic examination of the cells revealed that unexposed HeLa cells to sage oil were described by a regular polygonal development with an organized monolayer. The cells treated with sage oil and exposed to UV at 30 and 60 min became rounded and showed cellular shrinkage and nuclear condensation (Fig. 9). Strong anticancer activity was attributed α-humulene, a constituent of sage oil against human prostate carcinoma (Loizzo *et al*. 2007). Generally, some detected compounds in sage oil exhibited biological activities. For example, ethyl linoleate was applied as antidiabetic (Rayar and Manivannan 2015), anticancer (Heredia *et al.* 2022), antibacterial and anti-inflammatory (Ko and Kim 2018). According to Jayakumar *et al.* (2017) 9,12-octadecadienoic acid (Z,Z), methyl ester exhibited anticancer and hypocholesterolaemic potential. Elgorban *et al*. (2019) reported that bis(2-methylpropyl) benzene-1,2-dicarboxylate has antimicrobial activity. As mentioned in other study, anticancer, anti-inflammatory, antifungal, antiviral, antioxidant and antibacterial properties were attributed to octadec-9-enoic acid, n-hexadecanoic acid, and methyl octadecanoate (Khan and Javaid 2023).

CONCLUSIONS

1. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that ultraviolet (UV) radiation increased the chemical compounds as well as derivatives from sage oil.
2. The sage oil under controlled condition of UV radiation prevented protein denaturation particularly when sage oil was exposed to UV for 60 min.
3. Inhibition of BChE, an α-amylase as a marker of AD and diabetic infection, was higher when employed by sage oil exposed to UV than UV non-exposed sample.
4. Numerous yeasts, namely *C. albicans*, *C. tropicalis*, and *C. glabrata*, were more highly inhibited by sage oil exposed to UV than that non-exposed to UV.
5. The present research was limited to evaluating the effect of UV on the chemical profile of sage oil and its biological activities. It is necessary in future research to study the mechanisms of how UV changes the chemical constituents and biological activity of the oil.

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