Ozone Driven Modulation of Camphor Oil – Chemical Composition, Anti-Yeast Potential, and Anti-Ovarian Cancer Mechanistic Exploration

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Gas chromatography-mass spectrometry (GC-MS) analysis was used to examine the chemical constituents of crude camphor oil and its ozonized derivatives and to assess their *in vitro* anticancer and anti-yeast properties. In crude camphor oil, GC-MS profiling revealed 21 compounds from 15 different chemical classes, whereas ozonized oil included 22 compounds from fifteen various classes. Increase in the contents of dotriacontane and 2,2-dideutero octadecanal in the ozonized oil indicated chemical changes brought about by ozonation. Antifungal tests revealed that ozonation significantly reduced ($P \leq 0.05$) the Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) values while increasing the inhibition zones against *Candida albicans*, *Candida tropicalis*, and *Candida glabrata* (23 ± 0.6 mm, 26 ± 0.4 mm, and 25 ± 0.2 mm, respectively) when compared to crude oil. Both oils showed cytotoxic effects on SKOV3 ovarian cancer cells. However, ozonized camphor oil was more potent than crude oil with IC_{50}= 183.18 ± 2.29 µg/mL and IC_{50} = 152.04 ± 0.4 µg/mL, respectively. Following treatment with the IC_{50} of ozonized oil, cell-cycle analysis showed a notable decrease in S-phase cells and a notable increase in G2/M accumulation, suggesting inhibition of DNA production and triggering of G2/M arrest.

DOI: 10.15376/biores.21.2.4246-4262

Keywords: Camphor oil; Anti-yeasts; Anticancer; Cell cycle

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INTRODUCTION

Natural oils derived from plants have long been valued for their many technological, aromatic, and medicinal uses. These oils, which are made up of intricate blends of bioactive secondary metabolites, have garnered a lot of scientific interest because of their wide range of therapeutic properties, demonstrating antioxidant, antibacterial, anti-inflammatory, and

cancer-preventive effects (Qanash *et al*. 2023a; Alsolami *et al*. 2025; Al-Rajhi *et al.* 2025a). The revival of interest in plant-based products in the recent period has prompted a great deal of research into their biological activity and chemical makeup, with an emphasis on essential oils derived from aromatic plants (Yahya *et al*. 2022; Bazaid *et al*. 2025a,b).

From all common natural oils, camphor oil, derived mostly from the wood of *Cinnamomum camphora*, stands out due to its unusual chemical composition and several biological properties. Camphor and its terpenoid components have long been utilized in conventional medical treatment because of their pain-mitigating, anti-inflammatory, and antibacterial properties (Duda-Madej *et al*. 2024). Modern scientific research has verified several of these applications and increased the understanding of camphor's medicinal potential. Camphor's efficiency against numerous microbial infections, function in inflammation modulation, and ability to alter cellular mechanisms related to disease management have all been highlighted in the published research (Fazmiya *et al*. 2022).

Recent advances in natural product customization have identified ozonation of oils as a possible method for increasing biological activity (Vieira *et al*. 2025). These ozonated oils have better biological characteristics than their non-modified counterparts. The creation of oxygen-rich functional groups not only improves oils' ability to damage microbial membranes, but it may also enhance anticancer effects by increasing oxidative stress within malignant cells. Integrating ozonation into natural oil research is a valuable technique for improving therapeutic effectiveness and broadening the application spectrum of bioactive molecules obtained from essential oils like camphor (Al-Rajhi *et al.* 2025b). While natural products are often approved for human use, their ozonized derivatives may pose additional safety concerns due to the formation of new or poorly characterized compounds. Ozonization, as an oxidative process, can alter chemical composition and generate reactive or unknown constituents, potentially increasing medical risks. Therefore, careful toxicological evaluation is essential before considering ozonized natural products for human applications (Repciuc *et al.* 2025).

Accurate characterization of the chemical ingredients of natural oils is critical for determining their composition and biological function. Gas chromatography–mass spectrometry (GC–MS) is regarded as a highly effective and robust analytical approach for profiling essential oils, allowing for the exact recognition and measurement of volatile and semi-volatile components (Catarro *et al*. 2025). This technique enables researchers to associate individual terpenes, phenolics, and other botanical compounds with reported pharmacological impacts, hence boosting their comprehension of the mechanisms behind the medicinal benefits of natural oils such as camphor (Arzani *et al*. 2025).

Beyond antimicrobial effects, growing data suggests that several natural oils have anticancer properties, functioning through a variety of molecular and cellular pathways. One of the primary processes is the activation of apoptosis, a programmed cell death process required to prevent excessive proliferation (Al-Rajhi *et al*. 2022; Quintero-Rincón *et al*. 2025). Essential oil constituents may induce apoptosis by stimulating mitochondrial pathways, influencing caspase functioning, or inducing oxidative damage. Furthermore, these compounds could impact the cell cycle, thereby inhibiting cancer cells at particular stages and thus inhibiting tumor growth. Elucidating these pathways is vital for appraising the curative effects of natural oils in cancer treatment (Ben Miri 2025).

Despite the wide use of camphor oil in traditional medicine, limited studies have examined how controlled ozonation may alter its chemical composition and biological performance. In particular, the relationship between ozone-induced chemical

transformations and enhanced antifungal and anticancer activities remains poorly understood. Therefore, the present study aimed to compare crude and ozonized camphor oil through GC-MS chemical profiling and to evaluate their anti-yeast activity against Candida species as well as their anticancer potential against SKOV3 ovarian cancer cells, with particular attention to cell-cycle modulation as a possible mechanistic pathway.

EXPERIMENTAL

Oil and Chemicals

Camphor oil (Therapeutic Grade, white color, Natural and 100% Pure) was purchased from Handcraft Blends, Saudi Arabia (Code: B0DB619ZK9). The reagents and chemicals utilized throughout the investigation were purchased from the (Sigma-Aldrich, Egypt) manufacturer.

Ozonization of Camphor Oil

Ozone gas was produced *via* an electrical boundary shockwave plasma generator (Ozone Generator: OZ-5000, OzoneTech Systems, USA) at the Plasma Center, Al-Azhar University. A 2.1 L Drechsel cylinder containing 1.0 L of camphor oil was submerged in a cooling bath at −8 °C at the plasma device's outlet. Ozone was bubbled in the camphor oil for seven hours, generating a semisolid state at a steady rate of 0 to 10 L/min. After ozonation, the ozonated camphor oil was withdrawn from the Drechsel apparatus, moved to an empty glass bottle, and kept at 9 °C (Alsalamah *et al*. 2025a).

Detection of Various Components by GC-MS

To investigate the chemical composition of camphor oil and its ozonized form employing gas chromatography, the GC tests were conducted using a flame-ionization detector device (FID), an automatic sampling device (Agilent Technologies, USA), and a Rt-580 column (105.0 m × 0.28 mm × 0.23 µm; Agilent Technologies, USA). The FID data were collected using a chromatographic system (X-caliber data capture and software, Agilent Technologies, USA). The carrier gas, helium, was given by rip delivery (100:1). The pre-run interval lasted 10 min. The process took 0.7 min to attain equilibrium. There were two constructed ramps: ramp 1 had a pace of 7 °C/min with an ultimate average temperature of 205 °C, and ramp 2 had a rate of 6 °C/min. It reached a maximum temperature of 285 °C after starting at 49 °C. The experiments were conducted in controlled heat mode, with temperatures ranging from 100 to 286 °C and isothermal periods of 14.1 min, 1.7 mL/min was the medium's rate of action. The chemicals were identified by comparing their mass spectra to data from NIST 14 and 14s (National Institute of Standards and Technologies, Mass Spectra Libraries). Internal standards were utilized to carry out and alter the profiles of calibration (Al-Rajhi *et al*. 2025b).

Determination of Anti-Yeasts Action

The oil forms' anti-yeasts action was tested using the cup-plate agar diffusion approach versus *Candida albicans* (ATCC 90028), *Candida tropicalis* (ATCC66029), and *Candida glabrata* (ATCC 90030), as pronounced by Alsalamah *et al*. (2023) following some adjustments.

The examined yeasts were standardized (0.6 McFarland scale), sown in melted sterile sabouraud dextrose agar media, and placed in petri dishes. Four cups were removed after solidification using a sterile cork borer with a radius of 6.0 mm. To allow diffusion of the tested sample through the agar medium, 100 µL of the ozonized camphor oil solution (20 µg/mL) was carefully dispensed into each well using an automatic micropipette. The plates were placed in a refrigerator for approximately 2 h prior to incubation to facilitate the diffusion of the oil from the wells into the surrounding medium. The sample was then incubated at 36 °C for 48 h. A ruler calibrated in millimeters was employed to measure the observed inhibition zones.

Illustration of Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC)

To detect MIC of the examined oils *versus* every yeast species, a dilution of 0.5 McFarland was made. Each tested oil sample was diluted to 1.0 mg/mL. Utilizing 96-well plates, 100 µL of RPMI 1640 broth set at pH 7 with a MOPS buffer were put into each well. To execute serial dilution, 100 µL of each examined oil was combined with RPMI in every well of the first column. To evaluate the yeast suspensions, 100 µL (0.5 McFarland) was applied to each well. Negative control wells were those lacking yeast cell suspensions. Next, the specimens were incubated for one day at 34 ± 2 °C. The MIC was established as the smallest amount of tested oil that resulted in a 50% decrease in growth when contrasted with controls.

To evaluate the MFC, 50 µL of the clear blended well solution (devoid of visible growth) was cultured on Sabouraud Dextrose Agar plates and incubated for 48 hours at 35 °C. MFC was the smallest dose of the oil samples that inhibited growth (99.9%) when compared to growth in the control group (without treatment). The number of every yeast colony (CFU/mL) at various doses was compared to the number of each yeast colony in the control (no treatment) (Borman *et al*. 2017).

Evaluation of Antineoplastic Impact

SKOV3 cells were utilized to assess the anticancer properties of the evaluated oils, employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Initially, 5 mg of MTT was mixed in 1 mL of phosphate buffer saline (PBS). The produced cells (10^5 cells/mL) were then injected into a 96-well tissue culture plate and cultured for one day at 37 °C to form a complete monolayer film. Upon completion of incubation, the medium was withdrawn from the wells, and the cell monolayer was gently washed twice with sterile phosphate-buffered saline (PBS) to remove any residual medium and unattached cells.

Various dilutions of the specimens were prepared in Human Plasma-like Medium supplemented with 2% fetal bovine serum. Each concentration (0.10 mL) was evaluated in a different well, with three wells serving as a control that obtained only maintaining media. The samples then underwent incubation at 38 °C and examined for any physical indicators of toxicity. Next, 20.0 µL of MTT solution was injected to each well to mix it into the medium. The plate was shaken on a shaking workstation for 5 min at 150 rpm before being incubated at 38 °C with 6.0% CO_2 for 1 to 6 h to allow MTT to metabolize. MTT metabolized output was reconstituted in 20.0 µL of DMSO and shaken at 150.0 rpm for 5 min (Qanash *et al*. 2023b).

Cell Cycle Testing

To investigate the mechanism underlying the cytotoxic potential of ozonized camphor oil, its influence on cell-cycle progression was assayed employing SKOV3 ovarian cancer cells. SKOV3 cells (4.0×10^6 cells per T-75 flask) were treated with ozonized camphor oil at its IC_{50} dose for 24 h under standard cell culture conditions (37 °C and 5% CO_2). After treatment, the cells were harvested by trypsinization, washed twice with PBS, and fixed in ice-cold 70% ethanol at 4 °C. The fixed cells were then stained using the Cycletest™ Plus DNA Reagent Kit (BD Biosciences, USA) following the manufacturer's instructions. In the final step, cell cycle progression was evaluated utilizing a FACSCalibur flow cytometer (BD Biosciences, USA), and the distribution of cells across G0/G1, S, and G2/M phases was quantified (Zaher *et al*. 2016).

Statistical Testing

Each analysis was performed in triplicate for accuracy, and the results are expressed as mean ± standard deviation (SD). Following the Student's *T*-test, the results were analyzed using Graph Pad Prism V8 (San Diego, USA) tool.

RESULTS AND DISCUSSION

Volatile and Semi-Volatile Composition of Crude and Ozonized Camphor Oil

The diverse chemical constituents of crude camphor oil and its ozonized form were examined using GC-MS analysis. Twenty-one molecules from 15 different classes could be seen in crude camphor oil, while twenty-two compounds from fifteen various classes could be perceived in the ozonized camphor oil (Figs. 1A and 1B). It could be noticed that 15 compounds were observed in both oil forms including: eucalyptol; ç-terpinene; (+)-2-bornanone; methyl salicylate; 1-hexadecanol, 2-methyl-; 9-octadecanoic acid (z)-; 12-methyl-e,e-2,13-octadecadien-1 ol; 14-.a.-h-pregna; cyclopentane, (4-octyldodecyl)-; heptyl tetracosyl ether; 1-heptacosanol; ethanol, 2-(octadecyloxy)-; 17-pentatriacontene; dotriacontane; and 2,2-dideutero octadecanal. Besides, it could be noticed that eucalyptol and methyl salicylate were the major compounds in crude camphor oil, and both of them were slightly decreased upon exposure of oil to ozone.

The results indicate that ozonization induced the formation of several new compounds that were absent in crude camphor oil, including α-pinene, cis-11-eicosenoic acid, cis-vaccenic acid, dodecanoic acid (3-hydroxy-), and carbonic acid esters. In addition, the relative abundances of existing compounds changed noticeably after ozone treatment. For instance, 2,2-dideutero octadecanal increased from 6.94% to 11.13%, and dotriacontane rose from 4.55% to 11.88%. The increase in these compounds may be the result of the transformation of other susceptible components during ozonization, highlighting their particular susceptibility to ozonization. Furthermore, significant levels of pentalene, octahydro-1-(2-octyldecyl) (6.11%), heptyl hexacosyl ether (5.36%), and carbonic acid, eicosyl vinyl ester (4.25%) were observed in the ozonized oil. Minor amounts of α-pinene, dodecanoic acid (3-hydroxy-), cis-11-eicosenoic acid, and cis-vaccenic acid were also detected. Overall, these results demonstrate that ozonization both generates new chemical species and alters the composition of existing compounds in camphor oil (Tables 1 and 2).

The exposure to ozone causes oxidative alteration of unsaturated fatty acids in oils, resulting in the development of key ozonides, aldehydes, ketones, and secondary byproducts of oxidation that affect their chemical structure and perceived value. These reactions frequently follow a specific mechanism, resulting in reactive intermediates that break down into volatile chemicals (Coffaro and Weisel 2022; Freis and Vemulapalli 2025). GC-MS is commonly used to detect these ozonation products due to its great sensitivity in recognizing low-molecular-weight aldehydes, epoxides, and short-chain acids produced during oxidation.

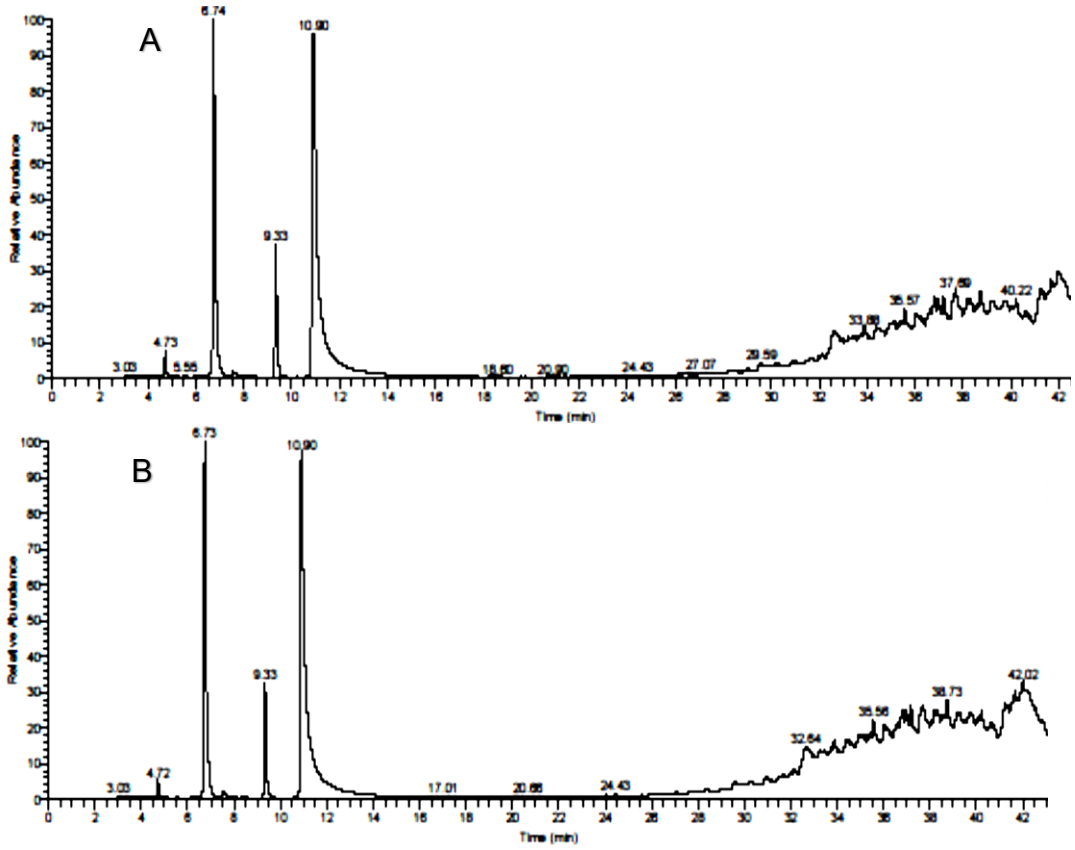

Fig. 1. GC-MS pattern illustrating various molecules for (A) Crude camphor oil, (B) Ozonized camphor oil

Distinctive mass-fragmentation patterns permit the identification of structurally identical oxidation products, whereas chromatographic separation eliminates complex mixtures formed during ozonolysis (Gu *et al*. 2021; Rontani *et al*. 2022). Ozonation of oils has the potential to profoundly change their chemical structure by oxidizing sensitive portions of lipids and upsetting the balance of essential bioactive molecules. During this process, ozone combines with double bonds in fatty acids, producing ozonides, peroxides, and secondary products of oxidation that may impair oil quality and usefulness. The major constituents of camphor oil, identified by GC-MS, include eucalyptol, methyl salicylate, (+)-2-bornanone, and oleic acid, among others. Many of these compounds contain functional groups prone to oxidative reactions. For instance, double bonds in monoterpenes (*e.g*., ç-terpinene, 1R-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene) and unsaturated fatty acids (*e.g*., oleic acid, 9-octadecanoic acid) are susceptible to ozonization, forming peroxides,

aldehydes, and other oxygenated derivatives. Additionally, oxygen-containing groups in terpenoids (eucalyptol, bornanone) and esters (methyl salicylate) may also undergo oxidative transformations, affect the chemical structure and associate biological activities of the ozonized camphor oil (Repciuc *et al*. 2025). The compounds listed in Tables 1 and 2 are expected to serve as anti-yeast and anticancer agents. Among the identified compounds, eucalyptol (1,8-cineole) represents one of the most relevant compounds.

Table 1. Detected Molecules in Crude Camphor Oil *via* GC-MS Testing

Retention Time (min)	Compound Name	Area %	Class	Molecular Formula	Molecular Weight
4.72	(1R)-2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene	0.67	Unsaturated hydrocarbon	$C_{10}H_{16}$	136
6.74	Eucalyptol	19.04	Terpenoid	$C_{10}H_{18}O$	154
7.54	ç-Terpinene	0.30	Monoterpene	$C_{10}H_{16}$	136
9.33	(+)-2-Bornanone	4.86	Terpenoid	$C_{10}H_{16}O$	152
10.91	Methyl salicylate	29.05	*o*-Hydroxyben-zoic acid ester	$C_8H_8O_3$	152
29.58	1,2-15,16-Diepoxyhexadecane	0.61	Epoxide	$C_{16}H_{30}O_2$	254
30.95	1-Hexadecanol, 2-methyl-	1.7	Fatty alcohol	$C_{17}H_{36}O$	256
32.57	9-Octadecanoic acid (Z)-	2.97	Fatty acid	$C_{18}H_{34}O_2$	282
32.64	12-Methyl-E,E-2,13-octadecadien-1 ol	2.30	Fatty alcohol	$C_{19}H_{36}O$	280
32.98	Oleic acid	1.21	Fatty acid	$C_{18}H_{34}O_2$	282
34.09	2-Dodecen-1-yl(-) succinic anhydride	0.56	Cyclic dicarboxylic anhydride	$C_{16}H_{26}O_3$	266
35.56	14-.a.-H-Pregna	5.38	Steroid	$C_{21}H_{36}$	288
36.16	Cyclopentane, (4-octyldodecyl)-	1.19	cycloalkane	$C_{25}H_{50}$	350
36.80	Docosyl octyl ether	3.47	Alkyl ether	$C_{30}H_{62}O$	438
37.19	Heptyl tetracosyl ether	5.35	Heptyl alcohol	$C_{31}H_{64}O$	452
38.59	1-Heptacosanol	2.22	Fatty alcohol	$C_{27}H_{56}O$	369
38.74	Ethanol, 2-(octadecyloxy)-	4.47	Fatty alcohol	$C_{20}H_{42}O_2$	314
40.22	Butyl dotriacontyl ether	1.13	Cyclic ether	$C_{36}H_{74}O$	522
40.41	17-Pentatriacontene	2.03	Alkane	$C_{35}H_{70}$	490
41.29	Dotriacontane	4.55	Alkane	$C_{32}H_{66}$	450
41.65	2,2-Dideutero octadecanal	6.94	Fatty aldehyde	$C_{18}H_{34}D_2O$	270

It has been reported to exhibit antibiofilm activity against *Candida albicans*, either alone or in combination with antifungal agents, indicating its role in disrupting fungal virulence and enhancing drug efficacy (Keymaram *et al.* 2022). In addition, eucalyptol has demonstrated promising anticancer properties, particularly through inhibition of metastasis in skin cancer models under both *in vitro* and *in vivo* conditions (Rahaman *et al.* 2022). Salicylate derivatives identified in the analyzed oils may also contribute to the anticancer activity. These compounds have been shown to suppress the proliferation of metastatic

breast cancer cells by inducing cell cycle arrest without triggering apoptosis, suggesting a cytostatic mechanism that may complement other anticancer pathways (Karalis *et al.* 2020). Furthermore, dotriacontane, a long-chain hydrocarbon detected in the current oils, has been implicated in potential therapeutic activity. Molecular docking analysis has suggested its ability to interact with biologically relevant targets, supporting its possible contribution to the observed biological effects such as antimicrobial and anticancer activities (Qanash *et al.* 2022).

Table 2. Detected Molecules in Ozonized Form of Camphor Oil *via* GC-MS Testing

Retention Time (min)	Compound Name	Area %	Class	Molecular Formula	Molecular Weight
4.71	α-Pinene	0.34	Monoterpene	$C_{10}H_{16}$	136
6.73	Eucalyptol	11.30	Terpenoid	$C_{10}H_{18}O$	154
7.53	ç-Terpinene	0.31	Monoterpene	$C_{10}H_{16}$	136
9.33	(+)-2-Bornanone	3.37	Terpenoid	$C_{10}H_{16}O$	152
10.90	Methyl salicylate	27.83	*o*-Hydroxyben-zoiacid ester	$C_8H_8O_3$	152
29.58	Dodecanoic acid, 3-hydroxy-	0.30	Hydroxy acid	$C_{12}H_{24}O_3$	216
29.71	*cis*-11-Eicosenoic acid	0.14	Fatty acid	$C_{20}H_{38}O_2$	310
30.18	1-Hexadecanol, 2-methyl-	1.17	Fatty alcohol	$C_{17}H_{36}O$	256
32.64	12-Methyl-E,E-2,13-octadecadien-1 ol	2.18	Fatty alcohol	$C_{19}H_{36}O$	280
32.85	9-Octadecanoic acid (Z)-	0.9	Fatty acid	$C_{18}H_{34}O_2$	282
33.12	*cis*-Vaccenic acid	0.20	Fatty acid	$C_{18}H_{34}O_2$	282
33.24	14-.α.-H-pregna	5.25	Steroid	$C_{21}H_{36}$	288
35.42	1-Heptacosanol	0.47	Fatty alcohol	$C_{27}H_{56}O$	369
35.56	Cyclopentane, (4-octyldodecyl)-	1.60	cycloalkane	$C_{25}H_{50}$	350
36.01	Carbonic acid, eicosyl vinyl ester	4.25	Vineyl ester	$C_{23}H_{44}O_3$	368
36.92	17-Pentatriacontene	1.69	Alkane	$C_{35}H_{70}$	490
37.18	Heptyl tetracosyl ether	1.93	Heptyl alcohol	$C_{31}H_{64}O$	452
37.67	ene, octahydro-1-(2-octyldecyl)	6.11	Sesquiterpene	$C_{26}H_{50}$	362
38.35	Ethanol, 2-(octadecyloxy)-	2.29	Fatty alcohol	$C_{20}H_{42}O_2$	314
38.73	Heptyl hexacosyl ether	5.36	Heptyl alcohol	$C_{33}H_{68}O$	480
39.69	2,2-Dideutero octadecanal	11.13	Fatty aldehyde	$C_{18}H_{34}D_2O$	270
41.28	Dotriacontane	11.88	Alkane	$C_{32}H_{66}$	450

Anti-Yeast Pattern of Camphor Oil and its Ozonzied Form

Testing of crude and ozonized camphor oil forms was carried out towards *C. albicans*, *C. tropicals*, and *C. glabrata* (Fig. 2). The present results revealed that ozonation of camphor oil ressulted in a slight elevation of the inhibition zone towards *C. albicans*, *C. tropicals*, and *C. glabrata* from 20±1 mm, 22±0.9 mm, and 22±0.5 mm, repectivley, for the crude camphor oil to 23±0.6 mm, 26±0.4 mm, and 25± 0.2 mm, subsequently for the ozonzied oil (Table 3). Camphor oil and its ozonized form have strong anti-yeasts activity against *C. albicans*, *C. tropicalis*, *and C. glabrata*. The native oil has mild growth inhibitory properties, most likely due to its naturally bioactive fatty acids and subsequent metabolites (Ivanov *et al*. 2021; Argüelles *et al*. 2024). Ozonation boosts this activity by producing reactive oxygen species and ozonides, which efficiently damage fungal cell membranes and metabolic activities. As a result, the ozonized version has a higher antifungal effectiveness, indicating its potential as a complementary or alternative way to treating *Candida*-related infections. Recognizing these comparative impacts is critical to optimizing therapeutic uses (Augello *et al*. 2024). These outcomes are in line with prior research that has confirmed the antimicrobail properties of ozonized oil (Al-Rajhi *et al*. 2024, 2025a; Alsalamah *et al*. 2025b). A notable decrease was recorded ($P \leq 0.05$) in MIC and MFC levels of ozonized camphor oil relative to crude oil towards *C. albicans*, *C. tropicals*, and *C. glabrata* (Table 3). The production of ozonides, peroxides, and other reactive oxygen species, which exacerbate rupture of membranes and obstruct vital metabolic pathways in Candida cells, is probably responsible for this increase in activity (Yu *et al*. 2016; Wang *et al*. 2023). The presnt resuts revealed that smaller quantities of the ozonized oil were sufficient to limit growth and produce fungicidal effects, according to lower MIC and MFC levels. These results imply that the chemical profile of the oil was changed by ozonation into a more bioactive form with greater antifungal effectiveness, as reported by other invitigators (Augello *et al*. 2024). Thus, the notable enhancement in performance validates the viability of ozonized camphor oil as a viable option for substitute antifungal tactics in accordance with other previusly publised resports illustating the effective role of ozozntion to imaprove antimicrobail imapct of natural oils (Cho *et al*. 2021; Travagli and Iorio 2023).

Table 3. Anti-yeasts, MIC, and MFC Impact of Crude Camphor Oil and Its Ozonized Form Relative to Fluconazole (25 µg/mL) as a Standard Drug

Test microbes	Inhibition zone (mm)			MIC		MFC	
	Crude oil	Oil+ O_3	Standard drug	Crude oil	Oil+ O_3	Crude oil	Oil+ O_3
C. albicans	20±1.0^{a}	23±0.6^{a}	20±0.4^{a}	62.5±0.2^{a}	31.25±0.2^{b}	125±0.4^{a}	62.5±0.2^{b}
C. tropicalis	22±0.9^{a}	26±0.4^{a}	20±0.5^{a}	62.5 ±0.3^{a}	15.62±0.2^{b}	125±0.2^{a}	15.62±0.3^{b}
C. glabrata	22±0.5^{a}	25±0.2^{a}	19±0.8^{a}	62.5±0.5^{a}	15.62±0.4^{b}	125±0.5^{a}	15.62±0.4^{b}

Results of Inhibition (zones mm) are written as means ± SD; Similar letters above numbers denote to non-significant difference $P > 0.05$), where distinct letters denote significant differences $P \leq 0.05$).

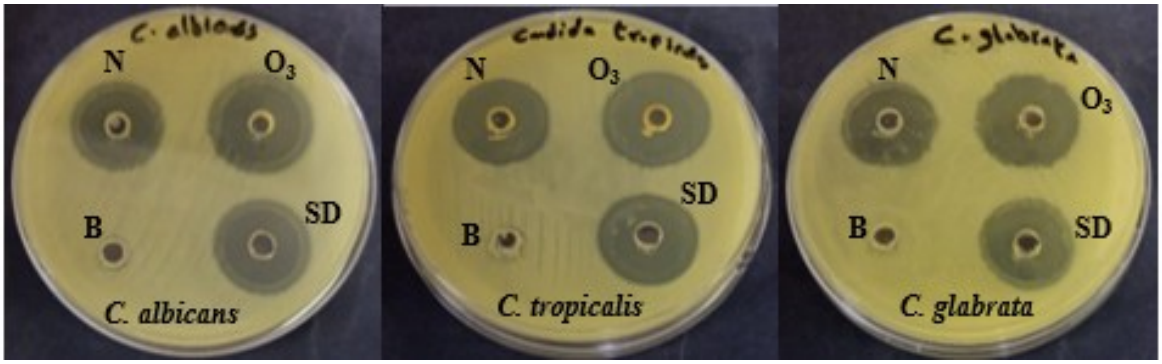

Fig. 2. Agar diffusion assay to test anti-yeasts imapct of camphor oil and its ozoznied forms towards *C. albicans*, *C. tropicals*, and *C. glabrata* (B: Blank, SD: standard drug (Fluconazle, N: crude oil, and O_3: ozonzied oil).

Anti-cancer Impact of Camphor Oil and its Ozonzied Form

In this study, both crude camphor oil and its ozonzied form were assessed over a range of 31.25–1000 µg/mL toward SKOV3 cells and the cells were examined under inverted microscope to comapre the imapct upon using various levels of both treatments (Fig. 3). Both crude camphor oil and its ozonized version have shown anticancer properties in *in vitro* tests, largely by causing apoptosis and reducing cell proliferation. Camphor crude oil demonstrated an inhibitory effect against SKOV3 cells (IC_{50} = 183.18 ± 2.29 µg/mL), while ozonized camphor oil showed a small increase in cancer action (IC_{50} = 152.0.4 ± 0.4 µg/mL) (Fig. 4).

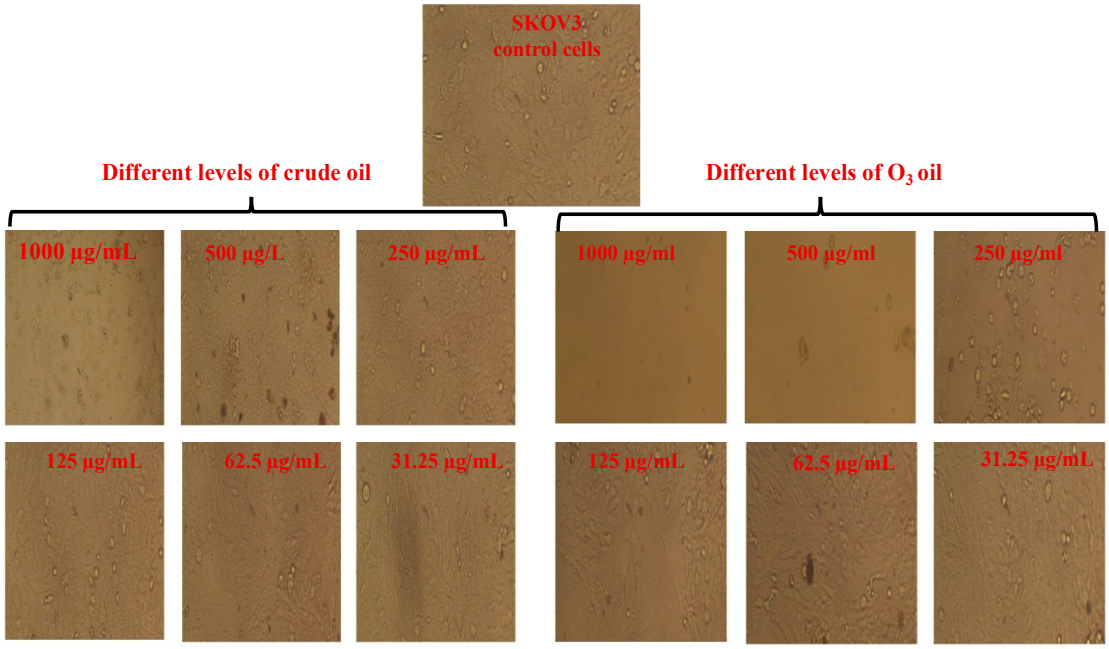

Fig. 3. Microscopic evaluation for various levels (31.25 - 1000 µg/mL) of camphor oil and its ozonized form on SKOV3 cells *versus* control cells was analyzed through an inverted microscope (Magnification = 400X)

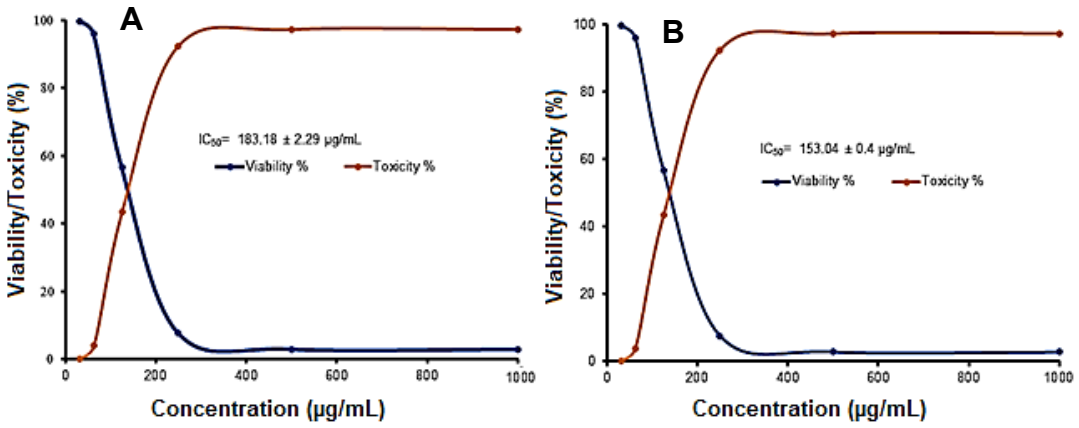

Fig. 4. Statistical testing for anticancer impact of camphor oil and its ozonized form towards SKOV3 cells (Data are represented as means ± SD.)

Ovarian cancer is an understudied malignancy with a dismal prognosis and few viable treatments (Tan *et al*. 2021). Both crude camphor oil and its ozonized version were cytotoxic to SKOV3 ovarian cancer cells in an dose-dependent manner, with the ozonized oil displaying a slightly higher efficacy. The higher anticancer effect of the ozonized version could be associated with the production of reactive oxygen species (ROS) and peroxidic species, which can cause mitochondrial dysfunction and apoptosis, as has been documented for ozonized oils in other cancer models (Izzotti *et al*. 2022). Furthermore, essential oils containing monoterpenes, such as camphor, are known to reduce the growth of cancer cells by triggering intrinsic death pathways. These data imply that ozonization may improve camphor oil's pro-oxidant and pro-apoptotic characteristics, leading to an even more successful *in vitro* anticancer substance (Machado *et al*. 2022; Al-Rajhi *et al*. 2025b).

Comparative Impact for Camphor Oil and its Ozonzied Form on Cell Cycle of SKOV3 Cells

Flow cytometry research revealed that crude camphor oil disrupted the normal cell cycle characteristics of SKOV3 cells. Most untreated cells were in G0/G1 (59.63%), with a significant percentage in S phase (39.06%), indicating active DNA synthesis. After treatment with IC_{50} of ozonized camphor oil, S-phase cells dropped to 24.24%, while G2/M phase cells rose from 1.3% to 14.69%. This suggests that the therapy inhibits DNA replication and causes a G2/M arrest, which is a frequent method by which cytotoxic molecules stop proliferation and promote death (Fig. 5).

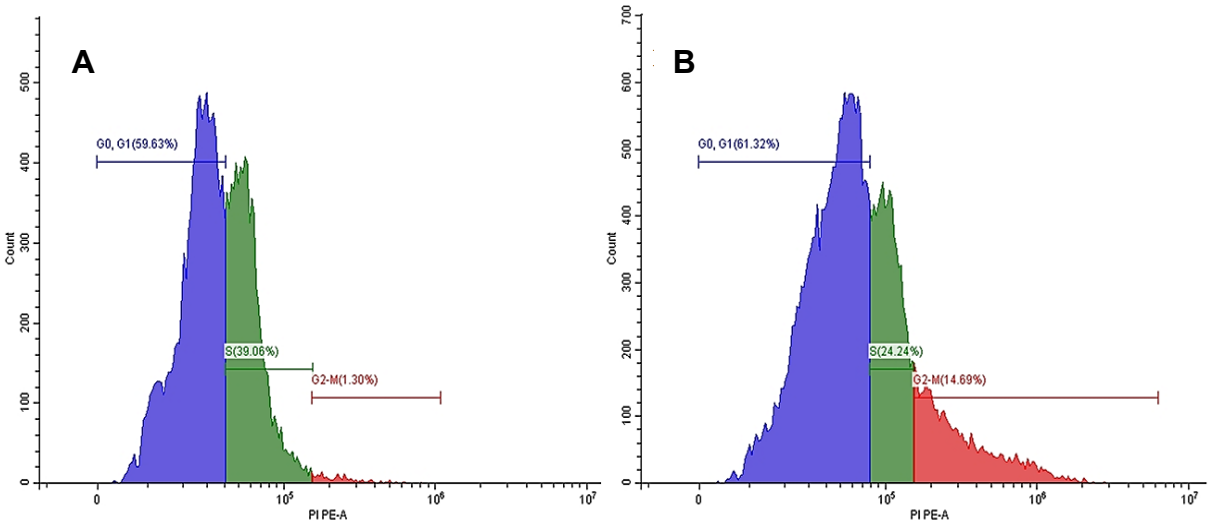

Fig. 5. Flow cytometric analysis evaluation of various cell cycles of SKOV3 cells (A) Control untreated SKOV3 cells; (B) Treated SKOV3 cells with IC_{50} of ozonized camphor oil. The blue, green, and red areas represent the G0/G1, S, and G2/M phases, respectively.

Thus, the rise in G2/M population, combined with a decrease in S-phase cells, supports an approach in which ozonized camphor oil causes replication stress, limits cell division, and induces death in SKOV3 ovarian cancer cells. The observed change from S phase to G2/M phase shows that ozonized camphor oil inhibits DNA synthesis, activating checkpoint mechanisms that prevent mitotic progression. Other essential-oil constituents including monoterpenes have been shown to cause G2/M arrest by inducing oxidative stress and destruction of DNA, triggering ATM/ATR signaling, and inhibiting cell-cycle progression (Kello *et al*. 2020; Liu *et al*. 2023). Ozonized oils are known to produce peroxidic chemicals that promote ROS generation, resulting in mitochondrial malfunction and cell-cycle blockage (Chirumbolo *et al*. 2023). Camphor-based drugs have also been demonstrated to inhibit the cyclins and CDKs essential for S-phase completeness (Sánchez-Martínez *et al*. 2019).

It is important to point out that, although ozonized camphor oil verified promising biological activities *in vitro*, its safety and efficacy in humans have not been established. Regulatory approval would require compliance with governmental standards for testing, including rigorous toxicological and clinical evaluations. The generation of new characterized constituents during ozonization may pose additional risks, and so, careful assessment of potential adverse influences is essential before any consideration for human applications (Leon *et al.* 2022).

CONCLUSIONS

1. Camphor oil's chemical profile and composition were altered after ozonation. Gas chromatography – mass spectrometry (GC-MS) analysis revealed the formation of additional compounds and an increase in the levels of certain constituents, which may have contributed to the observed biological activities.
2. Wider inhibition zones and markedly lower MIC and MFC values against *C. albicans*, *C. tropicalis*, and *C. glabrata* were also observed using ozonized camphor oil,

indicating that ozonation is a viable method to increase camphor oil's medicinal efficacy

3. A lower IC_{20} value and the inducement of G2/M cell-cycle arrest, which indicates interruption of DNA replication and development to mitosis, demonstrated the ozonized oil's better anticancer properties against SKOV3 cells.
4. A limitation of this study is that the safety of ozonized natural products in humans is unknown, as ozonization can generate new or reactive compounds. Further toxicological evaluation is needed before human use

FUNDING

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2601)

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Article submitted: November 23, 2025; Peer review completed: March 14, 2026; Revised version received and accepted: March 16, 2026; Published: March 27, 2026.
DOI: 10.15376/biores.21.2.4246-4262